THE UNIVERSITY OF MANITOBA

EVALUATION OF RESISTANCE TO *CLAVICEPS PURPUREA* IN TWO WHEAT CULTIVARS AND EFFECT OF FERTILIZATION ON THE DISEASE REACTION OF RESISTANT AND SUSCEPTIBLE CEREAL CULTIVARS

by

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EVALUATION OF RESISTANCE TO *CLAVICEPS PURPUREA* IN TWO WHEAT CULTIVARS AND EFFECT OF FERTILIZATION ON THE DISEASE REACTION OF RESISTANT AND SUSCEPTIBLE CEREAL CULTIVARS.

ABSTRACT

Plants of the spring wheat cultivar Kenya Farmer and durum wheat cultivar Carleton which possess some resistance to ergot, were inoculated in the greenhouse along with two susceptible cultivars, with each of 58 isolates of *Claviceps purpurea* and one isolate of *C. zizaniae*.

Considerable variation in disease severity was induced by the ergot isolates within as well as among the four cultivars. The reactions induced by some of the isolates appeared to be due to genetic rather than environmental factors since some of the isolates tested 2 and 3 times gave similar results. Differential interaction of the isolates with the resistant and susceptible cultivars indicate that resistance is vertical and that the isolates then differ in virulence. Isolates which consistently induced a disease rating of 1 only on the cultivars Kenya Farmer or Carleton, and in a few cases in all four cultivars, were recognized for the first time.

The resistance of Kenya Farmer was shown to be different and greater than that of Carleton. None of the isolates tested induced the production of visible honeydew and three isolates only induced a moderately susceptible reaction in the cultivar Kenya Farmer, whereas

many isolates produced honeydew and 19 isolates induced a susceptible reaction in Carleton. Both cultivars were more resistant than either Stewart 63 or Manitou and Stewart 63 was found to be highly susceptible to more isolates than Manitou.

Changes occurred in the disease reaction of five cereal cultivars, when inoculations were made on or shortly after the day of anthesis. Following fertilization, there was a reduction in the number of sclerotia as well as in the amount of honeydew produced. This effect was expressed sooner in the cultivars Kenya Farmer, than in the cultivars Manitou, Rosner and Conquest, and only gradually on the cultivar Prolific. In the cultivars Kenya Farmer, Manitou and Rosner, the number of partially infected kernels increased as the number of sclerotia decreased. Fertilized ovaries were susceptible to infection and could remain susceptible for 3 to 6 days after anthesis.

The format for this thesis is outlined below and has been approved by the Council of the Faculty of Graduate Studies and Research of the University of Manitoba.

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INTRODUCTION

Ergot disease of barley, rye, wheat and of many wild and cultivated species of grasses is caused by the fungus Claviceps purpurea (Fr.) Tul. This fungus has world-wide distribution. Of the crops mentioned, rye is the most susceptible.

The disease is characterized by the formation of black purplish ergot bodies in place of kernels. These bodies are termed "sclerotia". Because the ergotized heads also produce blasted kernels, empty glumes and a reduction in head size, the quality and per acre yield of infected grain and hay is also reduced (36).

Sclerotia contain potent poisonous alkaloids that if ingested even in small amounts are harmful to the circulatory system of most if not all animals. For this reason ergot disease of plants is of particular significance to the world's livestock industry. This condition characterized by circulatory malfunction is generally referred to in livestock circles as ergotism. Susceptible animals include cattle, horses, mules, sheep, hogs and poultry.

Ergot disease of crops has been reported from all provinces of Canada. In 1953 an extensive field survey of the Prairie Provinces revealed the presence of ergot on rye, wheat and barley. In fields of rye, ergot was about 10 times more prevalent than in those of wheat or barley. The incidence of ergot infection in cereal crops varies considerably from year to year. Data collected by grain

inspectors illustrates this with respect to ergot on rye. In the 1949-1950 crop year 1.3% of the railway cars inspected were graded "ergoty" whereas in the 1950-1951 year 7.7% were designated "ergoty". In the 1951-1952 crop year only 0.2% were down-graded for ergot. On the other hand, the incidence of ergot in 1952-1953 was 10.1% (11). The incidence of ergot is greatly influenced by the wide host range of the fungus as well as by environmental factors. There are 38 different host species of C. purpurea in Western Canada (9). Heavy infection is usually associated with weed grasses in or adjacent to fields of cereal crops (13).

Recently, male sterile wheat cultivars, triticale (Triticum sp. x Secale cereale), and lines used in the development of hybrid spring wheat at the University of Manitoba were found to be highly susceptible to ergot. Studies designed to locate possible sources of resistance for use in the triticale and hybrid wheat breeding programs are in progress. During these investigations two wheat cultivars were found to possess some resistance to ergot (30).

The objectives of the study here reported were to further evaluate the two resistant wheat cultivars and to determine the variability of the fungus with respect to virulence. The response of selected cereal cultivars when inoculated prior, during, and after anthesis was also investigated.

LITERATURE REVIEW

History of ergot

When the fungus Claviceps purpurea (Fr.) Tul. infects rye it is given the common name of "ergot" (18). The common name of this disease is of French origin being taken from "argot or ergot", meaning "spur" (16). The fungus affects flowers of the host and produces ergot sclerotia instead of kernels. The sclerotia are the dark-colored, horn-like structures which are so characteristic of the disease (8).

The Greeks and Romans recorded their observations on ergot, making it one of the oldest known plant diseases (41).

The sclerotia contain alkaloids which have a powerful action on the nervous system of man and animals and can produce gangrene or convulsions and, in severe cases, death (42). Thousands died from ingestion of ergot in cereal grain in Europe and Russia during the 10^{th}, 11^{th}, and 12^{th} centuries (43). "Holy Fire", "St. Anthony's Fire", and "St. Martial's Fire" were names that these early plagues of ergotism were known by (43) and it was one of the dreaded diseases in Europe prior to 1800 (18). These events occurred long before the cause became known (42).

Many workers were involved in revealing the nature of ergot. The ergots had been previously named Sclerotium clavus by De Candolle

in 1815. Levielle, in 1827, recognized the conidial or honeydew stage as a fungus (16).

The first one to note the function of the sclerotia in the production of the asigerous stage was Tulasne in 1852. The full life history of the fungus was established by Kuhn in 1863 (41).

Recognizing the disease

Ergot is localized, not systemic, and attacks single florets. The actual number of ergotized kernels varies from one to several in a head (16).

The symptoms of ergot disease have been discussed (3, 5, 8, 13, 16, 35, 36, 41). There are three main symptoms: 1). Honeydew - The first evidence of the infection is in the conidial "honeydew" stage. At this stage, the conidia of the fungus are produced on the infected florets and released in a sugary exudate which is known as honeydew. The exudate accumulates in droplets or adheres to the surface of the floral structures, depending upon the concentration of the exudate. The exudate which contains a large number of minute conidia is attractive to insects. 2). Blast - As healthy kernels fill and mature, affected spikes will have a number of sterile florets in which kernels do not develop. Butler and Jones (8) stated that the infection of a flower and sclerotial development is usually followed by sterility of neighbouring flowers, which remain empty without the production of sclerotia. These blasted kernels are always greater in number in the ergotized spikes than in the unergotized ones.

Heald (16) explained that the blasting or sterility of adjacent florets is due to incipient infections or to exhaustion of food supply. 3). Sclerotia - Sclerotial formation is the conspicuous stage of the disease. Sclerotia are dark or violet-colored, spur-like hard bodies, which replace the kernel in infected flowers. They are more or less cylindrical, straight or frequently curved, smooth or longitudinally furrowed. When the sclerotia are fully developed, they tend to protrude beyond the floral bracts. The interior of a sclerotium consists of a hard white mass of fungal tissue. Seaman (35) suggests that sclerotial size is determined by the host species. Certain grasses show quite inconspicuous sclerotia whereas other grass species produce ergot bodies many times larger than the kernels.

The causal organism

The fungus Claviceps purpurea (Fr.) Tul. is the causal agent of the disease. The classification is as follows (2): Class: Ascomycetes, Sub-class: Euascomycetidae, Series: Pyrenomycetes, Order: Clavicipitales, Family: Clavicipitaceae.

There are 2 stages of growth in its life cycle, sexual and asexual. These stages of growth have been discussed (3, 5, 8, 13, 16, 41).

Sexual stage - After passing through a resting period, sclerotia in the soil develop stromata and stipes. Up to 30 stromata may form from a single sclerotium. Stipes are cylindrical, and the length varies depending upon the depth of sclerotium below the soil

surface. Stromatal heads are spherical, and pale fawn. The flask-shaped perithecia are embedded within the head with ostioles protruding to upper surface of the head. Asci are produced in the perithecia; they are long, curved, hyaline, narrow at both ends and surrounded by paraphyses. Each ascus contains eight slender, filiform, slightly curved, hyaline, and continuous or septate ascospores.

Asexual stage - It combines conidial and sclerotial stage. In infected florets, the spores germinate into mycelium and form the absorbing structure at the base of the ovary. The mycelium ramifies into the ovary wall and produces the conidia which are held in a sugary exudate secreted by the stroma of the fungus. The conidial bearing mycelium soon differentiate into sclerotial hyphae below the conidial layer. Then, the sclerotia are gradually developed from sclerotial hyphae. At the maturity of the florets, the fully developed sclerotia are formed, and they are attached to the absorbing organ in the rachilla. The sclerotia are composed of a hard outer surface layer, a fertile hyphal mass, and the central large storage cells. In the germination of the sclerotia, the fertile hyphae develop into stromata and stipes.

Disease cycle

The fungus overwinters by means of sclerotia that remain in the soil from a previous crop or from wild or volunteer grasses in the headlands or ditches. Sclerotia may also be disseminated with seed

grain. Sclerotia which fall to the ground before or at harvest time do not germinate until the following spring. They germinate into stromata in which ascospores are produced. Before germination, sclerotia require a period of cold temperature to complete their resting stages followed by a period of higher temperature to germinate. An exposure of several weeks to cold followed by a similar period at a higher temperature favours germination of sclerotia (3). The sclerotia naturally can withstand very low temperature; thus, freezing is conducive to germination. According to Kirchhoff (19) freezing is not essential for the germination of sclerotia. He reported that the maximum germination could be induced at a temperature close to 0^{o} C for 30 to 40 days. The minimum temperature for germination was found to be slightly above 10^{o} C and the optimum is 18^{o} to 22^{o} C. Krebs (20) found that the germination of sclerotia following its exposure to freezing temperatures for 1 month tends to occur rapidly between 9^{o} and 15^{o} C. At 18^{o} C and above sclerotial germination was inhibited.

According to Barger (3) sclerotia do not germinate when dry. This same concept is indicated by Cook and Mitchell (12) when they stated that in their germination trials sclerotia were permitted to imbibe water prior to placing them on damp filter paper in closed dishes.

When conditions of moisture and temperature are favorable sclerotia germinate into stromata from which asci and later ascospores are produced. Ascospores serve as a primary inoculum for the

production of ergot disease. Barger (3) reported that ascospores may be disseminated by either wind or insects.

Butler and Jones (8) stated that the discharge of ascospores from the perithecia occurred in nature at the same time as the flowering of grains and grasses. The infected florets of the susceptible plants produce sweet sticky honeydew containing thousands of fungal conidia. These conidia are known as the secondary inoculum which can be carried to healthy florets by splashing rain, contact, and by the numerous insects which the honeydew attracts. Within the infected florets the fungus continues to grow. It replaces the ovule and gradually forms the sclerotium. It is in the latter stage that the fungus survives the winter.

Host relation

Pammel (28) described several hosts of C. purpurea. These include rye (Secale cereale L.), winter wheat (Triticum aestivum L.), oats (Avena sativa L.), wild ryes (Elymus spp.), quackgrass (Agropyron repens L. Beauv.), timothy (Phleum pratense L.), blue grass (Poa pratensis L.), annual bluegrass (P. annua L.), bluejoint (Calamagrostis canadensis (Michx.) Beauv.), redtop (Agrostis alba L.), and mannagrass (Glyceria fluitans (L.) R. Br.). Among the cultivated cereals rye was indicated as being most susceptible to the disease. He also reported the occurence of ergot on wild rice (Zizania aquatica L.). Pantidou (29) recently classified the ergot on wild rice as Claviceps zizaniae (Fyles) Comb. nov. and designated it a

distinct and separate species from C. purpurea.

McFarland (27) demonstrated cross inoculation of ergot on different hosts. He found that inoculations from rye to rye were easily obtained. Inoculation of rye was secured successfully from the honeydew conidia of each of the following: Bromus inermis Leyss., Agropyron repens (L.) Beauv., Poa pratensis L., and Arrhenatherum elatius (L.) Mert. & Koch. Successful infection of wheat occurred when the conidia from either rye, A. elatius, P. pratensis, or A. repens were used. Cross inoculations have given a higher percentage of infection when made from one of these grass species to another than when made from grass to rye.

Barger (3) reviewed Stager's work on the biological races of Claviceps spp. The specialized races of C. purpurea mentioned by Stager are summarized as follows:

1. The race attacking rye also infects wheat, barley (Hordeum vulgare L.), meadow fescue (Festuca elatior L.), Bromus sterilis L., and a few Poa spp.

2. The ergot from sweet vernal grass (Anthoxanthum odoratum L.) attacks rye and some other grasses but not barley.

3. Wood brome (Brachypodium sylvaticum (Huds.) Beauv.) and mannagrass (Glyceria fluitans) each have their exclusive single race.

4. The race on perennial rye-grass (Lolium perenne L.), infects meadow brome (Bromus erectus Huds.) and other Lolium spp., but not rye.

Brentzel (6) reported that honeydew conidia from barley

have the ability to infect barley, rye, quackgrass, Western wheat grass (Agropyron smithii Rydb.), crested wheat grass (A. desertorum (Fish.) schult.), and smooth brome (B. inermis). Wheat (Quality variety) did not become infected. Sphacelia spores from Poa sp. also infected Virginia wild rye (Elymus virginicus L. and Canada wild rye (E. canadensis L.). The honeydew conidia of both barley and Poa sp. failed to infect oats.

Campbell (9) successfully inoculated 421 isolates of C. purpurea, from 38 different host species, on rye, wheat, and barley growing in the greenhouse. He reported that one of three isolates from Glyceria borealis (Nash) Batch. did not infect these three hosts. However, ergot from rye infected all of the grass species tested, both in the field and in the greenhouse. These results differ from those of Stager (37) who failed to secure infection in many hosts in cross inoculation studies between species of the Gramineae. Furthermore, Campbell found that ergot from perennial rye-grass infected rye. This contradicted Stager's results, but it supported the findings of Bekesy (4) who infected rye with ergot from L. perenne L. and L. perenne L. with ergot from rye. Because of the existence of variability in the conidial colonies, Campbell concluded that physiologic races of C. purpurea as proposed by Stager do exist but only in the cultural sense of the term. However, he found no evidence that these races were species specific.

Riggs et al. (33) studied cross infectivity of isolates of C. purpurea. They used conidia of the isolates obtained from sclerotia

collected from rye, tall fescue (Festuca arundinacea Schreb.), and annual rye grass onto the flowers of Balbo rye, a clone of tall fescue, and annual ryegrass-tall fescue hybrid in the greenhouse. The results revealed that the sclerotia were produced in some plants of each host group by some cultures of each pathogenic group. No consistent evidence of host-pathogen specificity was obtained.

Frauenstein (14) inoculated 14 grasses and rye with an isolate of C. purpurea from timothy in East Germany. He obtained infection on all of the inoculated grasses, except rye.

Mantle (25, 26) experimenting in this field with Spartina townsendii H. & J. Groves and reed grass (Phragmites communis Trin.) reported that these two species of grasses were hosts of C. purpurea.

Loveless (24) recorded a number of hosts of C. purpurea that he located in Britain.

Seaman (35) reported some hosts of C. purpurea in Canada. They are species of the following plants: Agropyron, Agrostis, Avena, Bromus, Calamagrostis, Dactylis, Elymus, Festuca, Hordeum, Lolium, Phalaris, Phleum, Poa, Setaria, Stipa, and Triticum.

Production of ergot inoculum

Ascospores and honeydew conidia were used successfully as the inocula for ergot infection of rye flowers (3). In the commercial production of ergot sclerotia described by Hecke (17), ascospores obtained from germinating sclerotia were used to infect some blossoming rye to obtain honeydew conidia. These conidia were added

to water then sprayed on the crop in the field. Ergot conidia can also be obtained by growing the honeydew conidia in pure culture on suitable media.

Lewis (22, 23) demonstrated the technique of production, storage, and germination of conidia of C. purpurea. His first technique was reported in 1943. He grew the culture on sterilized wheat. After the spores formed, the cultures were blended with a minimum of water and screened. The spore suspension was thickened by adding an equal weight of beet sugar. Spores prepared by this way remained viable in cold storage for 3-4 months. In 1959, he developed a new method for producing ergot inoculum. The conidia of the fungus were produced in liquid shake cultures on potato extract-sucrose broth. These conidia could be preserved in 60% sterile sugar solution in cold storage for months.

Campbell (9) grew surface-sterilized sclerotia on an acidified agar medium. The mycelium growing out of the ergot fragment was transferred to slants of potato dextrose agar on which satisfactory sporulation occurred. The spores from these tubes were used as inocula.

Mantle (25) described two methods of isolating ergot fungus from its sclerotium. In his first method, sclerotia were surface sterilized in 0.1% mercuric chloride solution for 5 minutes, washed in several changes of sterile-distilled water, and cut into fragments which were incubated on potato dextrose agar at 24° C. Pure cultures were transferred to and maintained on agar slopes of the standard

culture medium. He also obtained cultures from single ascospores. For this method he germinated sclerotia by incubating surface-sterilized sclerotia on moist sand at 2° C for 4 weeks followed by incubation at 20° C until stromatal emergence. Then, single ascospores were collected and maintained on the agar medium.

Platford (31) also obtained cultures of ergot isolates in two ways. However, his methods and those employed by Mantle (25) differ in the techniques used. Platford's techniques are described as follows:

1. Sclerotial cultures were isolated by removing the rind from sclerotia and soaking these peeled sclerotia in 2% sodium hypochlorite for 1 minute. Following 4 washings in sterile distilled water, each sclerotium was cut aseptically into several pieces and plated onto 4% malt agar plates that had been acidified with 1 drop of 25% lactic acid. After germination occurred, the cultures were maintained on 4% malt agar slants.

2. Single ascospore cultures were obtained by placing unsterilized sclerotia on moist sterile vermiculite in covered containers and storing at 3° C for approximately 6 weeks. At that time, the containers were placed at room temperature (24° C) until stromata were fully developed. Then, ascospores were collected by allowing the germinated sclerotia to discharge onto 4% malt agar plates for timed intervals. Single ascospores were transferred to 4% malt agar plates until they germinated. These single ascospore cultures were maintained on agar slants of the same medium.

The conidia were increased in liquid shake culture using potato sucrose broth. These conidia were harvested and stored at 3° C in concentrated sugar solution (23). The conidial suspension was adjusted by diluting with sterile-distilled water to produce the required inoculum concentration (30).

Inoculation technique for evaluating ergot infection

Stager (37) used three methods of inoculation of ergot disease: 1). spraying the heads with an inoculum of spores suspended in water, 2). prying the glumes apart and spraying the inoculum into the florets, and 3). dipping the heads into the inoculum. In experiments with ergot of wheat (39), he inoculated the plant by injecting the conidial suspension with a hypodermic syringe.

Campbell (9) tested Stager's four methods of inoculation and finally adopted the method of spraying the inoculum onto the heads after clipping away the tips of the glumes. He also pointed out that clipping the tip of the glumes so that spores could be blown in with automizer and injecting the inoculum into the floret with a hypodermic syringe are fully successful in securing infection. Dipping the head into the inoculum was found to be useful with only few species and is not successful with hosts having stiff glumes, ones in which glume were hairy or waxy, and those hosts possessing narrow opening of the glumes which tend to trap air in the florets and exclude the spore suspension. The method of spraying the heads with a suspension is also ineffective because the florets are closed and the spores were

excluded.

Platford and Bernier (30) used the hypodermic syringe technique of inoculation in determining the resistance of cereal cultivars to ergot in the greenhouse. This technique allows the inoculum to readily reach the ovary of the inoculated florets, and furthermore, the desired amount of inoculum can be applied.

Stage of inoculation

Kirchhoff (19) reported that infection by ergot is generally more successful in the first stage of flowering than later.

Campbell (9) inoculated wheat and rye by removing the tip of the glumes at anthesis and spraying with an atomizer. He found that barley had to be inoculated as the heads were emerging from the leaf sheath in order to produce infection. The sheath was cut back, the awns and glume tips clipped off, and the spore suspension was sprayed on the heads.

Campbell and Tyner (10) later compared 12 barley varieties in the greenhouse by injecting florets with a conidial suspension of C. purpurea by hypodermic syringe and demonstrated that maximum susceptibility occurred before fertilization and that resistance appeared to exist after fertilization.

Butler and Jones (8) stated that the infection of ergot ascospores is more successful when the flowers have just opened, because there is no hindrance from a previous deposit of pollen for the spores to germinate on the stigmatic bushes of the flowers.

Assessment of the disease

Campbell and Tyner (10) determined the degree of infection of C. purpurea on barley by using percent infection of florets as indicated by the presence of sclerotia.

In comparing the infectivity of ergot isolates on some cereals and grasses, Riggs et al. (34) determined the amount of infection by the percent sclerotia formed.

Platford (31) investigated the variability of the reaction to infection by C. purpurea in cultivated cereals aiming to find possible sources of resistance. Inoculated florets were rated for the amount of honeydew produced on a 1-4 scale (1 = no visible honeydew, 4 = large drops of honeydew). The number of sclerotia formed was recorded and the sclerotia were rated for size on a 1-3 scale (1 = sclerotia very small, 3 = sclerotia larger than kernel). The spring wheat cultivar Kenya Farmer and the durum wheat cultivar Carleton were found to possess some resistance and were tested further with four ergot isolates (30). In both cultivars resistance is expressed by a decrease in the number of florets that develop sclerotia and a reduction in the amount of honeydew produced and in the size of the sclerotia. In addition, the majority of florets without sclerotia contain small, discolored, and shrivelled ovaries or undeveloped kernels rather than normal kernels. Therefore, in Kenya Farmer and Carleton the number of sclerotia produced per head is not an accurate index of the amount of infection.

SECTION 2

Results of research in publication form

1. An evaluation of the resistance to Claviceps purpurea in two wheat cultivars and of variation in the virulence of the pathogen.

ABSTRACT

Plants of the spring wheat cultivar Kenya Farmer and durum wheat cultivar Carleton which possess some resistance to ergot, were inoculated in the greenhouse along with two susceptible cultivars, with each of 58 isolates of Claviceps purpurea and one isolate of C. zizaniae. The amount of honeydew produced was rated visually, and the number of inoculated florets with an aborted ovary, a partially infected kernel and sclerotial size was recorded. A system of rating disease severity was developed on the basis of the above criteria and each host-isolate combination was assigned to one of six disease reaction classes.

Considerable variation in disease severity was induced by the ergot isolates within as well as among the four cultivars. The reactions induced by some of the isolates appeared to be due to genetic rather than environmental factors since some of the isolates tested 2 and 3 times gave similar results. Differential interaction between the isolates and resistant and susceptible cultivars indicated that resistance is vertical and that the isolates differed in virulence. Isolates which consistently induced a disease rating of 1 on the cultivars Kenya Farmer or Carleton, and in a few cases in all four cultivars, were recognized for the first time.

The resistance of Kenya Farmer was shown to be different and greater than that of Carleton. None of the isolates tested induced the production of visible honeydew and three isolates only induced a moderately susceptible reaction in the cultivar Kenya Farmer, whereas many isolates produced honeydew and 19 isolates induced a susceptible reaction in Carleton. Both cultivars were more resistant than either Stewart 63 or Manitou. Stewart 63 was found to be highly susceptible to more isolates than Manitou.

INTRODUCTION

The spring wheat, (Triticum aestivum L.) cultivar Kenya Farmer and the durum wheat, (T. durum Desf.) cultivar Carleton, were reported by Platford and Bernier (30) to possess some resistance to Claviceps purpurea (Fr.) Tul. In both cultivars, resistance is expressed by a decrease in the number of florets that develop sclerotia and a reduction in the amount of honeydew produced and in the size of the sclerotia. In addition, the majority of florets without sclerotia contain small, discolored and shrivelled ovaries or undeveloped kernels rather than normal kernels. Thus, resistance is not expressed as resistance to infection. The resistance of the cultivars Kenya Farmer and Carleton was expected to be adequate. Honeydew production is very small and secondary infection should therefore be minimal. Furthermore, the fact that only low numbers of very small sclerotia are produced should reduce the amount of primary inoculum the next spring.

The first reports by Stager (37, 38) concerning host specificity within C. purpurea are still commonly cited (5, 13) even though they were not supported by more recent research (4, 9). In a thorough study Campbell (9) tested 421 isolates of C. purpurea from 38 different host species and found that all indigenous and forage

grasses constitute a reservoir of ergot inoculum for rye, wheat, and barley. He noted variability in the conidial colonies and acknowledged the existence of physiologic races, in the cultural sense of the term, but found no evidence that these races were species specific.

Recently, Platford (31) compared two ergot isolates on a number of cultivated cereals and found that they differed in aggressiveness. We know of no other studies concerning aggressiveness or virulence in this fungus.

Since four isolates only were used in the original tests, the present study was undertaken to evaluate 1). the stability of the two resistant cultivars when tested against a wider range of isolates, and 2). the variability of the pathogen with respect to virulence.

MATERIALS AND METHODS

Selection of isolates and preparation of inoculum

One isolate of *Claviceps zizaniae* (Fyles) comb. nov. from wild rice and fifty-eight isolates of *C. purpurea* (Fr.) Tul. from various cultivated cereals and native or cultivated grasses were selected for this study, from amongst the permanent cultures of the fungus maintained on malt agar. The isolates were obtained by culturing either surface-sterilized sclerotial pieces on acid malt agar (sclerotial isolates) or single ascospores (ascospore isolates) (31) (Appendix 1). The host source, number and type of isolate are listed in Table 1. Conidia were increased as required on liquid shake culture (23) (Appendix 2). The mycelium was removed by filtration through a coarse sintered glass filter. The conidia were recovered from the filtrates and washed twice in sterile distilled water by low-speed centrifugation. They were resuspended in sterile 60% solution of sucrose and stored at 3° C. Although stock conidial suspensions prepared in this way have been reported not to show any appreciable loss in germination after 3 months in storage (23), they were used within 6-8 weeks in this study. Inoculum was prepared by diluting the stock conidial suspensions with sterile distilled water and unless otherwise stated, the spore density was adjusted to approximately 10^4 conidia/ml.

Plant materials and inoculation technique

The resistant wheat cultivars Kenya Farmer and Carleton as well as the susceptible cultivars Manitou (spring wheat) and Stewart 63 (durum wheat) were evaluated. Stewart 63 was found by Platford (31) to be more susceptible than Manitou and these cultivars were included to provide a check on the infectivity of the inoculum as well as to allow a broader assessment of virulence amongst the isolates tested. The experiment was done once. However, inoculations were repeated with some isolates which induced low levels of infection. Test plants were grown in the greenhouse in a 3:1:1 loam:peat:sand mixture in 6 in. pots, four plants/pot. Artificial light was used when necessary to provide a minimum intensity of about 700 ft-c. and a constant day length of 16 hr. A sufficient number of plants of the cultivars to be tested were grown at one time to test five to eight isolates. Approximately two days before anthesis, heads were selected for uniformity and after removing the outer glumes, 10 florets/head were inoculated by hypodermic syringe, injecting about 0.02 ml of the conidial suspension into each floret (30). Three heads of each cultivar were inoculated with each isolate and immediately covered with a small glassine envelope.

Assessment of the disease reaction

Approximately 10 days after inoculation, the amount of honeydew produced was rated visually as follows: 1 - no visible honeydew; 2 - honeydew confined within glumes; 3 - honeydew exuding from florets

in small drops and 4 - honeydew exuding from florets in large drops and running down the head (30). After 21 days, the reaction of each inoculated floret was determined and for each host-isolate combination, the number of florets with a kernel, an aborted ovary, a partially infected kernel and a sclerotium of size 1, 2 and 3 was recorded. The term "aborted ovary" denotes florets with a small, discolored and shrivelled ovary or undeveloped kernel. The term "partially infected kernel" denotes partial kernel development with mycelium at the base and usually with some discoloration of the adjacent tissue. These two reactions appear to be due to a high degree of host-parasite incompatibility and in assessing disease severity induced by individual isolates, they were grouped together and referred to as "abortive reaction". Sclerotial size was rated visually as follows: 1 - sclerotia very small; 2 - sclerotia approximately the size of a normal kernel and 3 - sclerotia larger than kernel, extending beyond the lemma and palea (30). A system of rating disease severity was developed using the above criteria, in order to compare the resistance of the cultivars and the virulence of the ergot isolates. In the rating system outlined in Table 2, emphasis is placed on the infection type and the amount of honeydew produced. A resistant reaction (2 rating) is based on the reaction of Kenya Farmer as described by Platford and Bernier (30).

Table 1. Host source, type, and number of isolates of *Claviceps purpurea*.

Host	Host code	Type of isolate: Sclerotia	Type of isolate: Single ascospore	Total
Rye (*Secale cereale* L.)	R	7	11	18
Quackgrass (*Agropyron repens* (L.) Beauv.)	C	8	3	11
Spring wheat (*Triticum aestivum* L.)	M	7	-	7
Smooth brome (*Bromus inermis* Leyss.)	B	2	5	7
Triticale sp.	T	3	2	5
Ryegrass (*Lolium temulentum* L.)	L	3	-	3
Timothy (*Phleum pratense* L.)	P	2	-	2
Durum wheat (*Triticum durum* Desf.)	D	1	-	1
Oat (*Avena sativa* L.)	O	1	-	1
Reedgrass (*Calamagrostis canadensis* (Michx) Beauv.)	CT	1	-	1
Common reed (*Phragmites communis* trin.)	PM	1	-	1
Tall fescue grass (*Festuca arundinacea* Schreb.)	F	1	-	1
Total		37	21	58

Table 2. Rating system for disease caused by Claviceps purpurea.

Varietal reaction	Infection			
	Rating	Type[1/]	Frequency of Sclerotia	Amount of Honeydew
Immune (I)	0	No infection; seed in all florets.	-	1
Very resistant (VR)	1	Abortive reaction (AR) mainly; no sclerotia.	-	1
Resistant (R)	2	AR and sclerotia of size 1 & 2 only.	Not greater than 40%	1-2
Moderately resistant (MR)	3	Sclerotia mainly size 1 & 2; 1 to 3 sclerotia of size 3.	Not greater than 60%	1-2
Moderately susceptible (MS)	4	Sclerotia of size 2 & 3.	Not greater than 80%	3
Susceptible (S)	5	Sclerotia mainly size 3.	Greater than 80%	4

1/ For explanation, see text.

RESULTS

Two of the 59 ergot isolates tested were found to be non-pathogenic, (i.e. all florets contained seed), one on Kenya Farmer and one on Manitou. Furthermore, a number of pathogenic isolates induced lower levels of infection on one or more of the four wheat cultivars than the four isolates used originally by Platford and Bernier (30). Although it seemed unlikely that the results were due to low infectivity of the inoculum, the isolates which induced the lowest levels of infection on a given host were tested again to confirm their reaction. Thus, 30, 25, 12 and 16 isolates were retested on plants of Kenya Farmer, Carleton, Manitou and Stewart 63 respectively. The results, on the basis of the previous host order, were as follows: 8, 13, 6 and 8 isolates respectively showed an increase; 8, 2, 1 and 6 isolates respectively showed a decrease and 14, 10, 5 and 2 isolates respectively, showed no change in disease rating. The extent of the increases in the disease ratings was greater in Carleton, Manitou and Stewart 63 than in Kenya Farmer. In the first three cultivars, 5, 5 and 6 isolates respectively induced a susceptible rather than a resistant reaction as in the previous test, whereas no isolate induced a disease rating greater than 2 on Kenya Farmer. Of the two isolates previously found to be nonpathogenic, one induced a disease rating of 1 on Kenya Farmer and the other a rating of 2 on Manitou. The highest disease rating induced by

each isolate in either test was used in presenting the data.

The wide range of disease severity induced in the wheat cultivars by the 59 ergot isolates is clearly shown, when the isolates are ranked in order of decreasing disease severity (Table 3). Variation in disease severity is observed within the individual cultivars as well as among the four cultivars inoculated with individual isolates. In each cultivar some isolates could not induce the formation of sclerotia and gave only an abortive reaction (1 rating). This reaction was particularly striking in Kenya Farmer in which 19 isolates, 13 of which were tested twice, gave a 1 rating. Although the relative performance of individual isolates is explicit, the more relevant types of isolate-cultivar interactions are shown below. Five isolates (R-17, P-1, Z-1, PM-1, and B-2) induced a low disease rating (1 or 2) and two isolates (C-1 and F-1) induced a disease rating of at least 4 on all four cultivars. Thirteen isolates (R-2A, R-6A, R-7A, R-9A, R-10A, R-13A, R-15, T-2A, O-1, C-2, C-3A, C-4, B-3A) induced a very resistant to resistant reaction (1 or 2 rating) in Kenya Farmer but a susceptible reaction (4 or 5 rating) in Carleton. Seven of the isolates were amongst those tested twice on Kenya Farmer with identical results. Thirteen isolates (M-2, M-4, R-1, R-3A, R-11A, R-12A, R-14, L-3, B-5A, B-6A, C-7, C-11, T-1) induced a disease rating of 1 or 2 on both Kenya Farmer and Carleton and of 4 or 5 on both Manitou and Stewart 63. Five isolates (R-13A, T-2A, C-2, C-3A, C-4) induced a resistant to very resistant reaction on the spring wheat cultivars Kenya Farmer and Manitou and a susceptible

reaction on the durum wheat cultivars, Carleton and Stewart 63. Isolates R-13A, T-2A and C-3A were amongst the isolates tested a second time on Kenya Farmer with identical results. Isolates R-13A and T-2A were also tested twice on Manitou and the results were the same.

There was no apparent effect of host source on the performance of the isolates with the exception that 9 of 18 isolates from rye induced a disease rating of 4 on Carleton. This is about half the total number of 4 ratings induced on this cultivar. The isolate from wild rice was one of two isolates which gave an abortive reaction (1 rating) on all four cultivars. However, both isolates induced a certain number of infected florets particularly in Kenya Farmer, and these isolates and others that induced similar reactions should be considered as capable of infecting.

Six isolates (P-1, C-5A, Z-1, PM-1, B-2, B-6A) which had induced a disease rating of 1 on Kenya Farmer and of 1 or 2 on Carleton when tested twice at a spore density of 10^4 conidia/ml, were tested again at a spore density of 10^6 conidia/ml on both Kenya Farmer and Carleton. Isolates P-1, Z-1, and B-2 were also tested on Manitou and Stewart 63 and isolate PM-1 on Manitou. All the isolates induced a disease rating of 1 on both Kenya Farmer and Carleton with the exception of isolate C-5A which induced a rating of 2, as it had done in one of the previous tests. All the isolates tested on Manitou and Stewart 63 induced a very resistant or resistant reaction with the exception of isolate B-2 on Stewart 63 which induced a disease rating of 4 (susceptible) instead of 1 as in both previous tests. However,

only 10 sclerotia, nine of size 1 and one of size 2 were produced and the reaction received a 4 rating because of a honeydew rating of 3.

None of the ergot isolates induced the production of visible honeydew (3 or 4 rating) in Kenya Farmer. Seven isolates only induced a rating of 2 and the remaining isolates induced a rating of 1. On Carleton, Manitou and Stewart 63, the results were variable and 15, 40 and 48 of the 59 isolates respectively, induced a honeydew rating of 3 or 4. Generally, isolates that induced a disease rating of 4 or 5 (susceptible reaction) also induced a susceptible honeydew reaction (3 or 4 rating) and isolates that induced a disease rating of 1 or 2 induced a low honeydew rating. However, a few exceptions were encountered. In Carleton and Manitou four and two isolates respectively induced a susceptible disease reaction (4 rating) but only a honeydew rating of 2. This was not observed in Stewart 63. On the other hand, six isolates (R-9A, T-2A, O-1, C-1, F-1, B-3A) induced the production of visible honeydew in Carleton even though the size and the number of the sclerotia produced did not exceed the limits of a 2 disease rating. None of the isolates induced such a reaction on Manitou or Stewart 63.

The response of the four wheat cultivars to the 59 ergot isolates is summarized in Table 4. Kenya Farmer was clearly more resistant to a greater number of isolates than Carleton. Furthermore, three isolates only induced a 4 reaction on Kenya Farmer as compared to 19 in Carleton. On the other hand, both cultivars were more resistant than Manitou and Stewart 63 and Manitou was highly susceptible to fewer isolates than Stewart 63.

Table 3. Disease ratings induced by 58 isolates of *Claviceps purpurea* and one isolate of *C. zizaniae* in each of four wheat cultivars.

Isolate[1/]	Disease rating[3/]			
	Kenya Farmer	Carleton	Manitou	Stewart 63
F-1[2/]	4[4/]	4	4	5
C-1	4	4	4	4
L-12	4	3	4	5
M-9	3	4	5	4
R-4A	3	4	5	4
R-18	3	4	4	5
C-8	3	4	4	4
C-6A	3	3	4	5
P-2	3	3	4	4
M-8	3	3	2	4
L-9	3	2*	4	4
B-1	3	2*	4	3
R-15	2	4	5	5
R-7A	2*	4	4	5
R-2A	2	4	4	4
R-6A	2	4	4	4
R-9A	2*	4	4	4
R-10A	2	4	4	4
C-2	2*	4	2	5
C-4	2*	4	2	4
D-1	2	3	5	4
M-6	2*	3	4	5
M-10	2*	3	4	5
C-9	2	3	4	5
C-10	2	3	4	5
M-5	2*	3	4	4
R-16	2	3	4	3
T-4A	2*	3	3	4
R-12A	2*	2	4	5
R-14	2	2	4	5
M-4	2	2	4	5
M-2	2	2*	4	4
R-1	2	2	4	4
R-3A	2*	2	4	4
L-3	2*	2*	4	4
R-5A	2*	2	4	3

Table 3. (Continued)

Isolate	Disease rating			
	Kenya Farmer	Carleton	Manitou	Stewart 63
CT-1	2	2*	3	4
B-4A	2	2	3	4
B-5A	2	1	4	5
T-7	2*	1*	4	3
B-3A	1	4	3	5
O-1	1	4	3	4
C-3A	1*	4	2	5
R-13A	1*	4	2*	4
T-2A	1*	4	2*	4
R-8	1*	3	5	5
T-6	1*	3	5	4
B-7A	1	3	4	5
C-11	1	2	4	5
R-11A	1*	2*	4	4
T-1	1*	2*	4	4
C-7	1	2	4	4
C-5A	1*	2*	4	2
R-17	1*	2*	2*	2*
B-6A	1	1	4	4
P-1	1*	1*	1*	2*
PM-1	1*	1*	1*	2*
B-2	1*	1*	1*	1*
Z-1	1*	1*	1*	1*

1/ Isolates ranked in order of decreasing disease severity on Kenya Farmer.

2/ The first letter indicates host source, the number indicates the isolate, and "A" indicates a single ascospore isolate.

3/ 1 = very resistant; 2 = resistant; 3 = moderately resistant; 4 = moderately susceptible; 5 = susceptible.

4/ * indicates isolate-cultivar combinations which were tested twice and induced a disease rating of 1 or 2 in both tests.

Table 4. Response of four wheat cultivars to 58 isolates of *Claviceps purpurea* and one isolate of *C. zizaniae* as indicated by the number of isolates in each disease rating class.

Host	Disease rating class[1/]				
	1	2	3	4	5
Kenya Farmer	19	28	9	3	0
Carleton	7	18	15	19	0
Manitou	4	7	5	37	6
Stewart 63	2	4	4	29	20

1/ 1 = very resistant; 2 = resistant; 3 = moderately resistant; 4 = moderately susceptible; 5 = susceptible.

DISCUSSION

The most striking aspect of this study was the great variability of the isolates with respect to virulence. Variability can be caused by both environmental and genetic factors and some of the variability in this study must be attributed to environmental factors. However, the fact that about 50 host-isolate combinations, some of which were single ascospore isolates, (Table 3) gave an infection type of 1 or 2 when tested twice, indicates that the performance of these isolates is likely due to genetic factors.

By definition, vertical resistance is greater resistance to some races than to others and differential interaction between races and varieties must occur (40). In this study differential interaction between ergot isolates and the four wheat cultivars is clearly evident when the isolates are arranged in order of decreasing disease severity on Kenya Farmer (Table 3). The isolates then differ in virulence sensu Van der Plank, and the resistance is vertical (40). The results also indicate that the gene(s) governing resistance in Kenya Farmer and Carleton are different. The existence of vertical resistance in a given host does not mean that horizontal resistance could not be present as well, and there are indications that such a situation might exist in the present study.

The disease rating system which was developed and used here for the first time combines the various aspects of the disease

reaction (Table 2). However, the reaction classes, with the exception of the very resistant reaction (class 1), were determined arbitrarily and on the basis of practical considerations as to what might constitute an acceptable level of resistance. The characters involved are quantitative and appeared in some cases to be influenced by environmental conditions. Furthermore there are groups of isolates in Table 3, ie. the first nine isolates, which show no evidence of differential interaction when Kenya Farmer is excluded. If the rating system was changed so that the reaction classes 2 to 5 were considered to be susceptible, and to reflect levels of susceptibility, the data in Table 3 would now be subject to a different interpretation. Evidence for differential interaction would now be found among the last 21 isolates where disease ratings of 1 are found. On the other hand, the remaining isolates would now be considered to induce susceptible reactions. Whether the differences in the reactions induced among the cultivars by these individual isolates are significant or not could not be determined from this study, which was exploratory and designed primarily to evaluate the stability of the two resistant cultivars. In view of the above remarks, it would appear worthwhile to compare small groups of selected isolates of different reaction types, in replicated trials and under controlled environmental conditions. There are indications from the results with Carleton, that honeydew production might not be controlled by the same gene(s) that regulate the infection type and this aspect should also be considered in future studies.

The existence of isolates which induce an abortive reaction only (1 rating) in Kenya Farmer or Carleton and in a few cases in the susceptible cultivars as well, was revealed for the first time. In previous studies (30) this reaction had been observed only in a few florets from inoculated heads of Kenya Farmer and Carleton and other florets with small sclerotia were also present. The abortive reaction appears to result from a highly incompatible host-parasite interaction and might be analogous to the hypersensitive reaction induced in leaves by various pathogens. Isolates of this type, which give a very resistant reaction on Kenya Farmer and a susceptible reaction on Manitou, should prove more useful in selecting individual plants from the progeny of crosses between Kenya Farmer and susceptible cultivars, than isolates that produce a few small sclerotia (2 rating) on this resistant cultivar.

The results obtained with the isolate from wild rice are in agreement with the meaning of the results reported by Brown (7), who found that the strain of ergot from wild rice did not infect cereals and other grasses. However, the results indicate that infection does occur in many florets, and it would appear more appropriate to state that the wild rice isolate cannot complete its cycle on these cereal cultivars.

The results of the present study indicate that the resistance of Kenya Farmer is different and considerably greater than that of Carleton. None of the isolates induced the production of visible honeydew and three isolates only incited a moderately susceptible

reaction in the former cultivar, whereas many isolates produced honeydew and 19 isolates induced a susceptible reaction in Carleton (Table 4). The resistance of Carleton would appear to be of limited value, whereas that of Kenya Farmer would still appear to be adequate and useful since the frequency of infection and the number of sclerotia obtained under the conditions of this experiment are probably high. Campbell's results (10) and those presented in the next paper indicate that the number of sclerotia can be expected to decrease to low levels when infection occurs at anthesis or shortly after.

2. The response of five cereal cultivars inoculated with *Claviceps purpurea* prior, during and after anthesis.

ABSTRACT

Changes occurred in the disease reaction of five cereal cultivars, when inoculations were made on or shortly after the day of anthesis. Following fertilization, there was a reduction in the number of sclerotia as well as in the amount of honeydew produced. This effect was expressed sooner in the cultivars Kenya Farmer, than in the cultivars Manitou, Rosner and Conquest, and only gradually on the cultivar Prolific. In the cultivars Kenya Farmer, Manitou and Rosner, the number of partially infected kernels increased as the number of sclerotia decreased. Fertilized ovaries were susceptible to infection and could remain susceptible for 3-6 days after anthesis.

INTRODUCTION

The ergot fungus, Claviceps purpurea (Fr.) Tul. infects the florets of cereals and grasses during the flowering period. It is generally believed that the low incidence of ergot infestation in self-pollinated cereal crops such as wheat and barley is due to the fact that the florets open only slightly and for a short time at anthesis. However, there are indications that ovaries develop resistance soon after fertilization (1, 10, 15, 32).

In seeking sources of resistance to ergot in the cultivated cereals, Platford (31) inoculated test plants about 2 days before anthesis, in order to allow the expression of the genetic resistance of the hosts without any interference from potential effects of fertilization. Two cultivars, one of spring wheat and one of durum wheat were found to possess resistance to ergot when tested in this way (30). The present study was made to determine the effects of fertilization on the expression of the disease reaction in resistant and susceptible cultivars.

MATERIALS AND METHODS

Plants of the following cereals were inoculated in the greenhouse with isolate M-4 (from wheat) of C. purpurea: spring wheat, Triticum aestivum L., cultivars Kenya Farmer and Manitou, triticale, Triticale sp., cultivar Rosner, barley, Hordeum vulgare L., cultivar Conquest and Secale cereale L., cultivar Prolific. The cultivar Kenya Farmer is resistant to isolate M-4 (previous paper) and the cultivar Manitou is less susceptible than the other cultivars (31).

Procedures for the preparation of the inoculum, the culture of the test plants, the method of inoculation and the assessment of the disease reaction were as described previously (previous paper).

Inoculations were started on the day the heads protruded from the boot for all the cultivars except Prolific for which inoculations were started when the entire spike was out of the sheath. This corresponded to stage 10.1 and 10.5, respectively, in the growth stage key for cereals prepared by Large (21). Inoculations were repeated on other emerged heads on each of the following 12 days. In each case, 10 florets/head on each of five main tillers/cultivars, were individually inoculated by hypodermic syringe with a conidial suspension at a spore density of 10^6 conidia/ml. The rye cultivar Prolific was inoculated during August and the other cultivars were

inoculated during the winter, one cultivar each month.

The date at which anthesis occurred in one or more florets was recorded for each head in the experiment and referred to as the date of first anthesis. In barley, anthesis occurred on the second day of inoculation. In this case the date of anthesis was determined by opening a few florets on successive days, since anthesis occurs within the florets, usually many days before the anthers are extruded. From the date of first anthesis, heads of rye were hand-pollinated daily during the period of anthesis. On the date of anthesis, the heads were pollinated prior to inoculation. Heads inoculated prior to anthesis were not hand-pollinated.

RESULTS

The results summarized in Table 5 show that pronounced changes occurred in the disease reaction of each cultivar when inoculations were made on or shortly after the day of anthesis. There was a marked decrease in the percent sclerotia formed on the day of anthesis in all cultivars except Prolific rye. No sclerotia were produced after this date in the cultivar Kenya Farmer, and only the cultivar Prolific produced sclerotia when inoculations were made later than the first day after anthesis. In Prolific, the percent sclerotia was reduced considerably on the first day after anthesis and decreased gradually until no sclerotia were formed on the seventh day after anthesis. There was no apparent relationship between the number of sclerotia of size 1 and 2 and the date of inoculation in any of the cultivars (Table 6). However, more sclerotia of size 3 were produced in all the cultivars on the first dates of inoculation, than at anthesis.

In the cultivars Kenya Farmer, Manitou and Rosner, the percent aborted ovaries began to decrease on the second or third day after anthesis and was reduced to zero by the seventh day after anthesis (Table 5). In the cultivar Prolific, the percent aborted ovaries remained low from the first date of inoculation until anthesis, increased by about a factor of five the day after anthesis and remained at about that level until the last day of inoculation. In

the cultivar Conquest, the percent aborted ovaries also remained fairly high until the last day of inoculation.

With the exception of the cultivar Prolific, in which few partially infected kernels (PI) were induced, the percent PI increased on the day of anthesis or on the following day. The increases were particularly pronounced in the cultivars Kenya Farmer and Rosner. The percent PI were greater in Kenya Farmer than in the other cultivars for each of the first 5 dates of inoculation. Few PI were encountered on the other cultivars until the second or first day before anthesis.

No visible honeydew was produced in Kenya Farmer regardless of the dates of inoculation. The amounts of honeydew produced in the cultivars Rosner, Manitou and Conquest when inoculated prior to anthesis received a rating of 3 or 4 (visible) whereas when inoculated on the day of first anthesis, all three cultivars received a rating of 2 (not visible). When inoculated on the day following anthesis, Rosner and Manitou both received a rating of 1 (not visible within floret). In the cultivar Prolific, the amount of honeydew produced decreased gradually from a rating of 4 on the first day of inoculation, to a rating of 2 on the third day after anthesis. It decreased to a rating of one on the fourth day after anthesis and remained at that level until the last day of inoculation.

Table 5. Disease reactions of five cereal cultivars inoculated with one isolate of Claviceps purpurea on successive days, prior, during and after anthesis.

Day of 1/ inoculation		Kenya Farmer 2/ % 3/				Manitou %				Rosner %				Conquest %				Prolific %			
		S	AO	PI	AH 4/	S	AO	PI	AH	S	AO	PI	AH	S	AO	PI	AH	S	AO	PI	AH
Before	6									58	36	0	4								
	5	26	20	30	2	68	20	0	3	76	22	2	4					92	4	0	4
	4	16	10	48	1	66	20	4	3	90	10	0	4					94	4	0	4
	3	20	36	42	1	80	12	0	3	94	6	0	4					96	2	0	4
	2	30	14	50	1	78	4	0	3	70	4	20	3					76	0	0	4
	1	34	16	46	1	54	26	10	3	38	18	42	3	44	28	0	3	82	8	0	3
Anthesis	0	6	24	64	1	40	22	26	2	24	18	40	2	6	22	0	2	80	6	0	3
After	1	0	4	72	1	4	10	22	1	8	14	70	1	16	16	2	2	48	28	0	3
	2	0	24	26	1	0	6	8	1	0	8	42	1	0	6	14	2	26	24	0	3
	3	0	4	20	1	0	4	0	1	0	12	30	1	0	12	8	2	6	30	2	2
	4	0	6	0	1	0	2	2	1	0	0	26	1	0	4	10	1	6	40	0	1
	5	0	6	8	1	0	0	0	1	0	6	0	1	0	28	0	1	2	44	0	1
	6	0	6	8	1	0	0	0	1	0	2	2	1	0	14	2	1	2	48	2	1
	7	0	0	10	1	0	0	0	1					0	12	0	1	0	36	2	1
	8													0	16	0	1				
	9													0	36	0	1				

1/ In relation to anthesis.

2/ For each cultivar, 10 florets/head on each of five main tillers/cultivar, were individually inoculated at each date.

3/ S = sclerotia; AO = aborted ovary; PI = partially infected kernel.

4/ AH = amount of honeydew: 1 = no visible honeydew; 2 = honeydew confined within glumes; 3 = honeydew exuding from florets in small drops; 4 = honeydew exuding from florets in large drops and running down the head.

Table 6. Effect of date of inoculation on size and number of sclerotia in five cereal cultivars.

Day of inoculation 1/		Kenya Farmer 2/ Sclerotia size 3/			Manitou Sclerotia size			Rosner Sclerotia size			Conquest Sclerotia size			Prolific Sclerotia size		
		1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Before	6							6	9	14						
	5	0	7	6	1	16	7	9	3	26				17	28	1
	4	5	3	0	3	17	13	9	22	14				23	23	1
	3	9	1	0	1	24	15	14	33	0				25	23	0
	2	13	2	0	8	13	18	10	23	2	0	11	11	14	24	0
	1	13	4	0	4	18	5	12	6	1	0	3	0	18	23	0
Anthesis	0	2	1	0	5	15	0	9	3	0	8	0	0	16	24	0
After	1	0	0	0	1	0	1	2	2	0	0	0	0	3	21	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	9	2	2
	3	0	0	0	0	0	0	0	0	0	0	0	0	1	2	0
	4	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0
	5	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
	6	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
	7	0	0	0	0	0	0				0	0	0	0	0	0

1/ In relation to anthesis.

2/ For each cultivar, 10 florets/head on each of five main tillers/cultivar, were individually inoculated at each date.

3/ 1 = sclerotia very small; 2 = sclerotia approximately the size of a normal kernel; 3 = sclerotia larger than kernel, extending beyond the lemma and palea.

DISCUSSION

The results of this study indicate clearly that following fertilization of the ovaries there is a pronounced effect on the two aspects of the disease reaction which are most important in the epidemiology of the disease, namely, the number of sclerotia and the amount of honeydew produced. This effect was expressed sooner in the cultivar Kenya Farmer, than in the cultivars Manitou, Rosner and Conquest, and only gradually in the cultivar Prolific. The results with the cultivar Conquest differ from those reported by Campbell and Tyner (10) who found that percent infection (sclerotia) in barley cultivars only decreased gradually from the time of heading until 10 days after. This might be due to differences in the virulence of the fungus or perhaps to the fact that Conquest possesses a greater level of resistance than the cultivars used by Campbell. During the period of anthesis (anthesis and 1 day after), the percent partially infected kernels increased as the percent sclerotia decreased, in the cultivars Kenya Farmer, Manitou and Rosner. Fertilized ovaries are thus susceptible to infection, and can remain susceptible from 3-6 days after anthesis, as previously shown for fertilized male-sterile barley (32).

Kenya Farmer was unique among the cultivars tested in that partially infected kernels were induced in fairly high numbers, on each of the five dates of inoculations prior to anthesis. While this

would seem to be due to the greater level of resistance of this cultivar, the reasons for this type of response are not clear. It would appear that in some florets, fungal invasion and development of the ovary proceed very slowly and that the pathogen has not progressed very much by the time fertilization occurs. Growth of the embryo could thus proceed for a short period of time before the pathogen became sufficiently established to inhibit any further development of the young kernel.

Since fertilization increases the resistance of the ovaries to some extent in all cultivars tested, it would appear that the most appropriate time to inoculate cultivars in order to determine their level of resistance would be about two days before anthesis. This would allow the complete expression of the host-parasite interaction without interference from the fertilization effect. In the field, the frequency of sclerotia formation would be reduced to very low level in cultivars that possess a resistance similar to that of Kenya Farmer, since ergot infection would occur at the time of anthesis only.

SECTION 3

Discussion of entire research program reported in Section 2

GENERAL DISCUSSION

Sources of resistance to disease must be tested adequately before being utilized in breeding programs, otherwise resistant varieties may not prove to be stable. This point is well illustrated by the results of this study.

The cultivars Kenya Farmer and Carleton were reported to be resistant to four ergot isolates and appeared to differ only slightly in their resistance (30). In this study, however, the resistance of Kenya Farmer was found to be genetically different from that of Carleton and effective against 56 of the 59 ergot isolates tested as compared to 40 for Carleton. Furthermore, none of the isolates induced the production of visible honeydew in Kenya Farmer, whereas many did in Carleton. The resistance of Carleton, thus appears to be of limited value. On the other hand, the resistance of Kenya Farmer might still be adequate, since this cultivar was shown to produce fewer sclerotia when inoculations were made on the day of anthesis rather than 2 days prior to anthesis.

The fact that the three isolates which were virulent on Kenya Farmer came from grasses, rather than from cultivated cereals, indicates that other isolates from these hosts should be tested in the future. It also indicates the continued importance of cutting grasses bordering fields of cereal crops before they head, in order to prevent the disease from spreading to the crop.

The results obtained indicate that considerable variability existed amongst the isolates with respect to virulence. Some of the isolates were virulent on three or four cultivars whereas others induced an abortive reaction only (1 rating) and were low in virulence in either of the resistant cultivars, and in a few cases, in the susceptible cultivars as well. Evidence of differential interaction between the isolates and the cultivars indicated that resistance was vertical.

Resistance of the type found in Kenya Farmer would not be effective by itself, since many infections still occur even though fewer sclerotia are formed. Thus, it would probably not be satisfactory in male-sterile wheat cultivars. However, it should be highly effective when present in wheat or triticale cultivars which are highly self-fertile, and which possess a good flowering habit, ie. tight glumes, and a short period of anthesis, since the frequency of infections would be reduced considerably in such cultivars.

There were indications that horizontal resistance might also be present in the cultivars. This should be confirmed in future studies, particularily for Kenya Farmer, since it would be desirable to transfer both types of resistances to new cultivars. Fortunately, the present study has revealed ergot isolates which would appear to distinguish between vertical and horizontal resistance and which could be used to determine whether both types are present in a host.

SECTION 4

Bibliography

Appendices

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Appendix 1. Method for obtaining pure isolate of Claviceps purpurea in culture.

1. Sclerotial isolates:

Peeled sclerotia were soaked for approximately one minute in a 2% sodium hypochlorite aqueous solution. This was followed by three washings in sterile distilled water. Each sclerotium was cut aseptically into several pieces and placed onto 4% malt agar plates that had been acidified with one drop of 25% lactic acid. After germination, mycelium was transferred to 4% malt agar slant held at 24° C. These sclerotial cultures were stored at 3° C for further use.

2. Single ascospore isolates:

Unsterilized sclerotia were placed on moist sterile vermiculite in covered plastic containers and stored at 3° C for approximately six weeks. For germination they were held at 24° C until the stromata were fully developed (approximately 4 weeks). Ascospores were collected by the technique of securing one stromatized sclerotia to the interior bottom of an aerobic culture dish (95 x 62). It was then inverted for a time interval over a 4% malt agar plate so that the ascospore population on the plate was invariably sparse. This facilitated a single ascospore transfer to an agar slant of the same medium. Germination was permitted at room

Appendix 1. (Continued)

temperature (24° C). Following germination, mycelium was transferred onto 4% malt agar slants and maintained at 3° C as a stock culture of a single ascospore isolate.

Appendix 2. Preparation of culture media for Claviceps purpurea.

1. Liquid medium for producing conidia:

Ingredients: 400 g peeled potato tubers, 200 g of commercial sucrose, and 600 g of distilled water.

Preparation: Peeled potato tubers are sliced and steamed for one hour in 600 ml of distilled water. Two hundred grams of commercial sucrose are added to 500 ml of the potato extract. One hundred and twenty five milliliters of the broth is autoclaved in a 500 ml flask for 15 min. at 15 lb/sq. in. Actively growing cultures are transferred to flasks and agitated on a gyrotory shaker for 10 to 20 days at a speed of 150 rpm. The conidia were harvested by filtering the culture through cheese cloth followed by a coarse sintered glass filter. The conidia were removed from the filtrate by low speed centrifugation. They were washed twice by resuspending in sterile distilled water and recentrifuging. The conidia were then preserved in 60% sterile sucrose solution and stored at 3° C.

2. Four percent malt agar medium (modified):

Ingredients: 15 g malt extract, 10 g maltose, 40 g agar, and water to make 1000 ml.

Preparation: Melt agar in water, add other ingredients, bottle and sterilized.