Inheritance of resistance to *Claviceps purpurea* in the *Triticum aestivum* cv. 6B364 and evaluation of methods of inoculation.

by

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A thesis
presented to the University of Manitoba
in fulfillment of the
thesis requirement for the degree of
MSc
in
'Plant Science'

Winnipeg, Manitoba

ISBN 0-315-48103-X

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A thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

MASTER OF SCIENCE

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ACKNOWLEDGEMENTS

Special thanks to Dr. C.C. Bernier for his guidance and patience throughout this study and to my advisory committee of Dr. Reid and Dr. L.E. Evans for reviewing this manuscript.

The technical assistance from Mr. R. Smith and help from Dr. K. Rashid in computer programing was also appreciated.

Special thanks to my wife, Karen, who encouraged me to complete my studies.

I would also like to thank the Department of Plant Science for the financial assistance I received during this study.

ABSTRACT

R.G. McLeod. M.Sc., The University of Manitoba.1988.

Inheritance of resistance to Claviceps purpurea in the Triticum aestivum cv. 6B364 and evaluation of methods of inoculation.

Major Professor:Dr. C.C. Bernier.

The Triticum aestivum cv. 6B364 with complete resistance (no sclerotia) to 12 different ergot isolates and with less than four sclerotia per spike when inoculated with two other ergot isolates was crossed with the susceptible wheat lines Chinese Spring, UM684 and Columbus to evaluate the inheritance of the resistance in different backgrounds.

The F_1 and F_2 progenies from each cross were assessed for complete resistance (no sclerotia) and susceptibility (one or more sclerotia/spike). When sclerotia were present, plants were also evaluated for reactions to three components of partial resistance ie. the number of sclerotia/ spike, the amount of honeydew, and sclerotial size. The F_1 plants from each of the three crosses displayed incomplete dominance for susceptibility to each of the three disease components.

The anaylsis of the Chinese Spring F_2 suggested that one gene controlled the complete resistance to isolate M-4. The F_2 populations of UM684 and Columbus were both small. Although the segregation patterns did not deviate from a 1:3 ratio, both appeared closer to a 1:2 ratio. This, as well as the fact that several plants in F_3 families developed sclerotia although they were derived from F_2 plants with zero sclerotia, suggested that more than one gene controlled complete resistance. Based on a sampling of F_3 families from F_2 plants that developed no sclerotia, it appeared that two genes from 6B364 were required for resistance to approach that of 6B364 (mean number of sclerotia per plant in a F_3 family less than one) in the UM684 and Chinese Spring backgrounds, whereas one gene from 6B364 in the Columbus background appeared to be required to reach this same resistance level.

There was evidence of the same gene(s) lowering the numbers of sclerotia and the amounts of honeydew produced as the observed number of F_2 plants that were either partially resistant for both disease components, or susceptible for both components, were higher than expected. Conversely, lower than expected numbers of single F_2 plants that were partially resistant for one disease component and susceptible for the other were observed.

To assess the ability to select for complete resistance as well as partial resistance, the data from F_3 families was tabulated according to the disease reactions of the F_2 plants. In the Chinese Spring cross, F_3 families were evaluated from F_2 plants having partial and susceptible reactions for all three disease components. It appeared that selection for lower numbers of sclerotia or amounts of honeydew were effective.

Two F_4 lines from the Chinese Spring cross that were identified as having resistance to the M-4 isolate were inoculated with the ergot isolates F-1 and P-2 to assess whether selections for resistance to M-4 resulted in resistance to other isolates. Reduced numbers of sclerotia occurred in both F_4 lines. 6B364 was completely resistant to these two isolates.

In a second study, the effectiveness of inoculating the susceptible wheat cultivars Manitou and Chinese Sping with ergot using a hypodermic syringe, an airbrush, a vacuum system, a pressure pump, and a mist bottle were evaluated indoors and in the field. The most effective methods were the hypodermic syringe and the vacuum system. The airbrush was also effective if glumes were clipped before inoculating.

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INTRODUCTION

Claviceps purpurea Tul. attacks the ovary of many cereal and grass species with the purplish-black sclerotia of ergot replacing the seeds.

Ergot reduces yield, not only by the absence of seeds in infected florets, but also by causing adjacent ovaries to abort (Seymour and McFarland, 1921). An average of 1.8 ergot sclerotia per spike in rye resulted in 19 percent reduction in the number of seeds per spike and a 31 percent reduction in yield when compared to the averages for the uninfected spikes (Harper and Seaman, 1980a). Despite the relatively high yield reduction in infected spikes, the estimate of direct yield losses from ergot in rye was calculated as insignificant because of the low field incidence.

Ergot sclerotia are often harvested with the grain since they are similar in size to grain kernels. This can cause serious problems as alkaloids in the sclerotia cause a convulsive or gangrenous form of ergotism when consumed by domestic animals (Burfening, 1973). Symptons of ergotism in swine are reduced rates of gain and feed efficiency, agalactia and stillborne piglets (Campbell and Burfening, 1972). In chicks, dietary ergot levels above 1.6% significantly depressed growth and increased the feed to gain ratio. When dietary levels were above 3.2% ergot, high chick mortality occurred (Bragg et al., 1970). In cattle, the gangrenous form of ergotism causes the loss of limbs, lameness and a decrease in milk production (Burfening, 1973).

Presently, human poisonings from ergot are rare, although there are occasional reports of mass poisonings (Alexopoulus and Mimms, 1979). During the Middle Ages, when the toxic nature of ergot was not appreciated and rye bread was a staple of the diet, thousands of people died agonizing deaths from a gangrenous ergotism known as St. Anthony's Fire (Barger, 1931). Ergotism has even been suggested to have contributed to the eratic behavior observed in the 'witches' of Salem in 1690 (Caporael, 1976). However, Spanos and Gottlieb (1976) dispute this theory.

Due to the toxic nature of ergot, there are strict guidelines regulating the amounts of ergot allowed in grain. The Canadian Grain Act contain regulations on the limits allowed in commercial grain. No.1 wheat must have less than four kernel-sized sclerotia per 500 grams, and No.1 rye must have less than .05% ergot by weight . Grains containing slightly higher levels of ergot even cannot be sold at lower Canadian Wheat Board Feed Grades which allow maximium limits of ergot by percent weight in rye, wheat, oats and barley of .33%, .15%, .25%, and .25% respectively (Seaman, 1980).

The consequence of these regulations is the downgrading of large amounts of grain; this results in lower prices being received by producers. Conners (1954) reported that the incidences of rye carloads graded ergoty was 1.3% in the 1949-50 crop year, 7.7% in the 1950-51 crop year and 10.1% in the 1952-53 crop year. Seaman and Harper (1974) found that spring wheat and durum wheat downgraded in 1972 to be 0.3 and 0.6 million bushels respectively. In 1973, 0.01 million bushels of spring wheat and 1.5 million bushels of durum were reported downgraded.

Harper and Seaman (1980b) compared the levels of ergot infections in rye determined from their surveys made between 1972-76, with the levels of ergot infections reported by Conners (1954,1956). The infected spikes in 1953 and 1955 were estimated at 4.4% and 3.3% respectively, while in the 5 year period from 1972-76 infection levels varied from 0.02 to 0.11% infected spikes. Harper and Seaman (1980b) suggested that the reduction in ergot incidence was due partly to the reduction in the acreage of spring rye.

The cultural methods used to reduce the quantity of primary inoculum (crop rotation, clean seed, and plowing), and spread of secondary conidial inoculum (mowing of grasses in headlands), are not consistently effective. The introduction of cultivars with tolerance or resistance to ergot would be desirable.

Platford and Bernier (1970) screened for ergot resistance using a syringe to inject ten florets per spike with a suspension of 10^4 conidia per mL. A durum wheat cv. Carleton and a spring wheat cv. Kenya Farmer were identified as partially resistance. Kenya Farmer and Carleton tended to have fewer than 30 and 50 percent of the inoculated florets developing sclerotia, respectively. In susceptible wheat cultivars, over 70 percent of the inoculated florets developed sclerotia. In the abence of sclerotia, normal kernels were found in most of the inoculated florets of the susceptible culativars, whereas in the partially resistant cultivars Kenya Farmer and Carleton, kernels did not usually develop in the uninfected florets. Such florets contained either small discoloured and shrivelled ovaries or undeveloped kernels. Carleton and Kenya Farmer also produced smaller sclerotia and less

honeydew in infected florets than a susceptible cultivar. Bernier (1978) screened a further 292 accessions of Triticum spp. Twelve accessions were identified as completely resistance (no sclerotia in any of the 10 inoculated florets) to all but one to four of the 14 ergot isolates. Generally, the sclerotia induced in these cultivars were small and no visible amounts of honeydew were present.

The ability to transfer the identified resistance into new backgrounds has met with mixed success. Platford (1976) reported success in transferring the partial resistance of Kenya Farmer and Carleton into susceptible wheats of the same species. Further studies on the inheritance of the resistant reaction of Kenya Farmer and Carleton suggested that more than one gene controlled the disease reactions and that the frequencies of sclerotia, sizes of sclerotia, and amounts of honeydew produced were controlled by separate genes. In other studies, incomplete expression of resistance of hybrids and amphidiploids occurred in crosses between the resistant T. timopheevi lines and susceptible rye lines (Riley, 1973; Kraker,1979) and durum lines (Krakar, 1979).

This study was initiated to determine the inheritance of the complete resistance of the hard red spring line 6B364 identified previously by Bernier (1978), and whether the resistance is expressed differently in the backgrounds of three susceptible wheat cultivars.

Chapter II

LITERATURE REVIEW

Disease cycle of ergot

The sclerotium acts as the resting body for overwintering ergot. The sclerotia may lie in the field or be harvested along with the seed and then be replanted in the spring along with the seed. Recently, Vizos et al. (1984) reported ergot conidia had survived an extremely mild winter in Britain but it is likely that conidia normally play a minor role in primary infections in the spring. The absence of any other reports on the survival of conidia overwinter supports this contention.

The sclerotia remain dormant overwinter and germinate in the spring. Germination takes place most freely at temperatures between 10-20° C (Mitchell and Cooke, 1968). As a precondition to germination, sclerotia must inbibe water (Barger, 1931;Mitchell and Cooke, 1968) and have been exposed to temperatures below 10° C for several weeks (Mitchell and Cooke, 1968). Alternating thawing and freezing temperatures also are reported to help stimulate sclerotial germination (Brentzel, 1947). The requirement of a chilling period to activate sclerotial germination would help to prevent germination on warmer days in the fall.

Alexopoulos and Mimms (1979) reviewed Killian's work (1919) on the sexuality of ergot. Several small mushroom-shaped stromata about 1

cm long are produced on the sclerotium during germination. On the surface of the stroma is several small cavities each containing a single, multinucleate ascogonium, and one or more multinucleate antheridia. Plasmogamy takes place between an atheridium and an ascogonium. The perithecium, which subsequently develop is imbedded in the stromal head, and has a long necked ostiole opening to the surface of the stromata. Each mature perithecium bears several asci each containinng eight thread-like ascospores. McCrea (1931) established ten single-spore cultures all from separate sources, and found no evidence of antagonism when cultures were intercrossed. This would suggest homothallism exist with ergot.

Ascospores are either extruded or forcibly ejected (Colotelo and Cook, 1977). The peak production of ascospores in Western Canada occurs in late June (Brown, 1947). The period of ascospore release has been reported to be in excess of 50 days in Britain (Mitchell and Cooke, 1968) and 60 days in Australia (Bretag and Merriman,1981a). Peak ascospore production of about 16,000 ascospores per sclerotium per day in Britain occurred about 12 days after the initial release of ascospores (Mitchell and Cooke, 1968). Sclerotia collected from one plant source germinated over a 6 week period (Mitchell and Cooke, 1968). This staggered germination would help to extend the period of ascospore release.

The ascospores are wind disseminated. About five to ten days after ascospore infection, honeydew is visually apparent on infected heads. This honeydew contains conidia which initiate secondary spread of the disease. Conidia are dispersed by splashing rain and insects.

Atanasoff (1920) reported of 40 different insect species were attracted to the honeydew.

There are conflicting reports as to how the ergot fungus penetrates the ovary of its hosts. Campbell (1958) studied the infection process by the ascospores of C. purpurea in barley, and found that pentration by the fungus occurred at the base of the ovary. Similarly, Kirchoff (1929) reported the point of infection in rye by C. purpurea occurred at the base of the ovary.

Luttrell (1980) sprayed conidia of C. purpurea on the florets of rye and placed plants in a dew chamber. Within 4 hours, hyphae were observed between the cells of the stigma. By the second day,hyphae extended into the apical tissue of the ovary and by eighth day, the hyphae had disintegrated the outer integument replacing it with hyphae. The discrepancy between the findings of Luttrell (1980) and Campbell (1958) may be due to differences in the type of spore used for inoculating, the host species and the higher humidity after inoculation in Luttrell's experiments. Any of these factors may influenced the initial pentration site(s) and further testing is needed to verify the infection site(s) and infection proccess.

Plants infected with ergot incorporate more CO_2 due to the larger energy sink (Bacon and Luttrell, 1981). The ergot sclerotium has a higher metabolioc activity and has the ability to preferentially divert host plant nutrients at the expense of adjacent florets (Corbett et al., 1974).

In late summer, a sclerotium forms in the infected floret completing the life cycle.

Control Measures

Many ergot control measures aim at eliminating the initial sources of inoculum produced by the sclerotia in the spring. Sclerotia can overwinter in the soil or be introduced along with the seed during planting. The use of certified seed helps to ensure the level of ergot in the seed is low as this is a requirement for seed certification. For example, No. 1 seed of wheat may contain only one ergot sclerotium per kilogram of seed (Seaman, 1980).

Mitchell and Cooke (1968) reported that sclerotia which had been allowed to imbibe water and were then exposed to temperatures below 10° C for at least 8 weeks, had final germinations of around 90%. This suggests most sclerotia would germinate after the first winter. The ungerminated sclerotia remaining in the the field would be subject to degradation by soil micro-organisms. Cunfer and Seckinger (1977) found that C. purpurea sclerotia did not survive longer than 6 months in the soil in Georgia. Thus, an one year rotation with a non-host of ergot would minimize inoculum carryover from sclerotia in the soil.

Plowing also helps to prevent the release of ascospores into the atmosphere. When the sclerotia were covered by 5-8 cm of soil, the spore-bearing stalks were unable to reach the surface (Brown, 1947; Bretag and Merriman, 1981b).

Burning trash in the fall helps to destroy sclerotia. More recently, pollution associated with burning of trash has resulted in this practise being banned in certain areas (Hardison, 1972). Attempts have been made to identify fungicides to prevent sclerotial germination. Hardison (1972) evaluated 27 fungicides and reported that while some fungicides reduced the formation of ascocarps, none gave complete control. Furthermore, his test`s were conducted in the greenhouse and he mentioned difficulties in the use of fungicides to prevent ascosarp formation may be encountered in the field due to crop residue interfering with spray contacting the sclerotia.

The control of grasses within a cereal field limits the secondary spread of ergot by conidia. Bretag and Merriman (1981a) reported that in Australia ryegrass flowered over a 2 month period while wheat flowered over a 6 day period. Peak ascospore release occurred during ryegrass flowering but prior to wheat flowering. In a field of male sterile wheat free from ryegrass, 10.5% of the spikes were infected while in a stand containing ryegrass, 25% of the spikes were infected. In England, the control of blackgrass in male sterile wheat caused the number of sclerotia per plot to drop from 138 to eight (Mantle and Shaw, 1976).

The incidence of ergot in the field often is the highest along the edge (Conners, 1954,1956; Harper and Seaman, 1980a). The outside edges of a field may not be tilled and can contain grasses infected with ergot. These grassy margins can provide an initial source of inoculum and encourage the secondary spread by conidia from grasses to the cereal crop. The cutting or tillage of grasses along the fields edges is recommended to reduce the incidence of ergot (Seaman, 1980).

Hyperparasites have been isolated from honeydews which attack the sphacelial stage of ergot. Mower et al. (1975) tested several of these hyperparasites isolated from honeydews. Fusarium roseum 'Sambucinum' appeared the most aggressive and further tests were conducted. F. roseum reduced ergot significantly in irrigated conditions but failed to control the development of sclerotia in either the greenhouse or non-irrigated situations. An experiment by Cunfer (1976a) revealed that the low osmotic potentials of honeydew in the greenhouse and non-irrigated situations prevented the spores of Fusarium spp. from germinating. Irrigation caused the osmotic potential to approach zero and then the inhibited would germinate, with subsequent parasitizing of the ergot mycelium. Compounds inhibiting spore germinations of some fungi have also been isolated from honeydews (Cunfer, 1976b), and these could also place a limitation on the use of hyperparasites to control ergot.

Wood and Coley-Smith (1980) evaluated the effectiveness of seven fungicides in controlling ergot in male sterile barley. None of the fungicides evaluated gave complete control, and timing of sprays were critical for disease reductions to occur. In 2 years of testing, Harper and Christensen (1986) found that none of the five systemic fungicides evaluated effectively controlled ergot of rye, all gave less than 50 percent control. There are no fungicides registered for control of ergot in Canada presently.

Physiological resistance to ergot has been identified in wheat (Platford and Bernier, 1970) and in barley (Cunfer et al., 1975) but the transference of this resistance into new commerical cultivars has

yet to be achieved. In the triticale breeding program at the University of Manitoba, lines having large numbers of sclerotia are discarded and this selection eliminates lines with sterility problems rather than identifing lines with physiological ergot resistances. However, Thakur et al. (1981) successfully developed inbred millet lines with physiological resistances to Claviceps fusiformis 'Loveless'.

Seaman (1980) described measures used to remove or reduce the percentage of sclerotia in harvested grains. Blending infected seed with non-infected seed could be used to reduce the percent sclerotia to a nonsignificant level. Sclerotia may be removed physically by screening or floating off in a brine solution. Sclerotia could also be removed from the grain during combining. There are no reports of how much ergot passes right through a combine (Harper and Seaman, 1980a).

Factors Affecting Infection

(i) Attributes of the Host

Field observations of cultivated cereals found that ergot incidences varied with crop species, with the order of decreasing incidences in cultivated cereals being rye, wheat, barley and oats (Weniger,1924;Dillion-Weston and Taylor,1942). When artifical inoculations using a hypodermic syringe to inject spores into florets were undertaken, lines from these four cereal species showed approximately equal susceptibility (Platford and Bernier,1976) with differences in infection levels between artificial inoculation and natural infection being attributed to escape mechanisms based on flower morphology and flowering habit rather than to physiological resistance.

One factor affecting the level of infection in the field is the timing of anthesis in relationship to spike emergence. Pollination increases cereals resistance to ergot (Abe and Kono, 1957; Campbell and Tyner, 1959; Rapilly, 1968; Ratanopas, 1973; Cunfer et al., 1975; Thakur and Williams, 1980). Anthesis in oats and barley occurs in the boot stage. Consequently, the ovary will have low susceptibility to ergot when the spike emerges. Anthesis in rye occurs after spike emergence. This increases the opportunity of ergot spores coming in contact with an unfertilized and still susceptible ovary. Since rye is a cross pollinating species, the florets open to allow entry of pollen. More open florets also increase the chance of ergot spores coming in contact with an unfertilized ovary.

The use of a hypodermic syringe to inject conidia into florets, allows one to distinquish between physiological resistance and reduced infection due to pollination effects. Pollination affects the infection level more on a line which exhibited incomplete resistance (lower number of sclerotia per spike) than a line that is susceptible (most inoculated florets having sclerotia). The delaying of inoculation until after pollination results in a reduction of the number of sclerotia/spike with complete resistance occurring in a partially resistant cultivar a couple days after pollination whereas a susceptible rye line did not develop complete resistance unless the inoculation was performed more than seven days after pollination (Ratanopas, 1973).

Cross pollinations between cultivars that exhibited differences in the lengths of time after pollination in developing complete ergot resistance, found that this resistant mechanism was maternally

controlled and not influenced by pollen parent (Darlington and Mathre, 1976).

Cereal cultivars have been identified which had reduced sclerotial size and produced lower amounts of honeydew when florets were inoculated using a syringe (Platford and Bernier, 1970).

(ii) Attributes of the Isolate

Conidia can be produced in culture and stored in a sugar solution until use (Lewis, 1959). Soos (1969) reported conidia stored 10 weeks in a sugar solution at room temperature did not significantly decrease in germination over this period. McCrea (1931) reported Claviceps produced by mycelia grown in continuous culture for two years were still infectious on rye.

Claviceps purpurea has a wide host range. The host species an isolate is collected from has little influence on its virulence on that or other species (Campbell, 1957; Riggs et al., 1968; Platford and Bernier, 1976; Darlington et al., 1977). Campbell (1957) collected 421 ergot isolates from 38 different host species and only one isolate from Glycera borealis Nash. failed to infect wheat, barley and rye. The Claviceps sp. occurring on wild rice is now considered an unique species (Pantidou, 1959).

Raising the concentrations of conidia up to 10^6 per mL increased the frequency of sclerotia produced per spike (10 florets inoculated using a syringe) on susceptible rye, triticale, spring wheat, barley,

and oat cultivars, and on the partially resistant spring wheat cv. Kenya Farmer and durum wheat cv. Carleton (Platford and Bernier, 1976). Although injecting a higher spore concentration of conidia raised the sclerotia frequency per spike, it did not affect the final sclerotium size or the amount of honeydew produced (Platford and Bernier, 1976).

Bernier (1978) screened 292 accessions of Triticum spp. using a moderately virulent isolate. Three plants per accession were inoculated using a syringe with ten florets per plant being injected with 10^4 conidia per mL. Accessions with less than 10 percent floret infection were retested with eight to 16 different ergot isolates. Accessions exhibited differential reactions with specific isolates with regards to the number of sclerotia per spike, suggesting that a gene for gene interaction may occur between ergot and its hosts.

(iii) Temperature

Temperatures between 10 and 30° C are required for mycelial extension in culture with an optinum temperature being around 25° C (McCrea, 1931; Mitchell and Cooke, 1968). Galapitage (1983) examined the optimum temperature for radial mycelial growth of ten isolates of Claviceps purpurea. Several had optima around 25° C although optima for the different isolates varied from 18 to 30° C. Further testing by Galapitage (1983) found there was no relationship between the optimum temperature for the extent of mycelial extension of an isolate in culture and the temperature which induced the highest percentage of sclerotia by syringe inoculation for the spring wheat varieties Manitou and Kenya Farmer.

Several researchers have noted greater infections by ergot in cooler growing seasons (Dillion-Weston and Taylor, 1942;Conners, 1954; Marshall, 1960). The effect of lower temperatures on cereals is to delay the occurrence of anthesis in relationship to spike emergence. This increases the period of plant susceptibility and would increase the possibility of spores coming in contact with an unfertilized ovary as well as the secondary spread of ergot by conidia from plant to plant.

Galapitage (1983) reported that the susceptible wheat variety Manitou generally had a higher percentage of sclerotia when inoculated at temperatures between 10 and 20° C than at 25° C. Conversely, the moderately resistant wheat variety Kenya Farmer tended to have a higher level of ergot infection at 25° C than at the lower temperatures; this indicates that the resistant genes are temperature sensitive. This work indicated complex three way interaction could occur between hosts, temperatures and isolates in determining the final levels of ergot.

Method of Inoculation

The level of natural infection of cereals by ergot in the field is generally low (Campbell, 1954; Harper and Seaman, 1980a). Several studies have evaluated different methods of inoculating to both increase the number of plants with sclerotia, and the number of sclerotia sclerotia per plant; this would increase efficiencies for screening cultivars for resistance, screening fungicides for the control of ergot, and producing of sclerotia for pharmaceutical purposes.

Lewis (1945) evaluated ergot inoculation of rye by catching flies, then exposing them to conidia of ergot and finally releasing them. This method of inoculating failed to cause any infections. Lewis (1945) also evaluated levels obtained by spraying of conidial suspensions of ergot onto field-grown rye in Michigan. The inoculated plots varied between 22 and 43 percent of the spikes infected while all the control plots had less than 6 percent of the spikes infected. Higher infection levels were obtained when inoculations were performed in the early morning to coincide with the opening of rye florets.

Puranik and Mathre (1971) evaluated several inoculation techniques on male sterile barley in the greenhouse. The use of an atomizer to spray conidia adjusted to 10^6 per mL resulted in low numbers of sclerotia even when plants were placed in a humidity chamber. Clipping the top 2 to 4 mm of the glumes and inoculating using a capillary tube or an atomizer resulted in 80 and 93%, respectively, of the florets being infected. If only the awns were removed, the use of these two latter techniques resulted in 81 and 83% infected florets respectively.

Campbell (1957) evaluated the virulence of ergot isolates by dipping spikes into a spore suspension, spraying inoculum onto spikes, by injecting a spore suspension with a syringe and by removing the glumes before spraying. Although he reported success in cross inoculations of isolates from different hosts, it was not clear in this paper which specific inoculation method was used to inoculate any one of the hosts.

The use of the hypodermic syringe to inoculate individual florets was used in studies to screen for disease resistance (Platford and Bernier, 1970;Bernier, 1978) and in inheritance studies (Riley, 1973; Platford 1976; Kraker, 1979). A high level of floret infection was obtained in susceptible plants by this method and differences in physiological resistance between lines were detected.

Peach and Loveless (1975) evaluated inoculation by syringe and spraying with an atomizer, when conidia concentration was adjusted to 3000 spores per mL. They used isolates collected from several grass species, and for each isolate approximately 10 plants were inoculated by each method and several grass isolates did not infect wheat when inoculated by spraying, yet the same isolates were infective when inoculated by syringe. They proposed that syringe inoculations did not represent natural infections and inoculations by this method would induce infections by some grass isolates that would not occur in in nature. However, the low number of spikes inoculated, the low spore concentration used, and the limited number of isolates collected from any one grass species make their conclusion questionable. Possibly, failure to induce infections using an atomizer was due to this method inducing low infection percentages, and not to some grass isolates being non-infectious on wheat. Thus, the inoculation of higher numbers of plants is necessary to verify that these grass isolates are unique.

Indirect methods have been evaluated in screening for ergot resistance. Lewis (1956) inoculated the coleoptile of two rye seedling lines with ergot conidia and found that differences observed in the seedling infection agreed with infection occurring in the field.

Platford (1976) used this coleoptile test to screen rye, triticale, wheat, oats and barley lines. The results from the coleoptile test were not always in agreement with results obtained from spikes inoculated by syringe. A high level of infection resulting from coleoptile inoculation occurred in rye and triticale lines found susceptible by syringe inoculations. However, oats, barley and wheat lines which were as susceptible as rye by the syringe method showed a marked decrease in infection following coleoptile inoculation, and no infection occurred in barley and oat seedlings. Although the coleoptile test may be a more rapid method of screening for resistance, the inconsistency between the coleoptile test and the adult plant susceptibility demonstrated by Platford (1976) places doubt on the value of this method.

Lewis (1962) evaluated the effects of guttation fluids collected from rye, wheat and barley on conidial germinations and germ tube growths. Rye guttation fluid was found to induced the most germ tube growth and that from barley the least. These results agreed with differences in resistance observed by the coleoptile test. The ability to use guttation fluid to measure resistance exhibited by the coleoptile test was questioned by Potbury and Drysdale (1969) who found no correlation between the reactions of wheat lines as determined in the coleoptile tests, and the reactions determined by germ tube growths in guttation fluid.

Methods of Measuring Physiological Resistance

The choice of a rating system will depend upon the objectives of the research and the resources available. Restrictions in time and labour may force the adoption of a more simpler rating system or a more

rapid inoculation system at the expense of accuracy in assessing of physiological resistance.

Many earlier researchers used the presence or absence of sclerotia to estimate infectivity of an isolate on a line (Stager, 1903; Campbell, 1957). This system was advantageous due to its simplicity but it would miss partially resistant plant types.

Futrell and Webster (1965) used the percentage of florets infected per spike to measure resistance in sorghum. This system would tend to identify intermediate resistant reactions.

Schmidt and Lucken (1976), using syringe inoculation, evaluated the number of sclerotia per spikelet, the weight of sclerotia per spikelet, and the size of sclerotia by passing through screens of different dimensions. They reported that for wheat, the number of sclerotia provided the most precise measurement of resistance.

Platford and Bernier (1970) and Riley (1973) used the number of sclerotia per spike, sclerotial size in relation to the seed and the amount of honeydew produced as parameters to measure the disease reaction in cereals. Riley formed a disease index which included all three disease parameters. Although the disease index developed would be helpful in deciding which plants to use in a breeding program, it would hinder a precise study on the inheritance of components of resistance as Platford (1976) found evidence that these three disease parameters were controlled by separate genes. This suggests it may be advantagous to look at all three disease components separately in an inheritance study.

Genetic Resistance to Ergot

The use of a hypodermic syringe to inject Claviceps spores into florets of cereals has revealed lines having lower numbers of sclerotia per spike (Platford and Bernier, 1970; Bernier, 1978).

The ability to transfer the identified resistance into new backgrounds has met with mixed succeess. The ergot resistant Triticum aestivum L. cv. Kenya Farmer (four or less of the ten inoculated florets per spike infected) and the T. durum desf. cv. Carleton (six or less of the ten inoculated florets infected) were crossed with susceptible wheats of the same species. In the resulting F_2 populations, plants were obtained with resistance equal to that of the resistant parent (Platford,1976). Riley (1973) found resistance was suppressed in F_1 produced from crosses between a resistant T. timopheevi Zhuk. line and a susceptible Secale cereale L. line. Simarly, Krakar (1979) found incomplete expression of resistance in hybrids and amphidiploids produced from crosses between ergot resistant T. timopheevi lines and susceptible rye and durum lines. Ergot resistance was also suppressed in progenies from crosses between wheat-rye, and wheat-agropyron (Galstjan-Avanesjan,1967). The lack of success in transferring resistance by the latter four researchers was likely associated with difficulty of transferring traits in interspecific crosses.

Schmidt and Lucken (1976) made diallel crosses between six wheat lines with varying resistance to ergot. They inoculated using a syringe and found general combining ability accounted for most of the variability for sclerotia number and weight of sclerotia per spikelet.

Platford (1976) investigated the number of genes controlling ergot resistance in Kenya Farmer and Carleton by crossing these two resistant lines to susceptible wheats of the same species and observing the F_1, F_2 and F_3 progenies. The disease reactions in the resulting progenies were measured by the numbers of sclerotia per spike, the sizes of sclerotia, and amounts of honeydew produced. Reduced sclerotia numbers appeared to be controlled by two recessive genes in Kenya Farmer and two dominant genes in Carleton. Analysis of F_2 progenies of both crosses suggested the three components of the disease reaction were controlled by separate genes. The genes for resistance appeared to be linked in Carleton, but assorted independantly in Kenya Farmer.

Platford (1976) et al. (1977) using Chinese Spring Monosomic Anyalsis of the resistance in Carleton and Kenya Farmer found chromosome 3B of Carleton and 6B of Kenya Farmer carried genes which conditioned the resistant response to all three disease components.

Strategies in developing disease resistant cultivars

The main objective of incorporating resistant genes into a cultivar is to reduce the level of disease when the crop is grown commercially. Johnston (1984) described durable resistance as resistance that remained effective during prolonged and widespread use in an environment favourable to disease development. Several different approaches have been successful in developing cultivars with durable resistance. Factors listed by Johnston (1983) as contributing to durability include:

A) The inherent durability of its resistance.

B)The variability of the pathogenicity of the pathogen.

C)The life cycle of the host and pathogen.

D)Extent of the deployment of the host cultivar and of other resistant and susceptible host cultivars or species.

E)Epidemiological factors affecting the size of the pathogen population.

Thus plant breeders must not only attempt to incorporate disease resistant genes into new cultivars but should also have a strategy in utilizing genes that will remain effective or durable over time.

Major genes conferring disease resistance.

There are some examples where single genes have conferred resistance over prolonged periods of time. Johnston (1984) quotes, as examples, single gene resistance to loose smut of wheat, leaf spot of maize, and Helminthosporium blight of oats. Johnson (1984) also suggested that a single gene was more likely to confer lasting resistance to a cultivar if the pathogen does not rely on the host for survival, but can survive either saprophytically or on other hosts.

Flor (1955) described the gene for gene relationship in which the host has genes for resistance and susceptibility and the pathogen has genes for virulence and avirulence. This theory helped explain why host resistance could be overcome by a pathogen.

The pyrimiding of several resistance genes into a single cultivar to make it more difficult for a pathogen to develop a gene combination for virulence has been utilized in developing a rust resistant cultivar. Green and Campbell (1979) reviewed the historical

development of resistant wheat cultivars to stem rust in Canada. The cultivar Selkirk (licensed 1953) and the cultivar Neepawa (licensed 1969) have maintained resistance over many years. In 1987, Neepawa was still grown on about 30% of the spring wheat acreage in the prairies although Selkirk was not widely grown. Green and Campbell attributed the durable resistance of these varieties to the number and combination of genes they possessed. Selkirk contains the resistance genes Sr6, Sr7b, Sr9d, Sr17 and Sr23. Neepawa is a Thatcher derivative of unknown genotype, but is presumed to carry the same geneas for resistance as Thatcher (Sr5, Sr9g, Sr12, Sr16) plus other genes as Neepawa has maintained resistance while Thatcher is rated susceptible in the field.

Certain resistance gene combinations have also provided more effective resistance to stem rust in Australia. Johnston (1984) reported that Australian wheat varieties with SR7a, Sr11, Sr17 and Sr36 genes, quickly became susceptible upon release, while the wheat varieties Timgalen, Timson, Cooke and Shortin with the gene combination Sr5, Sr6, Sr8 and Sr36 maintained their resistance over the years.

The gene for gene theory assumed independant action of major genes. On this basis, Van der Plank (1963,1968) argued that major genes for resistance would be eventually overcome and when this occurred, the rate of disease development would likely be more rapid due to the inability to select for genes which reduced the rate of disease development when backcrossing major genes. As an example, Van der Plank cites the susceptibility of the potato variety Vertifolia which was extremely susceptible to late blight when its resistance gene was overcome by virulent races.

Parlevliet (1983) questioned whether the Vertifolia effect was of common occurrence in a cultivar when the major genes were overcome by a pathogen. He cites an example of the potato cultivars with and without R-genes. The cultivars with the R-gene on average had a slightly higher field resistance to virulent isolates than cultivars without this gene. This would suggest minor genes were not necessarily lost during backcrossing. Reasons suggested for cultivars still maintaining field resistance even though the major genes were overcome by virulent pathogens were that commerical cultivars selected in backcrossing programs often have good field resistance, and there may have also been indirect selections of minor genes, if they enhanced the actions of the major gene(s).

Martin and Ellingboe (1976) developed isogenic wheat lines with and without the gene Pm4 which confers resistance to intial infection by powdery mildew. The presence of Pm4 in lines inoculated with isolates virulent to this gene had reduced rates of disease development. Nass et al. (1981) using isolines of winter wheat with Pm3c,Pm4 and MA demonstrated significant residual effects against an isolate of powdery mildew having virulence to all three genes. The resistance genes Pm2 and Pm5 were observed not to have any residual activity on disease development by this isolate. Similarly, Brodny et al. (1986) reported wheat lines with the defeated leaf rust genes Sr6, Sr8 and Sr9a had reduced number of pustules, pustule size and sporulation compared to wheat lines without these genes. These three examples suggest that major genes react epistatically to control more than one disease component although the lines used may not have been isogenic except for

the major genes and carried some rate reducing genes. The epistatic action of major genes would lessen the occurrence of the Vertifolia Effect.

Major genes have been observed to interact additively to enhanced resistance. Samborski and Dyck (1982) found that the wheat cultivar Columbus had enhanced resistance to leaf rust when the two genes Lr16 and Lr13 were present.

Van der Plank (1963) assumed that the only action of major genes was the independant action described by the gene for gene theory. On this basis, his warnings about utilization of major genes were warranted. However, it has been documented that genes that exhibit a gene for gene action with a pathogen may also react either epistatically to reduce the rate of disease development or react additively with other major genes to cause enhanced resistance. Therefore, the utilization of major genes should be considered in a breeding program when they are readily avaliable.

Resistance controlled quantitatively.

Van der Plank (1963,1968) suggested that resistance conferred by minor genes would be more durable because it was race non-specific. This generalization about minor genes has been questioned by Ellingboe (1982) who speculated that systems where the gene for gene theory has not been detected was due to inadequate work. Parlevliet and Zadoks (1977) modelled simulated gene for gene action based on the occurrence of small differential action between different polygenes on a computer program and showed that it would be difficult to recognize gene for gene

action when each gene had a small effect because their action would be masked by environmental effects.

Whether minor genes have race specific action will likely be debated for the next several years and probably never fully resolved. However, plant breeders have utilized polygenic resistance especially in cross pollinating crops using a recurrent selection program. Sharp (1983) quoted examples of leaf blight of maize, and bacterial wilt of alfalfa being under this control.

In self-pollinating species, polygenic resistance has not been utilized as often since it is simpler to use major genes. Resistance under polygenic control has been used to develop lines with effective resistance in self pollinating crops such as barley to leaf rust (Parlevliet et al.,1985), slower disease development to stripe rust of wheat (Krupinsky and Sharp,1978), and to barley net blotch and scald (Sharp, 1983).

Field Tolerance.

Roberts et al. (1984) reported that wheat lines which were of equal susceptibility to leaf rust demonstrated differences in percent yield loss for the same infection level. Crosses were made between these lines but selecting for this trait in derived F_2 populations failed. It was concluded this trait was under complex genetic control and it was doubted such tolerance could be used in a breeding program. Inability to select for this trait is not surprising as there is little response to yield selection from a F_2 plant to F_3 line (Knott, 1972).

Many lines classified as susceptible have been shown to process some genes for resistance. This is recognized by the occurrence of more susceptible F_2 plants than the susceptible parent used in a cross. Examples have been found with crown rust of oats (Simons, 1985), net blotch of barley (Sharp,1983), stripe rust of wheat (Krupinsky and Sharp, 1978) and rust of barley (Parlevliet and Kuiper, 1985). These observations indicate a minor disease could become more prevalent should background genes conferring field tolerance not be present in newly released cultivars.

Major genes are usually backcrossed into a susceptible cultivar with agronomically acceptable traits. Based on past experiences of major genes being overcome by a pathogen, a cultivar may last longer if major genes were incorporated into a line with some polygenic resistance. Should the resistance be overcome, at least the line will maintain yield by having genes that reduce the rate of disease development.

Chapter III

MANUSCRIPT 1

The inheritance of resistance to *Claviceps purpurea* in the *Triticum aestivum* cv. 6B364

ABSTRACT

The Triticum aestivum cv. 6B364 which exhibited complete resistance (no sclerotia) to 12 different ergot isolates and produced less than four sclerotia per spike when inoculated with two other ergot isolates when tested in a previous screening program, was crossed with the susceptible wheat lines Chinese Spring, UM684 and Columbus to evaluate its inheritance of the resistance in different backgrounds.

The F_1 and F_2 progenies from each cross were assessed for complete resistance (no sclerotia) and susceptibility (one or more sclerotia/spike). When sclerotia were present, plants were also evaluated for reactions to three components of partial resistance ie. the number of sclerotia/ spike, the amount of honeydew produced, and sclerotial size. The F_1 plants from each of the three crosses displayed incomplete dominance for susceptibility to each of the three disease components. The anaylsis of the F_2 populations suggested that one gene controlled the complete resistance to isolate M-4 in each cross. Many plants in F_3 families developed sclerotia even though they were derived from F_2 plants which failed to develop any sclerotia; this suggests that more than one gene controlled complete resistance. Based on sampling of F_3 families derived from resistant F_2 plants, it appeared that two genes from 6B364 were required for resistance to approach that of 6B364 (mean number of sclerotia per plant in a F_3 family less than one) in the UM684 and Chinese Spring backgrounds, whereas one gene from 6B364 in the

Columbus background appeared to be required to reach the same resistance level.

Narrow sense heritability values were calculated by regression of F_3 family means onto its F_2 parent in the progeny from the Chinese Spring cross. Positive heritability values were obtained for all three disease components when all F_2 plants were included, but values were significant for number of sclerotia and honeydew only when plants without sclerotia were excluded.

There was evidence that the same gene(s) lowered the number of sclerotia and the amount of honeydew produced as the observed numbers of F_2 plants that were either partially resistant for both disease components or susceptible for both components were higher than expected. Conversely, lower than expected numbers of single F_2 plants were observed to be partially resistant for one disease component and susceptible for the other.

To assess the ability to select for complete resistance as well as partial resistance, the data from F_3 families were tabulated according to the disease reactions of the F_2 plants. In the Chinese Spring cross, F_3 families were evaluated from F_2 plants having partial and susceptible reactions for all three disease components. There were indications that selection for either lower numbers of sclerotia or amounts of honeydew could be effective.

Two F_4 lines in the Chinese Spring cross identified as having resistance to M-4 (means less than 1 sclerotium/spike with 8 plants evaluated) were inoculated with the ergot isolates P-2 and F-1 to check

whether the selection for resistance with M-4 resulted in resistance to other isolates. Reduced numbers of sclerotia occurred in both F_4 lines. 6B364 was completely resistant to these two isolates.

This study indicated that it was important to select to the F_3 to ensure genes for ergot resistance were fixed.

INTRODUCTION

The incidence of the ergot fungus Claviceps purpurea Tul. on cereals in Western Canada is generally low (Campbell, 1954) and yield losses are not significant (Harper and Seaman, 1980a). The consumption of sclerotia harvested along with the grain can cause harmful side effects and poisoning (Burfening, 1973), and consequently there are strict guidelines regulating levels of ergot in grain. The standard for No. 1 hard red spring wheat is less than three kernel size sclerotia per 500 gm, and the lowest grade of Feed Wheat for the Canadian Wheat Board must have less than 0.15 percent sclerotia (Seaman,1980). A total of 2.4 million bushels of spring and durum wheat were downgraded in Western Canada in 1973 and 1974 (Seaman and Harper,1974).

Cultural methods such as rotation with non cereals and plowing are presently used to control ergot (Seaman,1980). The recent trends towards minimium tillage and the continous cropping of cereals in some regions could increase the carryover of inoculum causing ergot to become more prevalent. Attempts to control ergot with foliar fungicides have not been successful (Wood and Coley-Smith,1980; Harper and Christensen, 1986) and the development of resistant cultivars would offer an effective method of controlling this disease.

The order of decreasing incidences of ergot in fields among the cultivated cereals was reported to be rye (Secale cereale L.), wheat (Triticum aestivum L.) barley (Hordeum vulgare L.) and oats (Avena

sativa L.) (Weniger,1924; Dillon-Weston and Taylor,1942). The use of a syringe to inject inoculum into florets found that wheat, barley and rye lines were often similarly susceptible for the number of sclerotia developing per spike (Platford and Bernier, 1970). The differences observed between natural and inoculated infections were attributed to escape mechanisms based on flower morphology and flowering habit rather than a physiological resistance.

The number of sclerotia induced per spike when inoculations are performed before pollination are higher than the number of sclerotia induced per spike when inoculations are performed after pollination (Abe and Kono,1957;Campbell and Tyner,1959; Rapilly,1968; Ratanopas, 1973; Cunfer et al.,1975) and it is important to regulate the timing of inoculation prior to the occurrence of pollination to distinquish genetic resistance. The results of injecting of ergot spores into cereal florets, by hypodermic syringe, about 48 hours prior to anthesis found that lines having reduced numbers of sclerotia per spike, lower amounts of honeydew and smaller sclerotia (Platford and Bernier, 1970). The partially resistant cultivars identified, Kenya Farmer and Carleton, had either small, discoloured and shrivelled ovaries or undeveloped kernels in florets where sclerotia were absent. A susceptible cultivar usually had normal kernels in the absence of sclerotia in inoculated florets. Platford (1976) crossed the partially resistant Triticum aestivum L. cv. Kenya Farmer (less than four of the ten inoculated florets infected) and the T. durum desf. cv. Carleton (less than six of the ten inoculated florets infected) with susceptible wheats of the same species. In the F_2 populations, plants were obtained with a number of sclerotia equal to

that of their respective partially resistant parent. Anaylsis of F_2 progenies suggested that the disease components number of sclerotia per spike, amounts of honeydew produced, and sclerotial size were all controlled by separate genes. The partial resistance for reduced number of sclerotia appeared to be controlled by two recessive genes in Kenya Farmer and two dominant genes in Carleton. Attempts to transfer resistance to ergot in interspecific crosses have been less successful with resistance being suppressed in F_1 progeny from crosses between the resistant T. timopheevi Zhuk. lines and the susceptible lines of rye (Riley,1973; Krakar,1979) and wheat (Krakar,1979). These results indicate it would be advantageous to utilize resistance within a cereal species.

Further screening of 296 accessions of Triticum spp. identified several species and lines with few or no sclerotia when ten florets per spike were inoculated (Bernier, 1978). Generally, honeydew was not produced in amounts visible outside florets. Furthermore, inoculated florets without sclerotia contained small, discoloured and shrivelled ovaries or undeveloped kernels (Bernier,C.C. personnel communication). One of the several T. aestivum lines selected, line 6B364, demonstrated complete resistance to 12 ergot isolates and had less than four sclerotia per 10 inoculated florets with two other isolates. This study was initiated to determine the inheritance of the complete resistance of line 6B364, the influence of three different susceptible backgrounds on the expression of resistance, and whether, in the absence of complete resistance, the gene(s) involved might exert any influence on the three components of partial resistance identified by Platford and Bernier (1970,1976).

MATERIALS AND METHODS

Isolate selection and preparation of inoculum. All three ergot isolates were obtained from hyphal tips. The main isolate used in the study was M-4 which was isolated from a sclerotium collected from the spring wheat cultivar Manitou. The other two isolates used, P-2 and F-1, were isolated from sclerotia collected from Phleum pratense L. and Festuca arundinacea Schreb., respectivelty (Ratanopas,1976). All three isolates chosen were virulent on Chinese Spring and avirulent on 6B364, however, the three isolates were considered to each have a different combination of virulent genes since differences were displayed in their virulence/avirulence when several lines of Triticum spp. were inoculated (Bernier, personnel communication).

Conidia used for inoculation were obtained by placing mycelia of an isolate in a flask containing a modified potato sucrose broth (Lewis, 1959), and then the flask was placed on a gyrotory shaker for about ten days. The conidia produced were then separated from the mycelia by filtration, concentrated by centrifugation, stored in 60% sucrose at 4° C, and used within 4 months (Platford and Bernier, 1976). Inoculum was prepared daily by diluting the conidial suspension with sufficient sterile distilled water to adjust the concentration to 10^4 conidia/mL by using a spectrophotometer technique (Platford, 1976).

Plant materials and method of inoculation. The completely resistant spring wheat line 6B364 (no sclerotia in any of the ten florets

inoculated) was crossed reciprocally with the soft spring wheat cultivar Chinese Spring, the hard red spring wheat cultivar Columbus and the utility spring wheat line UM684 which has a Glenlea background. Three seeds of each of the parents, F_1, F_2 and selected F_3 families were planted in a 15 cm pot containing 2:1:1 (V:V:V) soil mixture of soil, sand and peat.

Plants were grown in the greenhouse and shortly before inoculation, plants were moved to a growth chamber to provide 16 hr illumination (190 mu/s/m^2), 8 hr dark period and day/night temperatures of 20/16° C. After inoculation, plants were kept in the growth chamber for 30 days until final scorings were completed. Ethirimol was applied as required to control powdery mildew.

Plants were inoculated as described by Platford and Bernier (1976). Individual spikes were inoculated with a syringe approximately 48 hours prior to anthesis to allow the expression of genetic resistance without interference from potential effects of fertilization (Ratanopas,1973). Each floret was injected with approximately 0.02 ml suspension of 10^4 conidia per mL with ten florets per spike being inoculated.

Assessment of the disease reaction. The number of sclerotia was recorded at 30 days. Plants having nine and ten sclerotia per spike were considered equally susceptible and combined into a single class. The days from planting to anthesis and the presence of awns were used as additional traits in assessing F_2 plants from the Chinese Spring cross.

The amount of honeydew produced, and the sclerotial size were evaluated on an individual spike 10 and 30 days after inoculation

respectively. The scales used were slightly modified from scales developed by Ratanopas (1973) (Table 1).

Plants were further classified as having either no sclerotia or honeydew in any of the 10 inoculated florets, or as having at least one sclerotium or visible honeydew on a spike. Plants having five or fewer sclerotia per spike were considered to have partial resistance for reduced number of sclerotia, whereas plants with more than five sclerotia per spike were considered susceptible. In plants considered to have at least one sclerotium, the amount of honeydew and sclerotial size could be assessed. Such plants were considered to have partial resistance for reduced amounts of honeydew if only small droplets were produced on a spike (ratings of 0,1,3,5) and susceptible if larger droplets were produced (ratings of 7 to 9). Plants were classified as having partial resistance for reduced sclerotial size if all sclerotia were smaller than the seed (ratings of 1,3), whereas plants developing seed size or larger sclerotia (ratings of 5 to 9) were classified as susceptible.

Results from F_2 families of the same cross were pooled only after the chi-square tests for homogeneity and independance (contingency chi-square) showed them to behave similarly. The chi-square test for goodness of fit was used to anaylyze the data for all F_2 progenies.

In tabulating the frequencies of plants in the different partial resistance categories in th F_2 progenies of the Chinese Spring cross, plants without any sclerotia were excluded since the expression of genes reducing the amount of honeydew or sclerotial size could not be assessed

in these plants. The independant assortment of partially resistant classes, intermediate classes and susceptible classes for each disease component was tabulated and compared with the number of plants expected assuming random assortment of these traits.

The expression of the complete resistance in each of the crosses was further evaluated by inoculating at least 10 F_3 families from F_2 parents having complete resistance. Due to space limitations, the F_3 progeny of the Chinese Spring cross was evaluated prior to the Columbus and UM684 F_3 progenies. The reactions of F_3 plants in the Chinese Spring cross derived from a single F_2 plant had variable reactons. In an attempt to determine the variability in the disease reaction that occurs within a single plant, two spikes per F_3 plant were inoculated in the progenies from crosses between 6B364 X UM684 and 6B364 X Columbus. In the Chinese Spring cross, F_3 families from F_2 plants with intermediate and susceptible reactions were evaluated for each disease component. Narrow sense heritabilities for each disease component in the Chinese Spring cross were calculated by regression of the F_3 family means onto its F_2 parents (Falconer, 1981).

Two lines derived from different F_2 plants which did not develop any sclerotia when inoculated with isolate M-4, and had means of less than one sclerotium per plant in the F_3 families (more than eight plants per F_3 family inoculated with M-4) were inoculated with ergot isolates F-1 and P-2 to determine whether the selection for resistance with M-4 was also effective against other isolates.

Table 1. Rating scales for the ergot disease components amount of honeydew and sclerotial size.

Disease Scale	Sclerotial Size	Amount of Honeydew
0	No sclerotia	No honeydew
1	All sclerotia smaller than seed.	Honeydew within the floret.
3	One sclerotium seed size, the rest smaller.	A single small droplet outside floret.
5	More than one sclerotia of seed size.	Several droplets outside floret.
7	One sclerotium larger than seed.	One large droplet outside floret(s).
8	Two sclerotia larger than seed.	Several large droplets outside several florets.
9	More than three sclerotia larger than the seed.	Copious amounts on many florets.

RESULTS

There were no differences between F_1 plants from each of the crosses and their reciprocals with regards to the number of sclerotia per spike thus allowing the data from F_2 families of the same cross to be pooled. The number of sclerotia per spike was not correlated to either the number of days to anthesis, or to the presence of awns.

The mean reactions for the number of sclerotia per spike developing in Chinese Spring, UM684 and Columbus were 8.3, 7.6 and 7.2 respectively (Table 2). The F_1 in all three crosses had slightly lower susceptible reactions than its respective susceptible parent for the numbers of sclerotia. The order of the ranking for susceptibility in the F_1 was the same as the order of the ranking for susceptibility in the susceptible parents.

Chinese Spring and UM684 were both rated susceptible for the amounts of honeydew produced (Table 2). The F_1 progeny of the Chinese Spring was also rated susceptible for the amount of honeydew (rating of 5.9), whereas the F_1 progeny of UM684 produced lower amounts of honeydew than its susceptible parent. Columbus produced low amounts of honeydew, and similarly, its F_1 progeny also produced lower amounts of honeydew.

The sclerotia developing on inoculated spikes of UM684 and Chinese Spring were larger than the seed (ratings of 7,8,9) (Table 2). In

Columbus, sclerotia ranged from seed size to larger sizes. In the Chinese Spring F_1 progeny, the sclerotia were all larger than the seed whereas in both the UM684 and Columbus crosses, the size of the sclerotia in the F_1 progenies were variable, ranging from small to larger sizes.

The numbers of plants in the F_2 populations of the UM684 and Columbus crosses were low due to the inadvertent application of triadimefon to some plants. The sprayed plants were excluded since triadimefon reduces infection levels (Harper and Christensen, 1986).

Plants having no sclerotia developing per spike were observed in the F_2 progeny of each cross. The number of F_2 plants resistant (no sclerotia) and susceptible (one or more sclerotia per spike) in the Chinese Spring, UM684 and Columbus crosses were 49 to 152, 28 to 62, and 20 to 39 respectively (Table 3). Although these segregation patterns did not deviate from a 1:3 ratio in each of the three crosses indicating that the resistance to M-4 may be controlled by a single gene, the sizes of the populations in the UM684 and Columbus crosses were low, and the segregation ratios for these crosses are closer to a 1:2 segregation pattern. This would suggest that more than one gene may be controlling resistance.

The hypothesis that resistance was controlled by a single recessive gene was further tested by evaluating F_3 families derived from F_2 plants with zero sclerotia: 24 families were tested from the Chinese Spring cross and 10 families from each of the other two crosses. Two separate segregation patterns for F_3 families derived from F_2 plants with zero

sclerotia were observed to occur in all crosses. On the basis of significant differences in the mean reactions for the number of sclerotia per F_3 family, an F_2 plant with zero sclerotia was confirmed to be resistant if the reaction of the derived F_3 family averaged less than one sclerotium. This level of resistance appeared to be reached in the Columbus cross by the accumulation of one resistant gene from 6B364, whereas in the UM684 and Chinese Spring crosses, it appeared two genes from 6B364 were needed to approach this same resistance level (Table 3).

There were F_3 families in the Chinese Spring and Columbus crosses in which none of the inoculated plants developed sclerotia. None of the 10 F_3 families derived from F_2 plants producing zero sclerotia in the UM684 cross had all F_3 plants in a family with zero sclerotia. This could have been due to the limited number of F_3 families sampled rather than to an inability to fix the resistance for zero sclerotia in this background. In the Chinese Spring cross, the number of F_3 plants evaluated per family was low, so it could not be concluded whether these families were homozygous for zero sclerotia. However, the occurrence of F_3 families with zero sclerotia would suggest that this reaction of 6B364 can be transferred into new backgrounds. In the F_3 families derived from F_2 plants with zero sclerotia, two of the 24 families evaluated in the Chinese Spring cross, and one of the 10 families evaluated in the Columbus cross had no sclerotia developing on any of the plants inoculated within a family. It was not possible to do chi-square tests for the goodness of fit for zero sclerotia because F_2 populations were not large enough.

Table 2. Reactions of parents and F_1 progenies of crosses between the resistant wheat line 6B364 and three susceptible wheat cultivars inoculated with ergot isolate M-4.

	Number of plants evaluated	Number of sclerotia		Amount of Honeydew		Sclerotia Size	
		Mean	Range	Mean	Range	Mean	Range
Parents							
Chinese(CH) Spring	16	8.3	6-9	8.4	8-9	7.9	7-9
UM684 (UM)	9	7.6	6-9	7.0	5-9	8.0	7-9
Columbus(Co)	5	7.2	6-9	2.8	0-7	5.6	5-8
6B364	25	0.0	-	0.0	-	0.0	-
F1 Progeny							
6B364 X CH	12	4.8	1-8	5.9	5-8	7.8	7-9
6B364 X UM	10	4.0	1-8	2.3	0-5	4.8	3-7
6B364 X Co	25	3.7	0-7	1.7	0-8	4.6	0-8

a - The number of sclerotia per spike with 10 florets inoculated.
b - No honeydew=0; Large droplets outside floret=8. See Table 1 for further explaination of ratings.
c - Smaller sclerotia than seed < 5; Sclerotia seed size =5; Sclerotia larger than seed > 5.

Table 3: Segregation for complete resistance to the ergot isolate M-4 in the F_2 populations and selected F_3 families from completely resistant F_2 parents from crosses between 68364 and three susceptible wheats.

Parents[d]	F2 Progeny		F_3 Families from different F_2 Plants with Complete Resistance[2]						
	Observed R:S[b]	Chi-Square 1:3	Number Evaluated	Number Averaging less than One Sclerotium per Spike	Predicted R:S of F_3 Families[c]	Expected R:S One Gene	Chi-Square One Gene	Expected R:S Two Genes	Chi-Square Two Genes
68364 x Chinese Spring	49:152	0.01[a]	24	6	12:189	50.3:150.7	38.90	12.6:188.4	0[a]
68364 x UM684	28:82	1.93[a]	10	3	9:82	22.8:68.2	11.10	5.7:85.3	1.47[a]
68364 x Columbus	20:39	2.04[a]	10	6	12:47	14.8:44.2	0.46[a]	3.7:55.3	17.50

a Not significantly different at P=.05 (Chi-square).

b A resistant plant had no sclerotia or honeydew with ten florets per spike inoculated. Susceptible plants had one or more sclerotia. All susceptible parents used tended to have 7 or more sclerotia while 68364 was completely resistant.

c Based on the sub sample of F_3 families. 6/24, 3/10 and 6/10 of F_2 plants in the families from the crosses between Chinese Spring, UM 684 and Columbus averaged less than one sclerotium per family. This ratio was used to estimate the number of F_2 plants not segregating for resistance.

d Ten florets per spike were injected with 10^4 conidia per mL.

Table 4: The average number of sclerotia per spike in F_3 families derived from F_2 plants with different classes for the number of sclerotia.

68364 Crossed With	Disease Reaction F_2 number of Sclerotia	Number of F_3 families evaluated[a]	F3 Family Means: Average Number of Sclerotia per Family[a]	Average Amount of Honeydew per Family[b]	Average Sclerotial Size per Family[d]	0	Cumulative percent of F_3 family means for sclerotia number rating less than: <1	<2	<4	<5	<6
Columbus	0	10	1.4 ± 1.9	c	1.4 ± 1.5	10	60	80	90	90	90
UM684	0	10	1.7 ± 1.9	c	1.7 ± 0.9	0	30	60	100	100	100
Chinese Spring	0	31	3.8 ± 2.4	3.2 ± 2.7	4.2 ± 2.5	8	19	32	45	58	81
Chinese Spring	1.2	10	4.1 ± 1.5	3.4 ± 2.5	4.9 ± 1.7	0	0	10	40	60	90
Chinese Spring	3,4,5	8	5.1 ± 1.7	3.6 ± 2.5	6.0 ± 0.8	0	0	0	20	50	63
Chinese Spring	7,8,9	9	6.0 ± 1.7	5.5 ± 2.0	5.5 ± 1.1	0	0	0	0	22	33

a Four, 10 and ten florets per F_3 family were evaluated for the Chinese Spring, UM684 and Columbus crosses respectively. Plants were inoculated by injecting 10^4 conidia per mL into ten florets per spike. All F_3 plants included in calculating the families mean for number of sclerotia.

b 0, no honeydew; 5, small droplets outside florets; 8, large droplets outside florets. Rated at ten days after inoculation. F_3 plants without any sclerotia excluded from the calculation of families mean reaction for amount of honeydew.

c Large amounts of honeydew were not observed on the exterior of the florets on any of the plants; all plants had a rating of 0, 1 or 3 and the mean would be less than 3.0.

d 1, all sclerotia smaller than seed; 5, sclerotia approximately kernel size; 7, one sclerotia larger than kernel. Rated 30 days after inoculation. F_3 plants without any sclerotia excluded from the calculation of families mean reaction for sclerotial size.

Table 5: The average amount of honeydew produced per spike in F_3 families derived from F_2 plants with different classes for the amount of honeydew.

Line 68364 crossed with	Disease reaction F_2 rating for honeydew	Number of F_3 families evaluated	F3 Family Means: Average Amount of Honeydew per Family[a]	F3 Family Means: Average Number of Sclerotia per Family[b]	F3 Family Means: Average Sclerotial size per Family[c]	Cumulative Percentage of F_3 family means for amounts of honeydew that are less than: <1	<3	<5	<7
Chinese Spring	0, 1	30	3.1 + 2.5	4.1 + 1.7	4.6 + 1.6	30	46	70	93
Chinese Spring	3, 5	16	5.2 + 2.1	5.2 + 1.6	6.0 + 1.1	6	31	76	94
Chinese Spring	7, 8, 9	9	6.3 + 1.5	6.4 + 1.2	6.1 + 1.2	0	0	0	42

a 0, no honeydew; 1, Honeydew within floret; 3 one drop of honeydew outside floret; 5, small droplets of honeydew outside floret; 7 one large droplet outside floret; 8, several large droplets outside floret; 9, head oozing with honeydew. Plants without any sclerotia in F_3 families were excluded from the mean.

b Number of sclerotia per spike with ten florets being inoculated by the injection of 10^4 conidia per mL into individual florets.

c 1, sclerotia, smaller than kernel; 5, sclerotia approximately kernel size; 7, one sclerotia larger than seed.

Table 6: The average for sclerotial size rating in F_3 families derived from F_2 plants with different classes for sclerotial size.

Line 68364 crossed with	Disease Reaction F_2 Sclerotial[2] size	Number of F_3 families evaluated[a]	F3 Family Means[a]			Cumulative Percentage of F_3 family means for sclerotial size rating less than:			
			Average Sclerotia size per Family[b]	Average Number of Sclerotia per Family	Average Amount of Honeydew per Family[c]	<1	<3	<5	<7
Chinese Spring	0	28	5.0 + 1.7	4.5 + 1.9	3.6 + 2.5	0	14	36	89
Chinese Spring	1, 3	6	4.9 + 1.6	4.3 + 1.1	3.3 + 2.5	0	16	33	83
Chinese Spring	5	6	6.3 + 1.4	5.7 + 1.7	5.4 + 1.6	0	0	16	66
Chinese Spring	7	4	4.6 + 1.8	4.4 + 2.0	3.3 + 2.9	0	25	50	100
Chinese Spring	8, 9	9	5.9 + 1.3	5.6 + 1.6	5.0 + 2.5	0	0	10	78

a At least four plants per F_3 family inoculated in the Chinese Spring evaluated and ten plants per F_3 family in the UM684 and Columbus crosses. Ten florets per spike were injected with 10^4 conidia per mL. Plants without any sclerotia in the F_3 were excluded from the mean.

b 1, all sclerotia smaller than seed; 5, sclerotia approximately kernel size; 7, one sclerotia larger than kernel.

c 0, no honeydew produced; 5, small droplets outside florets; 8, large droplets outside florets.

The averages of the mean reactions of F_3 families for all disease components were tabulated in each of the crosses for the F_3 families derived from F_2 plants which failed to produce any sclerotia. When these F_3 families in each of the three crosses were inoculated, there were tendencies for the numbers of sclerotia and the amounts of honeydew to be lower, and the sclerotia smaller (Table 4). The mean reactions of plants from these F_3 families for the numbers of sclerotia, amounts of honeydew and sclerotial sizes were lowest in the Columbus cross and highest in the Chinese Spring cross. Sixty percent of these F_3 families in the Columbus cross derived from F_2 plants without sclerotia had a mean number of sclerotia per spike of less than one whereas only 19 percent of the Chinese Spring F_3 families had a similar low mean. F_3 plants in the UM684 and Columbus crosses from F_2 plants with zero sclerotia were observed not to have any exterior honeydew (plants having ratings of 3 or lower) when plants with sclerotia were examined. Many F_3 plants in the Chinese Spring cross from F_2 plants without sclerotia had exterior honeydew . Reduced sclerotial sizes occurred in F_3 plants from resistant F_2 plants as none of the F_3 plants were observed with larger sclerotia (ratings of 7 or larger) in the UM684 and Columbus crosses. On the other hand, sclerotial size was not noticeably reduced in F_3 plants derived from F_2 plants with zero sclerotia in the Chinese Spring cross with many plants having larger sclerotia.

In the Chinese Spring cross, plants in F_3 families derived from F_2 plants with low and high numbers of sclerotia per spike were evaluated. F_3 families from F_2 plants with high numbers of sclerotia (7,8,9) produced on average a higher number of sclerotia (6.0) than those F_3

families derived from F_2 with one or two sclerotia (mean of 4.1) (Table 4).

Since the reaction for complete resistance masks the expression of genes reducing amounts of honeydew, F_3 plants with zero sclerotia were excluded from the tabulating of the average for family means in Table 5. F_3 families from F_2 plants with a low reaction for the amount of honeydew (ratings of 0,1) tended to produce families with lower means for the amounts of honeydew as well as the numbers of sclerotia and smaller sclerotia than F_3 families derived from F_2 plants with higher amounts of honeydew (Table 5). None of the F_3 families derived from susceptible F_2 plants with the honeydew ratings of 7,8,9 had means for the amount of honeydew less than five whereas 30 percent of the F_3 family means derived from plants with low amounts of honeydew (0,1) had means of less than five. This indicates an ability to select for lower or higher amounts of honeydew.

F_3 plants without sclerotia were excluded from the tabulation of family means for sclerotial size in Table 6, since it is not possible to assess genes reducing sclerotial size in these plants. The averages of the F_3 family mean reactions for sclerotial sizes derived from F_2 plants with sclerotial size ratings of 0,1 and 3 and 7 were slightly smaller than the averages of the F_3 families mean reactions derived from F_2 plants with ratings of 5,8 and 9 (Table 6). F_3 families derived from F_2 plants with a rating of 7 (one sclerotium larger than the seed) on average had smaller sclerotia than plants with a rating of 5 (all sclerotia being seed size). This discrepancy might be due to the fact that many of the F_2 plants with a rating of 7 were observed to have low

numbers of sclerotia (Table 6); this may have favoured larger sclerotia since more plant nutrients would be available for each sclerotium.

Further evidence of the association between numbers of sclerotia and lower amounts of honeydew was provided by the positive heritabilities obtained in the Chinese Spring cross by regression of the F_3 family mean onto its F_2 parent (Table 7). The heritabilities were positive and significant for the number of sclerotia and amount of honeydew when plants without sclerotia were excluded. The heritability was not significant in this population for sclerotial size. The heritability for honeydew increased from .35 to .57 when plants with no sclerotia were excluded. This suggested the presence of genes reducing honeydew that were independant from those that reduced the number of sclerotia.

It appeared from the data, that F_2 plants with reduced numbers of sclerotia also showed lower amounts of honeydew. To verify this observation, F_2 plants of the 6B364 X Chinese Spring cross were placed in three different classes for both the numbers of sclerotia and the amounts of honeydew produced, and the observed and expected frequencies of plants calculated in the different classes for these two disease components (Table 8). The observed frequency of F_2 plants with low numbers of sclerotia which also had low amounts of honeydew was greater than expected. Similarly, the observed frequency of F_2 plants with high numbers of sclerotia which also had high amounts of honeydew was greater than expected. Conversely, the frequencies of F_2 plants with high numbers of sclerotia and low amounts of honeydew, or the group of plants with low numbers of sclerotia and high amounts of honeydew produced were lower than expected. When plants were grouped on the basis of

intermediate numbers of sclerotia (3,4,5) with any of the three categories for the amount of honeydew produced, the observed and expected frequencies were equal. Similarly, when plants were grouped on the basis of intermediate amounts of honeydew produced (ratings of 3,5), with any of the three categories for the number of sclerotia, the expected and observed frequencies were equal.

The F_2 data also suggested that plants with reduced numbers of sclerotia also had smaller sclerotia. To verify this observation, F_2 plants from the Chinese Spring cross were grouped into classes for low, intermediate and high numbers of sclerotia, and into four classes for sclerotial sizes (Table 9). The observed frequency of plants having low numbers of sclerotia (1,2) that were all small sizes (1,3) was higher than expected. Conversely, the numbers of plants producing low numbers of sclerotia (1,2) that were of intermediate to larger sclerotial sizes (ratings of 5,7,8,9) were lower than expected. Similarly, lower than expected number of plants developed high numbers of sclerotia (6-9) which were of smaller sizes (ratings of 1,3). On the other hand, the predicted number of plants was higher than expected in the category for higher numbers of sclerotia (6-9) with large sizes (ratings of 8,9).

Thus, the gene(s) which reduce the number of sclerotia also seem to lower the amounts of honeydew produced and the size of sclerotia. This is also supported by the relationship between the reaction of F_2 plants with regards to the three disease components, and the reaction of the F_3 families (Tables 4,5,6), as well as the positive and significant hertiability values between disease components (Table 7). These results can be explained by the epistatic action of a gene(s) on all three disease components.

Two F_4 lines derived from F_3 families which averaged less than one sclerotium per spike when inoculated with isolate M-4, also had low numbers of sclerotia when inoculated with isolates P-2 and F-1 (Table 9). 6B364 showed complete resistance (zero sclerotia) to both isolates whereas Chinese Spring was susceptible (>8 sclerotia/spike).

Table 7. Calculation of the narrrow sense heritabilities by regression of the F_3 family means onto its F_2 parent from the cross 6B364 X Chinese Spring for the three disease components.

Components Regressed (F3 to F2)	All F_2 and F_3 plants (N= 58 families) reg h^2	F2 and F3 populations ignoring plants without sclerotia (N= 27 families) reg h^2
sclerotia no. to sclerotia no.	0.29 + .10**	0.26 + .11**
honeydew to to honeydew	0.35 + .10*	0.57 + .15*
size to size	0.19 + .07**	ns
sclerotia no. to honeydew	0.34 + .08*	.39 + .10*
honeydew to sclerotia no.	0.26 + .11**	ns
honeydew to size	0.16 + .09***	ns
size to sclerotia no.	0.23 + .08**	ns
size to honeydew	.23 +.08**	ns
sclerotia no.	0.21 +.08**	ns

* significant at .01.
** significant at .05.
*** significant at .10.

Table 8. The observed and expected frequency for association between the number of sclerotia per spike and amounts of honeydew in F_2 plants of the 6B364 x Chinese Spring cross.

Ratings for the Number of Sclerotia in F_2[a]	Ratings for the Amounts of Honeydew[b]	Frequency	
		Plants Observed[c]	Plants Expected[d]
1, 2	0, 1	14	6.0
3, 4, 5	0, 1	13	13.5
6, 7, 8, 9	0, 1	8	15.5
1, 2	3, 5	8	9.9
3, 4, 5	3, 5	31	22.4
6, 7, 8, 9	3, 5	19	25.7
1, 2	7, 8, 9	2	8.1
3, 4, 5	7, 8, 9	19	18.1
6, 7, 8, 9	7, 8, 9	35	20.8

a Number of sclerotia per spike. Spikes were inoculated using a syringe to inject 10^4 conidia/mL into 10 florets. Number of plants with low, medium and high numbers of sclerotia were 24/140, 44/140, and 62/140.

b Amount of honeydew at 10 days, 0 = none; 5 = small droplets; 8 = large droplets. Number of plants with low, medium and high amounts of honeydew were 35/140, 54/140 and 62/140.

c The number observed in a population of 140 F_2 plants. Plants without any sclerotia in the F_2 are excluded.

d The number expected assuming random assortment of plants with low numbers of sclerotia (1,2) and low amounts of honeydew (0,1) is calculated by: 140 x 24/140 x 35/140 = 6 plants expected.

Table 9. The observed and expected frequency for association between the number of sclerotia per spike and sclerotial size in F_2 plants of the 6B364 x Chinese Spring cross.

Ratings for the Number of Sclerotia in F_2 [a]	Ratings for the Size of Sclerotia [b]	Plants Observed [c]	Plants Expected [d]
1, 2	1, 3	20	3.9
3, 4, 5	1, 3	3	6.9
6, 7, 8, 9	1, 3	0	10.2
1, 2	5	1	6.8
3, 4, 5	5	27	15.5
6, 7, 8, 9	5	12	17.7
1, 2	7	2	4.5
3, 4, 5	7	14	10.0
6, 7, 8, 9	7	10	11.5
1, 2	8, 9	1	8.7
3, 4, 5	8, 9	10	19.7
6, 7, 8, 9	8, 9	40	22.6

a Number of sclerotia per spike. Spikes were inoculated using a syringe to inject 10^4 conidia/mL into 10 florets. Number of plants with low, medium and high numbers of sclerotia were 24/140, 44/140, and 62/140.

b Sclerotial size at 30 days, 1,3 - size smaller than seed, 5 - sclerotia seed size, 7,8,9 - sclerotia larger than seed. The number of plants with ratings of 1 or 3, 5, 7, and 8 or 9 sclerotia were 23/140, 40/140, 26/140 and 51/140.

c The number observed in a population of 140 F_2 plants. Plants without any sclerotia in the F_2 are excluded.

d The number expected assuming random assortment ie plants with low number of scleoria (1,2) and small sclerotia (1,3) is calculated by: 140 x 24/140 x 23/140

a

DISCUSSION

The F_1 plants from each of the three crosses displayed incomplete dominance for susceptibility to each of the three disease components. The means of the reactions for all three disease components were lower in the Columbus F_1 than in the Chinese Spring F_1. The F_1 in each of the three crosses also displayed a wide range in the number of sclerotia per spike. Ratanopas (1973) found that infection levels in Manitou dropped from 80 to 54 percent when time between inoculation and anthesis was decreased from 72 to 24 hours. Thus, attempts were made to inoculate approximately 48 hours prior to anthesis. However, it was likely that the time between inoculation and anthesis could have been from 30 to 72 hours in an individual spike, and these differences in the timing of inoculation could have contributed to the variability of the reactions for the number of sclerotia in the F_1. It is also possible that the expression of genes for resistance in the heterzygous condition may occur when the time between inoculation and anthesis is reduced ie. from 72 to 30 hours.

Some F_2 plants in each of the crosses were completely resistant (no sclerotia when inoculated). The F_2 segregation pattern in the Chinese Spring cross suggested that one gene controlled complete resistance (1 resistant:3 susceptible) to isolate M-4. The evaluation of F_3 families derived from F_2 plants lacking any sclerotia, revealed that segregation for resistance occurred in many families, and that there were

significant differences between families in the mean number of sclerotia per spike. On the basis of the reactions of F_3 families, it appeared that resistance to isolate M-4 in the Chinese Spring and UM684 backgrounds was controlled by two genes whereas in the Columbus background, only one gene from 6B364 was required to acheive this same resistance level. Differences in the mean reactions of F_3 families from F_2 plants that failed to produce sclerotia suggested that these F_2 plants consisted of more than one genotype. The inoculation of two spikes per plant in the F_3 families of the UM684 and Columbus crosses indicated that some genotypes could have from zero to five sclerotia per spike. This partially explains why many of the F_3 plants produced sclerotia even though they were from F_2 plants which failed to develop any sclerotia.

Sharp (1983) crossed two cultivars susceptible to stripe rust (Puccinia striiformis West.) and by repeated selection for resistance obtained some F_6 lines which exhibited resistance. These results indicated that susceptible cultivars may have minor genes for resistance, and through selection, they can be combined to contribute additively to the resistance. The results of the present study also suggest that the three susceptible cultivars used in the crosses may have minor genes which can affect the components of partial resistance. Chinese Spring, UM684 and Columbus were all similar in their susceptibility to the ergot isolate M-4 when the disease component number of sclerotia produced per inoculated spike was evaluated. The F_3 families derived from F_2 plants having zero sclerotia that averaged less than one sclerotium per spike in the crosses with Chinese Spring, UM684

and Columbus were 60, 30 and 19 percent, respectively (Table 3). One possible explanation for the differences found in the disease reactions of the progenies from the three crosses with 6B364 was that the three susceptible lines had different levels of background genes which reacted additively to enhance the major gene(s) expression from 6B364.

Either a single or two closely linked genes for resistance from 6B364 that reduced sclerotia number, also reduced the amount of honeydew and sclerotial size. A single gene influencing more than one disease component has been reported with powdery mildew (Erysiphe graminis f. sp. tritici E. Marshal) of wheat (Martin and Ellingboe, 1976) and leaf rust (Puccinia reconditia Rob. ex Desm.) of wheat (Brodny et al.,1986).

Tabulating the mean reaction of F_3 families according to the disease reaction of the F_2 plant for complete and partial resistance and susceptibility suggested that there was association of the mean number of sclerotia and amount of honeydew in an F_3 family and its F_2 parent.

There appeared to be genes reducing the amount of honeydew that were independant from the gene(s) that reduced the numbers of sclerotia since heritability values increased when plants without sclerotia were excluded from the calculation of heritabilities by regression (Table 7).

Line 6B364 was completely resistant to 12 of the 14 ergot isolates used in the orginal screening program (Bernier, 1978). F_4 lines from F_3 families having means of less than one sclerotium per plant when inoculated by to the ergot isolate M-4 also had low numbers of sclerotia when inoculated by the isolates P-2 and F-1 (Table 10). These results

suggest that the partial resistance for lower numbers of sclerotia to M-4 also reduced the average numbers of sclerotia which developed in response to inoculation by P-2 and F-1.

In the University of Manitoba breeding program, it has been observed that F_6 wheat lines were susceptible to ergot infection even though they were derived from a resistant F_2 parent. In this study, the instability of the numbers of sclerotia per spike observed in many F_3 families from F_2 plants having zero sclerotia explains the loss of resistance which occurred over several generations in the field. It also demonstrates the importance of selecting at least to the F_3 to ensure that the resistance genes are not segregating, and to differentiate plants with complete resistance from plants with low numbers of sclerotia per spike.

Chapter IV

MANUSCRIPT 2

Evaluating methods of inoculating wheat with *Claviceps purpurea*.

ABSTRACT

Several methods of inoculating wheat with ergot were evaluated. These include a hypodermic syringe, a vacuum inoculation system developed for inoculating wheat with loose smut, a mist bottle, an airbrush and a pump which forcefully ejected a fine stream of spore suspension. The ergot isolate M-4 was adjusted at concentrations of either 10^4 or 10^5 conidia/mL, and used to inoculate either the susceptible Triticum aestivum L. cultivars Manitou or Chinese Spring in all studies. Trials were conducted in the field, greenhouse and growth chamber. Each method was assessed by counting the number of sclerotia per inoculated spike. Inoculated spikes were further classified as having sclerotia (one or more sclerotia per spike) or having no sclerotia (plant escapes).

In the field tests, none of the inoculation techniques were completely effective in inducing the formation of sclerotia in all inoculated spikes. Temperatures in both years at the time of inoculation were above 30° C Inoculation by means of either a hypodermic syringe or a vacuum system in both the greenhouse, and growth chamber, induced development of at least one sclerotium/spike. The airbrush appeared to be also an effective inoculation system if glumes were clipped prior to inoculation. Inoculations with the pressure pump, the misting bottle and the airbrush with the glumes intact all resulted in low numbers of sclerotia per spike with some of the inoculated spikes failing to develop any sclerotia.

The hypodermic syringe, the vacuum system, the airbrush with glumes intact, and the airbrush with the glumes clipped prior to inoculation were evaluated using conidia concentrations of 10^4 and 10^5 conidia per mL in the growth chamber. Raising the spore concentration resulted in significantly higher number of sclerotia per spike using the airbrush when the glumes were not clipped prior to inoculation. The number of sclerotia on spikes inoculated with the syringe, the vacuum system, and the airbrush when glumes were clippped varied only slightly or not at all when the spore concentration was raised from 10^4 to 10^5 conidia/mL. The ability to screen for resistance in the field is also discussed.

INTRODUCTION

The incidence of ergot caused by Claviceps purpurea Tul., on cereals in the field is generally low (Weniger, 1924; Dillon-Weston and Taylor, 1942) and natural infection is not reliable in distinguishing between resistant and susceptible cultivars; thus a variety of methods have been used to inoculate cereals with conidial suspensions of ergot. These include misting the suspension onto rye florets in the field (Lewis,1945), coating insects with honeydew and releasing them (Lewis, 1945), injecting the suspension into individual florets with a hypodermic syringe (Platford and Bernier,1970), and spraying a suspension on florets with either glumes clipped or unclipped by means of an atomizer (Puranik and Mathre, 1971). The use of either a syringe or an atomizer to spray spores onto a susceptible cultivar with clipped glumes resulted in sclerotial development in most of the inoculated florets.

Factors other than the method of inoculation can influence the number of sclerotia developing per spike. As pollination greatly reduces the ability of ergot to infect cereals (Ratanopas, 1973), it is important to standardize the time of inoculation in relation to the occurrence of anthesis; this will aid in differentiating genetic resistance from the effects of pollination. Sclerotia developed in 78 percent of the florets of the susceptible wheat cultivar Manitou inoculated at 48 hours prior to anthesis whereas florets inoculated on

the day of anthesis developed sclerotia in only 30 percent of the florets. Inoculation at about 48 hours prior to anthesis was recommended to maximinize differences in the number of sclerotia produced by susceptible and partially resistant cultivars (Ratanopas, 1973).

The concentration of conidia in the inoculum can also affect the final number of sclerotia produced per spike as demonstrated when ten florets per spike are inoculated by a syringe. The average number of sclerotia developing per spike increased from 0.4 to 7.9 when the concentration was increased from 250 to 10^6 conidia per mL (Platford and Bernier, 1976). These authors concluded that 10^4 conidia per mL was adequate for differentiating resistant and susceptible wheat cultivars, since lower concentrations decreased the number of sclerotia per spike in susceptible cultivars, and higher concentrations increased the numbers of sclerotia in the partially resistant cultivar Kenya Farmer to levels equal to that of susceptible cultivars.

This study was undertaken to evaluate several methods of inoculating spikes of wheat in greenhouse, growth chamber, and field experiments. The influence of spore concentration was also assessed in a growth chamber experiment.

MATERIALS AND METHODS

Preparation of inoculum and disease assessment. The ergot isolate M-4 was used in all studies. It was obtained by isolating mycelial growth that developed from a sclerotium collected on the spring wheat cultivar Manitou (Bernier, personnel communication).

Conidia used for inoculation were obtained by placing mycelia of M-4 in a flask containing a modified sucrose broth (Lewis,1945), and then the flask was placed on a a gyrotory shaker for about 10 days. The conidia produced were separated from the mycelium by filtration, concentrated by centrifugation, stored in 60% sucrose at 4° C and always used within 4 months (Platford and Bernier, 1976). Inoculum was prepared daily by diluting the conidial suspension with sufficient sterile distilled water to adjust the concentration to either 10^4 or 10^5 conidia/mL using a spectrometer technique (Platford, 1976).

The Triticum aestivum L. cultivars Manitou or Chinese Spring were used in all studies. The efficiency of the inoculation methods under evaluation was assessed by counting the number of sclerotia produced per spike. An inoculated spike was further classified as having sclerotia (at least one sclerotium per spike) or having no sclerotia (plant escapes).

Methods of inoculation. The following methods of applying the conidial suspension were compared: 1) a hypodermic syringe to inoculate 10 florets per spike (Platford and Bernier,1970); 2) a vacuum system developed for inoculating spikes of wheat with loose smut (Neilsen, 1983); 3) an airbrush at a pressure of 276 KPa to atomize the spore suspension. Plants inoculated with either their glumes intact or clipped. 4) A plastic misting bottle. 5) A pressure pump which projected a fine stream of conidial suspension under pressure. All inoculations were performed not more than 48 hours prior to anthesis.

Experimental Protocols

Field Experiments. The following methods of inoculation were evaluated in 1983 and 1984 with the susceptible wheat cultivar Manitou: the hypodermic syringe, the airbrush, the vacuum system, and the pressure pump. In each year, 50 spikes were inoculated during the day by each method with a conidial suspension of 10^4 conidia/mL. Spikes inoculated were between 20 to 40 hours prior to anthesis.

In a further experiment conducted only in 1983, a factorial design was used to evaluate the interrelationship of the three inoculation methods (hypodermic syringe, airbrush and misting bottle), with time of inoculation (day versus night) and covering inoculated spikes with a glassine envelope after inoculation versus not covering, on the number of sclerotia which subsequently developed per inoculated spike. Spikes from twenty plants of Chinese Spring were inoculated for each treatment with a conidial suspension of 10^4 conidia/mL.

Indoor Studies. Inoculating by use of a hypodermic syringe, airbrush or vacuum system which were compared in the 1983-84 field experiments were also evaluated in the greenhouse in the fall of 1984 along with the misting bottle. Spikes from thirty plants of the wheat cultivar Manitou were inoculated at about 40 hours prior to anthesis with a conidial suspension of 10^4 conidia/mL.

In a separate experiment, the effect of spore concentration on the same four methods of inoculation ie. hypodermic syringe, the vacuum system, and the airbrush were evaluated using either 10^4 or 10^5 conidia/mL. Spikes from thirty Manitou plants were evaluated in the growth chamber at temperatures of 20/16° C day to night.

RESULTS

In the field experiments conducted in 1983 and 1984, none of the four inoculation methods evaluated were completely effective in inducing the development of at least one sclerotium on all the plants inoculated by a given method (Table 1). In the greenhouse tests, the vacuum system and hypodermic syringe techniques induced formation of at least one sclerotium on each of the inoculated spikes. The number of sclerotia developed per spike was the highest using the vacuum system and hypodermic syringe in both the field and greenhouse. The average number of sclerotia was consistently higher in spikes inoculated using the vacuum versus using the hypodermic syringe. Only 10 florets per spike were inoculated using the hypodermic syringe, whereas the vacuum system exposed the entire spike to inoculum which would allow more than 10 florets to contain sclerotia. An important aspect of the method of inoculation for plant breeders is the consistency of the method. The stardard deviations for the mean number of sclerotia induced by methods were similar when spikes were inoculated in the field or greenhouse. However, the mean number of developing sclerotia per spike was reduced when inoculations were made in the field with the hypodermic syringe and vacuum system.

Inoculation of florets by injecting with a syringe was generally more effective than spraying with the airbrush: the average number of

sclerotia produced per spike using syringe inoculation ranged from 2.6 to 6.1, while the number of sclerotia produced per spike using the airbrush ranged from 0.2 to 4.8 (Table 2). Spraying spikes by means of a mist bottle was completely ineffective. Bagging spikes with a glassine envelope after inoculation significantly reduced the number of sclerotia/spike when plants were inoculated by the syringe during either day or night (Table 2). When plants were inoculated with the airbrush, bagging again reduced the average number of sclerotia/spike when inoculations were made at night but not when inoculations were made during the day. The numbers of sclerotia were low when plants were inoculated during the day using the airbrush, and no significant differences occurred between bagged and unbagged spikes. It was observed that bagged spikes were covered with aphids; this could have stressed the spikes and caused many florets abort. As a consequence, fewer sclerotia would develop on a spike. There were no clear benefits to be derived by inoculating with the syringe in the evening compared to day inoculations. The inoculation in the evening using the airbrush was more effective in inducing higher numbers of sclerotia per spike. The higher temperatures that occur during the day may have resulted in more rapid evaporation during the inoculation of the atomized conidial suspension reducing the number of spores reaching the spike.

In the 1984 growth chamber study, at least one sclerotium was induced on all inoculated spikes using a vacuum system, a hypodermic syringe and an airbrush, when glumes were clipped before inoculation at both spore concentrations (Table 3). The higher conidial concentration resulted in a significant increase in the number of sclerotia per spike when

inoculations were performed with the glumes intact using the airbrush. The use of a higher conidial concentration had a minimal effect on the number of sclerotia per spike when inoculations were made with the hypodermic syringe, the vacuum system, and with the airbrush when the glumes were not removed. The clipping of the glumes resulted in a significant increase in the final number of sclerotia when plants were inoculated using the airbrush (Table 4).

Table 1: Comparison of the efficiency of five methods of inoculating the ergot fungus on the number of sclerotia induced in the susceptible wheat cultivar Manitou.

Method[a]	1983 Field Study		1984 Field Study		1984 Greenhouse Study	
	Average Number of Sclerotia per spike[b]	Percent of spikes with one or more sclerotia	Average Number of Sclerotia per spike[b]	Percent of spikes with one or more Sclerotia	Average Number of Sclerotia per spike[c]	Percent of spikes with one or more sclerotia
Hypodermic Syringe[d]	2.9 ± 2.4	82	3.3 ± 2.5	82	6.2 ± 1.5	100
Vacuum	5.3 ± 4.3	85	5.3 ± 4.4	85	14.1 ± 4.3	100
Mist Bottle	Nt[e]	Nt	Nt	Nt	0.3 ± 0.6	46
Pressure Pump	2.8 ± 2.8	77	2.9 ± 2.9	79	Nt	Nt
Airbrush	1.9 ± 2.2	70	1.9 ± 2.1	78	2.2 ± 1.9	76

a Inoculum concentration was 10^4 conidia/mL.

b 50 plants per treatment inoculated during the day.

c 30 plants per treatment.

d Ten florets per spike were inoculated.

e Nt = Not tested.

Table 2: The effect of inoculation technique with day or night inoculation and with bagging glumes after inoculation on the number of sclerotia induced in the susceptible wheat cultivar Chinese Spring.

Method			Average Number of Sclerotia/Spike[a]	Ratio Plants Infected/ Total Plants Inoculated[b]
Syringe	Bag	Day	2.6 d	0.6
Syringe	No Bag	Day	6.1 a	1.0
Syringe	Bag	Night	4.2 c	0.9
Syringe	No Bag	Night	5.8 ab	1.0
Airbrush	Bag	Day	1.3 e	0.5
Airbrush	No Bag	Day	0.2 e	0.1
Airbrush	Bag	Night	2.4 d	0.8
Airbrush	No Bag	Night	4.8 bc	1.0
Mist Bottle	Bag	Day	0.0 e	0.0
Mist Bottle	No Bag	Day	0.0 e	0.0
Mist Bottle	Bag	Night	0.0 e	0.0
Mist Bottle	No Bag	Night	0.3 e	0.3

a Numbers within a column followed by the same letter are not significantly different at P=0.05 (Duncan's multiple range test).

b Ratio of plants with at least one sclerotium per spike per total plants inoculated. For a ratio of 1.0, all plants inoculated had a least one sclerotia per spike. Twenty spikes were inoculated with 10^4 conidia/mL by each method.

Table 3: The effect of conidia concentration and inoculation methods on the number of sclerotia per spike induced in the wheat cultivar Manitou in the growth chamber.

Methods of Inoculation[b]	Spore conidia/mL	Number of Sclerotia per Spike[a]
Hypodermic syringe with ten florets inoculated	10^4	7.8 ± 0.99 c
Vacuum	10^4	15.9 ± 4.80 a
Airbrush with glumes intact	10^4	1.4 ± 1.70 d
Airbrush with glumes clipped	10^4	12.3 ± 3.40 b
Hypodermic syringe with ten florets inoculated	10^5	9.6 ± 0.70 bc
Vacuum	10^5	17.0 ± 5.20 a
Airbrush with glumes intact	10^5	7.3 ± 3.60 c
Airbrush with glumes clipped	10^5	12.6 ± 3.70 b

a Numbers within a column followed by the same letter are not significantly different at P=0.05 (Duncan's multiple range test).

b None of the methods had the glumes clipped except for the Airbrush.

DISCUSSION

In the field, none of the five methods evaluated were completely effective in inducing at least one sclerotium in all inoculated spikes of Manitou wheat. However, when inoculations were performed indoors, all the spikes developed one or more sclerotia when inoculated with the hypodermic syringe and vacuum system (Table 1). Galapitage (1983) found that at temperatures of 25° C, Manitou became less susceptible to ergot infection. This might explain why lower numbers of sclerotia per spike developed when inoculations were made in the field. Higher temperatures also reduce the time between spike emergence and pollination and the occurrence of pollination also can cause an appreciable reduction in the number of inoculated florets which develop sclerotia (Abe and Kono, 1957; Campbell and Tyner, 1959; Ratanopas, 1973). These two factors would complicate screening in the field as lines would likely differ in maturity which would necessitate the inoculation over several days, and varying daily temperatures would impact on infection levels in susceptible cultivars. Several plants per line could be inoculated to compensate for the lower infection levels observed to occur with field inoculations under high temperatures. As well, temperatures could be lowered by shading plots with the use of netting. Since some plant escapes may occur with field inoculations, lines identified to be resistant in the field could be rechecked indoors at a later date.

Inoculations with the airbrush or the misting bottle failed to induce sclerotia in many inoculated spikes, whereas inoculation with the high pressure pump raised the number of sclerotia induced per spike slightly. Problems occurred using the pump such as the high volume of inoculum required and the tendency for inoculum to heat up as it passed through the machine. The use of the syringe and vacuum system both resulted in a high number of sclerotia per spike in the greenhouse and growth chamber. In the field, these two methods induced about half the number of sclerotia induced per spike as in the greenhouse (Table 1). Clipping the glumes and then using the airbrush also induced sclerotia in all inoculated spikes and a high number of sclerotia per spike (Table 3). This method was not evaluated in the field with the glumes removed.

Increasing the spore concentration from 10^4 to 10^5 conidia/mL induced a higher number of sclerotia per spike when inoculations were performed using the airbrush on plants having glumes intact, and to a lesser extent the hypodermic syringe. The number of sclerotia per spike was affected only slightly with the raising of spore concentration when inoculations were performed using the vacuum system or the airbrush with the glumes clipped. There would be little advantage with the higher spore concentration using the hypodermic syringe, the vacuum system and the airbrush with glumes clipped as all spikes inoculated at the lower spore concentration developed sclerotia and had a high number of sclerotia per spike. The use of 10^5 conidia/mL would also necessitate the production of a larger quantities of conidia. Bagging spikes after inoculation appeared to be of no value as higher sclerotia number per spike did not occur.

Chapter V

GENERAL DISCUSSION

This study suggests that the complete resistance (no sclerotia in an inoculated spike) of 6B364 can be transfered into new backgrounds and that it should be feasible to develop commerical spring wheat cultivars with ergot resistance approaching that of 6B364. This study also found that it was important to characterize at least to the F_3 to ensure the reactions of plants with complete resistance as several susceptible plants occurred in F_3 families derived from F_2 plants that had failed to develop any sclerotia. Some F_3 families were identified in the Chinese Spring and Columbus crosses that appeared to be homozygous for zero sclerotia. There were no families that were homozygous for zero sclerotia in the UM684 cross, however, this may be due to the limited number of F_3 families sampled rather than an inability to transfer resisitance of 6B364 into this background.

The gene(s) of 6B364 which contribute to partial resistance for lower number of sclerotia also appeared to reduce the amount of honeydew and to a lesser degree sclerotial size. This suggests that either a single gene has an epistatic effect on all three disease components or linkage of genes controlling each disease component. Platford (1976) reported that genes reducing the number of sclerotia and the amount of honeydew appeared to be linked in Kenya Farmer and to assort independantly in Carleton.

The loss of F_2 plants from the Columbus and UM684 crosses in this present study was unfortunate as larger populations may have allowed a more accurate genetic anaylsis and intrepretation of the the number of genes controlling partial resistance. In a future study, it would be desireable to evaluate some of the F_3 families in the Columbus and UM684 crosses derived from F_2 plants with partial and susceptible reactions for all three disease components. The data on the F_3 families derived from F_2 parents without sclerotia suggested that the Columbus and UM684 backgrounds resulted in derived F_3 plants not having large sclerotia or large amounts of honeydew (ratings of 7 or larger). On the other hand, many F_3 plants from the Chinese Spring cross had large sclerotia or large amounts of honeydew even though their F_2 parent had no sclerotia. The apparently more stable reactions for partial resistance in the Columbus and UM684 backgrounds may allow estimation of the number of gene(s) controlling the partial resistant

The expression of genes for complete resistance from 6B364 appears to be influenced by the background of the susceptible parent with which it is crossed. Complete resistance was acheived more readily in the background of Columbus than in the background of Chinese Spring. The choice of susceptible parent in this study influenced the intrepretation of the number of genes controlling complete resistance to ergot. The complete resistance of 6B364 (average of less than one sclerotium per plant in a F_3 family) appeared to be controlled by two genes in the Chinese Spring and UM684 backgrounds whereas in the Columbus background, this resistance appeared to be controlled by one gene.

Furthermore, there was evidence that background resistance genes were present in Columbus and not in Chinese Spring since a higher percentage of resistant plants was observed in the F_2 and F_3 generations of the Columbus cross. The presence of genes for resistance in progeny derived from crosses with two susceptible parents has been noted in other host-pathogen interactions such as net blotch of barley (Sharp, 1983), stripe rust of wheat (Krupinsky and Sharp, 1978) and rust of barley (Parlevliet and Kuiper, 1985). Plant breeders often must backcross disease resistance into susceptible cultivars. The results of this study suggest that the selection of a susceptible parent can influence the ability to transfer ergot resistance, and that consideration of the choice of the susceptible parent in a disease resistance breeding program could be advantageous.

The reactions for the numbers of sclerotia in Columbus and Chinese Spring were similar when inoculated 48 hours prior to anthesis. Thus, the evaluation of potential wheat cultivars for their reactions to ergot by injecting 10^4 conidia/mL, as used in the present study, would not in itself reveal which cultivar to use in a crossing program. Perhaps the development of a screening methodology to differentiate the relative susceptibility would be feasible. It would be desireable to test the effects of spore concentration and the time of inoculation in relation to anthesis on the reaction of susceptible cultivars. The partially resistant wheat cultivar Kenya Farmer became completely resistant when inoculations were performed one day after anthesis whereas complete resistance did not occur in the susceptible rye cultivar Prolific until 7 days after anthesis (Ratanopas, 1973). It appears that pollination

induces changes in the ovary that prevent or delay the infection process and that this occurs more rapid in resistant cultivars than in susceptible cultivars (Ratanopas, 1973). In this study, the occurrence of either aborted ovaries or shrivelled seeds in inoculated florets without sclerotia in 6B364 suggests that a similar mechanism may be responsible for the reduced number of sclerotia in both lines. The importance of changes induced by pollination on the complete resistance of 6B364 is not known, and a study on the effect of time of inoculation before anthesis on the reaction of 6B364 would appear desireable.

The hypodermic syringe, the vacuum system, and the airbrush when glumes were clipped were all relatively effective in inducing higher numbers of sclerotia/spike. On the otherhand, the plant misting bottle and airbrush when glumes were intact were not effective methods of inoculation. Reduced numbers of sclerotia occurred when inoculations were made in the field compared to levels in the greenhouse with the syringe, vacuum system and airbrush. Temperatures on the days of inoculation in the field were above 30° C in both years. Galapitage (1983) reported that the average number of sclerotia per spike dropped when inoculations were made at 25° C versus lower temperatures. The use of the syringe, the vacuum system, and airbrush were effective in inducing at least one sclerotium/spike in the greenhouse whereas almost 18 percent plant escapes occurred by both of these methods in the field. The airbrush was not evaluated in the field on plants with the glumes clipped.

The hypodermic syringe has been utilized in most screening studies (Platford and Bernier, 1970; Bernier, 1978) and inheritance studies

(Riley, 1973; Platford, 1976; Kraker, 1979) at the University of Manitoba. This method is relatively accurate, however, it is both tedious and slow. Should the screening for ergot resistance become an objective in a large breeding program, it would be advantageous and likely necessary to have a system that is both rapid and efficient in inducing sclerotia. As well, a large program would necessitate the screening in the field. This study found that the vacuum system and airbrush used on plants with clipped glumes would be more rapid than the syringe, and induce acceptable numbers of sclerotia/spike. Under field conditions, the efficacy of the inoculation techniques dropped. To compensate for the high number of susceptible plant escapes in the field inoculations, several plants per line could be inoculated. High temperatures at the time of inoculation were likely responsible for decreasing the number of infected florets, and the use of nets to shade plots would likely increase the efficacy of field inoculations.

VI. LITERATURE CITED

Abe, T. and Kono,M. 1957. On the relationship between the infection and fertilization of cereal crops inoculated with a conidial suspension of ergot fungus,Claviceps purpurea(Fr.) Tul. Sci. Rep. Fac. Agric. Saikyo Univ. 9:34-40.

Alexopoulus, C.J. and C.W. Mimms.1979. Introductory Mycology. John Wiley and Sons. 3rd. ed. p.340,341.

Atanasoff, D. 1920. Ergot of grains and grasses. Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture 127pp.

Bacon,C.W. and Luttrell,E.S. 1981. Competition between ergot of Claviceps purpurea and rye seed for photosynthates. Pytopathology 72:1332-1336.

Barger,G. 1931. Ergot and Ergotism. Gurney and Jackson, London.279pp.

Bernier, C.C. 1978. Evaluation of resistance to Claviceps purpurea in the genus Triticum. 3rd International Congress of Plant Pathology,p296 Abstract.

Bragg, D.B.,Salem, H.A. and Develil, T.J. 1970. Effect of dietary Triticale ergot on the performance and survival of broiler chicks. Can. J. of Anim. Sci. 50:259-264.

Brentzel, W.E. 1947. Studies on ergot of grains and grasses. North Dakota Agr. Exp. Sta. Bull. 348 20pp.

Bretag, T.W. and Merriman, P.R. 1981a. Epidemiology and cross-infection of Claviceps purpurea. Trans. Brit. Mycol. Soc. 77:211-213.

Bretag, T.W. and Merriman, P.R. 1981b. Effect of burial on the survival of sclerotia and production of stromata by Claviceps purpurea. Trans. Br. Mycol. Soc. 77:658-660.

Brodny, U., Nelson, R.R. and Gregory, L.V. 1986. The residual and interactive expressions of "defeated" wheat stem rust genes. Phytopathology 76:546-549.

Brown, A.M. 1947. Ergot of cereal and grasses. Abstr in Proc. Can. Phytopath. Soc. 15:15

Burfening, P.J. 1973. Ergotism. Jour. Am. Vet. Med. Assoc. 163: 1288-1290.

Campbell, W.P. 1954. Ergot in cereals in Western Canada in 1954. Can. Plant Dis. Sur. 34:30-32.

Campbell, W.P. 1957. Studies on ergot infection in gramineous hosts. Can. J. of Bot. 35:315-320.

Campbell, W.P. 1958. Infection of barley by Claviceps purperea. Can. J. of Bot. 36:615-619.

Campbell, W.P. and Tyner, L.E. 1959. Comparision of degree and duration of susceptibility of barley to ergot and loose smut. Phytopathology 49:348-349.

Campbell, C.W. and Burfening, P.J. 1972. Effects of ergot on reproductive performance in mice and gilts. Can. J. of Anim. Sci. 52:567-569.

Caporael, L.R. 1976. Ergotism the satan loose in Salem. Nature (NY) 194:21-26.

Colotelo, N. and Cook, W. 1977. Perithecia and spore liberation of Claviceps purpurea:scanning electron microscopy. Can. J. of Bot. 55:1257-1259.

Conners, I.L. 1954. Ergot in cereals in Western Canada in 1953. Can. Plant Dis. Sur. 33:23-27.

Conners,I.L. 1956. Ergot in cereals in Western Canada in 1955. Can. Plant Dis. Sur. 35:29-31.

Corbett, K.,Dickerson, A.G. and Mantle,P.G. 1974. Metabolic studies on Claviceps purpurea during parasitic development on rye. J. of Gen. Microbiol. 84:39-58.

Cunfer,B., Mathre, D.E. and Hockett, E.A. 1975. Factors influencing the susceptibility of male-sterile barley to ergot. Crop Sc. 15:194-196.

Cunfer, B. 1976a. Water potential of ergot honeydew and its influence upon colonization by microorganisms. Pytopathology 66:449-452.

Cunfer, B. 1976b. Inhibition of conidial germination by Claviceps purpurea honeydew fluid. Can. J. of Botany 54:2541-2545.

Cunfer, B.M. and Seckinger, A. 1977. Survival of Claviceps purpurea and C. paspali sclerotia. Mycologia. 69:1137-1140

Darlington, C.C. and Mathre, D.E. 1976. Resistance of male sterile wheat to ergot as related to pollination and host genotype. Crop Sci. 16:728-730.

Darlington, C.C., Mathre, D.E. and Johnston, R.H. 1977. Variation in pathogenicity between isolates of Claviceps purpurea. Can. J. Plant Sci. 57:729-733.

Dillon-Weston,W.A. and Taylor,R.D.1942. Observations of ergot in cereal crops. J. of Agric. Sc. 32:457-466.

Ellingboe, A.H. 1982. Genetic aspects of active defense. In Active Defense Mechanisms in Plants. ed. R.K.S. Wood Plenum. New York. p179-192.

Falconer, D.S. 1981. Introduction to Quantitative Genetics. 2nd ed. Longman Inc. New York. 340 pp.

Flor, H.H. 1955. Host-parasite interactions in flax rust-Its genetic and other implications Phytopathology 45:680-685.

Futrell, M.C. and Webster, O.J. 1965. Ergot infection and sterility in grain sorghum. Plant Dis. Rep. 49:680-683.

Galstjan-Avanesjan, S.H. 1967. Susceptibility of some wheats, their hybrids and certain other Graminae to Claviceps purpurea. Plant Br. Abst. 38:4261 Abstract.

Galapitage,N.N. 1983. Effects of temperature on cultural characteristics and pathogenicity of ten isolates of Claviceps purpurea on two spring wheat cultivars.MSc Thesis. Univ. of Manitoba.72pp.

Green, G.J. and Campbell, A.B. 1979. Wheat cultivars resistant to Puccinea graminea f.sp. tritici in Western Canada: Their development, performance and economic value. Can. J. Plant Pathol. 1:3-11.

Hardison, J.R. 1972. Prevention of ascocarp formation in Claviceps purpurea by fungicides applied over sclerotia at the surface. Phytopathology 62:609-611.

Harper, F.R. and Seaman, W.L. 1980a. Ergot of rye in Alberta: Estimation of yield and grade losses. Can. J. of Plant Path. 2:222-226.

Harper, F.R.and Seaman, W.L. 1980b. Ergot of rye in Alberta: Distribution and severity 1972-1976. Can. J. of Plant Path. 2:227-231.

Harper, F.R. and Christensen, G.O. 1986. Control of ergot with systemic fungicides. Pesticide Research Report.p.285,286.

Johnston, R. 1983. Genetic background of durable resistance. In Durable Resistance in Crops, ed. F. Lamberti, J.M. Waller,N.A. Van der Graaff,p. 5-24.

Johnston,R. 1984. A critical analysis of durable resistance. Ann. Rev. Phytopathol. 22:309-330.

Killian, C. 1919. Sur la sexualite de l'ergot de siegle, le Claviceps purpurea Tulasne. Bull. Soc. de France,25:182-197.

Kirchoff, H. 1929. Contributions to the biology and physiology of the ergot fungus. Rev. of Appl. Mycol. 8:560-562.

Knott, D.R. 1972. Effects of selection for F2 plant yield on subsequent generations in wheat. Can. J. Plant Sci 52:72-76.

Krakar, P.J. 1979.Nature and inheritance of ergot (Claviceps purpurea) susceptibility in Triticum timopheevi (Zhuk) X Secale cereale and Triticum timopheevi X Triticum durum hybrid. Ph.d Thesis. Univ. of Man. 94pp.

Krupinsky, J.M. and E.L. Sharp. 1978. Additive resistance in wheat to Puccinea striiformis.Phytopathology 68:1795-1799.

Lewis, R.W. 1945. The field inoculation of rye with Claviceps purpurea. Pytopathology 35:353-360.

Lewis, R.W. 1956. Development of conidia and sclerotia of the ergot fungus on inoculated rye seedlings. Phytopathology 46:295-296.

Lewis, R.W. 1959. Production, storage and germination of conidia of C. purpurea. Acta. Botanica Acad. Sci. Hung. 5:71-77.

Lewis, R.W. 1962. Guttation Fluid:Effects on growth of Claviceps purpurea in vitro. Science 138:690-691.

Luttrell, E.S. 1980. Host-parasite relationships and development of the ergot sclerotium in Claviceps purpurea. Can. J. Bot. 58:942-958.

Mantle, P.G. and Shaw, S. 1976. Role of ascospore production by Claviceps purpurea in aetiology of ergot disease in male sterile wheat. Trans. Brit. Mycol. Soc. 67:17-22.

Marshall, G.M. 1960. The incidence of certian seed borne diseases in commercial seed samples. Ann. Appl. Biol. 48:19-26.

Martin, T.J. and Ellingboe, A.H. 1976. Differences between compatible parasite/host genotype involving Pm4 locus of wheat and the corresponding genes in Erysiphe graminis f.sp. tritici Phytopathology 66:1435-1438.

McCrea, H. 1931. The reactions of Claviceps purpurea to variations of environment. Amer. J. of Bot. 18:50-77.

Mitchell, D.T. and Cooke, R.C. 1968. Some effects of temperature on germination and longevity of sclerotia in Claviceps purpurea. Trans. Brit. Mycol. Soc. 51:721-729.

Mower, R.L. Snyder, W.C. and Hancock, J.G. 1975. Biological control of ergot by Fusarium. Phytopathology 65:5-10.

Nass, H.A. Pedersen, W.L.,Mackenzie, D.R. and Nelson, R.R. 1981. The residual effects of some "defeated" powdery mildew resistance genes in isolines of Chancellor winter wheat. Phytopathology 71:1315-1318.

Nielsen, J. 1983. Spring wheat immune or highly resistant to Ustilago tritici. Plant Disease 67:860-863.

Pantidou, M.E. 1959. Claviceps from zizantia. Can. J. of Bot. 37:1233-1236.

Parlevliet, J.E. and Zadoks, J.C. 1977. The integrated concept of disease resistance; a new view including horizonital and vertical resistance in plants. Euphytica 26:5-21.

Parlevliet, J.E. 1983. Durable resistance in self-fertilizing annuals. In Durable Resistance in Crops ed. F. Lamberti, J.M. Waller, N.A. Van der Graaff, pp347-362.

Parlevliet, J.E. and Kuiper, H.J. 1985. Accumulating polygenes for partial resistance in barley to barley leaf rust, Puccinia hordei. I. Selections for increased latent periods. Euphytica 34:7-13.

Parlevliet, J.E., Leijn, M. and Van Ommeren, A. 1985. Accumulating polygenes for partial resistance in barley to barley leaf rust, Puccinia hordei. II. Field evaluation. Euphytica 34: 15-20.

Peach, J.M. and Loveless, A.R. 1975. A comparision of two methods of inoculating Triticum aesticum with spore suspensions of Claviceps purpurea. Trans. Brit. Mycol. Soc. 64:328-331.

Platford, R.G. and Bernier, C.C. 1970. Resistance to Claviceps purpurea in spring and durum wheat. Nature 226:770.

Platford, R.G. 1976. Reaction of cultivated cereals, chromosomal location and inheritance of resistance in wheat to Claviceps purpurea. Ph.d Thesis. Univ. of Manitoba.81pp.

Platford, R.G. and Bernier, C.C. 1976. Reaction of cultivated cereals to Claviceps purpurea. Can J. Plant Sci. 56:51-58.

Platford, R.G., Bernier, C.C. and Evans, L.E. 1977. Chromosome location of genes conditioning resistance to Claviceps purpurea in spring and durum wheat. Can. J. Genet. Cytol. 19:679-682.

Potbury, M. and Drysdale, R.B. 1969. Effects of guttation fluid from rye and barley on growth of Claviceps purpurea in vitro. Brit. Mycol. Soc. 53:137-138.

Puranik, S.B. and Mathre, D.E. 1971. Biology and control of ergot on male-sterile wheat and barley. Phytopathology 61:1075-1080.

Rapilly, F. 1968. Etudes sur l'ergot du ble: Claviceps pupurea (Fr.) Tul. Annls Epiphyties 19:305-309.

Ratanopas, S. 1973. Evaluation of resistance to Claviceps purpurea in two wheat cultivars and effect of fertilization on the disease reaction of resistant and susceptible cereal cultivars.MSc. Thesis. Univ. of Manitoba. 73pp.

Ratanopas, S. 1976. Variability and nutrition of Claviceps purpurea in culture. Ph.d Thesis. Univ. of Manitoba 141pp.

Riggs, R.K., Henson, L. and Chapman, R.A. 1968. Infectivity of and alkaloid production by some isolates of Claviceps purpurea. Pytopathology 58:54-55.

Riley, K.W. 1973. A study of the inheritance of resistance to ergot Claviceps purpurea in two wheats: Triticum durum desf. and T. timopheevi Zhuk. MSc. Thesis. Univ. of Manitoba. 48pp.

Roberts, J.J., Hendricks, L.T. and Patterson, F.L. 1984. Tolerance to leaf rust in susceptible wheat cultivars. Phytopathology 74:349-351.

Samborski, D.J. and Dyck, P.L. 1982. Enhancement of resistance to Puccinia reconditia by interactions of resistance genes in wheat. Can. J. Plant Pathol. 4 :152-156.

Schmidt, H. and Lucken, K. 1976. Ergot resistance in spring wheats. Agronomy Abstracts, Annual Meetings of A.S.A. p61.

Seaman, W.L. and Harper, F.R. 1974. Incidence of ergot in wheat in Western Canada. Proc. Can. Phytopath. Soc. 41:32 Abstract.

Seaman, W.L. 1980. Ergot of grains and grasses. Can. Dept. Agri., Publication No. 1438 9pp.

Seymour, E.K. and McFarland, F.T. 1921. Loss from rye ergot. Phytopathology 11:41 Abstract.

Sharp, E.L. 1983. Experience of using durable resistance in the U.S.A. In: Durable Resistance in Crops ed. F. Lamberti, J.M. Waller, N.A. Van der Graaff, pp385-396.

Simons, M.D. 1985. Transfer of field resistance to Puccinia corontata from Avena sterilis to cultivated oats. Phytopathology 75 :314-317.

Soos, T. 1969. On the viability of Claviceps purpurea (Fr.) Tul. strains. ACTA Agron. Acad. Sci. Hung 18:49-54.

Spanos, N.P. and Gottlieb, J. 1976. Ergotism and the Salem village witch trials. Science 194 :1390-1394.

Stager, R. 1903. Infectionsversuche mit Gramineen-bewohnenden Claviceps. Arten. Botan Ztg. 61:111-158.

Thakur, R.P. and Williams, R.J. 1980. Pollination effects on Pearl Millet Ergot. Phytopathology 70:80-84.

Thakur, R.P., Williams, R.J. and Rao, V.P. 1982. Development of resistance to ergot in pearl millet. Phytopathology 72:406-408.

Van der Plank, J.E. 1963. Plant Diseases:Epidemics and Control. New York Academic. 349 pp.

Van der Plank, J.E. 1968. Disease Resistance in Plants. New York: Academic 206 pp.

Vizos, J.H., Coley-Smith, J.R. and Swetez, V. 1984. Overwintering of the conidial stage of Claviceps purpurea on Poa annea. Trans. Brit. Mycol. Soc. 82:342-343.

Weniger, W.1924. Ergot and its control. N.D. Agric. Stn. Bul. 176.23pp.

Wood, G. and Coley-Smith, J.R. 1980. The effectiveness of of fungicides against Claviceps purpurea attacking male-sterile barley. Ann. Appl. Biol. 96:169-175.

Table 1. Rating scales for the ergot disease components amount of honeydew and sclerotial size.

Disease Scale	Sclerotial Size	Amount of Honeydew
0	No sclerotia	No honeydew
1	All sclerotia smaller than seed.	Honeydew within the floret.
3	One sclerotium seed size, the rest smaller.	A single small droplet outside floret.
5	More than one sclerotia of seed size.	Several droplets outside floret.
7	One sclerotium larger than seed.	One large droplet outside floret(s).
8	Two sclerotia larger than seed.	Several large droplets outside several florets.
9	More than three sclerotia larger than the seed.	Copious amounts on many florets.

Table 7. Calculation of the narrrow sense heritabilities by regression of the F_3 family means onto its F_2 parent from the cross 6B364 X Chinese Spring for the three disease components.

Components Regressed (F3 to F2)	All F_2 and F_3 plants (N= 58 families) reg h^2	F2 and F3 populations ignoring plants without sclerotia (N= 27 families) reg h^2
sclerotia no. to sclerotia no.	0.29 + .10**	0.26 + .11**
honeydew to to honeydew	0.35 + .10*	0.57 + .15*
size to size	0.19 + .07**	ns
sclerotia no. to honeydew	0.34 + .08*	.39 + .10*
honeydew to sclerotia no.	0.26 + .11**	ns
honeydew to size	0.16 + .09***	ns
size to sclerotia no.	0.23 + .08**	ns
size to honeydew	.23 +.08**	ns
sclerotia no.	0.21 +.08**	ns

* significant at .01.
** significant at .05.
*** significant at .10.

Table 2. Reactions of parents and F_1 progenies of crosses between the resistant wheat line 6B364 and three susceptible wheat cultivars inoculated with ergot isolate M-4.

	Number of plants evaluated	Number of sclerotia		Amount of Honeydew		Sclerotia Size	
		Mean	Range	Mean	Range	Mean	Range
Parents							
Chinese(CH) Spring	16	8.3	6-9	8.4	8-9	7.9	7-9
UM684 (UM)	9	7.6	6-9	7.0	5-9	8.0	7-9
Columbus(Co)	5	7.2	6-9	2.8	0-7	5.6	5-8
6B364	25	0.0	-	0.0	-	0.0	-
F1 Progeny							
6B364 X CH	12	4.8	1-8	5.9	5-8	7.8	7-9
6B364 X UM	10	4.0	1-8	2.3	0-5	4.8	3-7
6B364 X Co	25	3.7	0-7	1.7	0-8	4.6	0-8

a - The number of sclerotia per spike with 10 florets inoculated.
b - No honeydew=0; Large droplets outside floret=8. See Table 1 for further explaination of ratings.
c - Smaller sclerotia than seed < 5; Sclerotia seed size =5; Sclerotia larger than seed > 5.

Table 10. The average number of sclerotia per spike in parents and selected F_4 lines from the Chinese Spring X 6B364 cross.

Line	Isolate F-1 Average number of sclerotia/spike	Isolate P-2 Average number of sclerotia/spike
Chinese Spring	8.5	8.4
Line 46	0.8	2.5
Line 165	0.8	2.0
6B364	0	0

a- at least four plants/line evaluated. Spikes were inoculated by injecting conidia/mL with a hypodermic syringe.
b- F_4 lines from the 6B364 cross which were completely resistant in the F_2 and the resulting F_3 family average less than 1 sclerotium when inoculated with the ergot isolate M-4.