THE UNIVERSITY OF MANITOBA

REACTION OF CULTIVATED CEREALS, CHROMOSOMAL LOCATION AND INHERITANCE OF RESISTANCE IN WHEAT TO CLAVICEPS PURPUREA

by

R. GARY PLATFORD

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ABSTRACT

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Reaction of Cultivated Cereals, Chromosomal Location and Inheritance of Resistance in Wheat to Claviceps Purpurea.

Major Professor; Claude C. Bernier.

The reaction of cereal species to Claviceps purpurea was investigated utilizing one current commercial cultivar from each of six cereal species. Seven inoculum concentrations of each of two isolates of C. purpurea, one from Triticum aestivum and one from X Triticosecale were used. Inoculations were performed by injecting individual florets with a hypodermic syringe with a measured volume of inoculum. Sclerotia frequency increased with increasing inoculum concentration. However, inoculum concentration had no effect on the average size of the sclerotia or on the average amount of honeydew produced. T. aestivum was less susceptible than the other cereal species. The C. purpurea isolate from Spring Wheat was more virulent than the isolate from Triticale. Further testing of individual cultivars within each cereal species revealed that T. aestivum cv. Kenya Farmer and T. durum cv. Carleton possessed higher levels of resistance than other spring and durum wheat cultivars. No resistant cultivars were detected in Hordeum vulgare, Avena sativa, X Triticosecale or Secale Cereale.

Further testing of T. aestivum cv. Kenya Farmer and T. durum cv. Carleton revealed that resistance in these two cultivars was expressed by a decrease in both the frequency and size of the sclerotia and a reduction in the amount of honeydew produced. Also, in the absence of sclerotial production, inoculated florets of the resistant wheat cultivars produced a shrivelled shrunken ovary or partially infected seed whereas that of the susceptible T. aestivum cv. Chinese Spring and T. durum cv. Stewart 63 normally produced seed.

A seedling coleoptile test was assessed to determine its usefulness in screening cereal cultivars for resistance to C. purpurea. S. cereale and X Triticosecale cultivars were more susceptible than T. aestivum cultivars as evidenced by the higher percentage of infection and the production of microsclerotia on the coleoptile of the former. Cultivars of T. aestivum differed in the degree of discolouration of the coleoptile rather than in percentage infected coleoptiles showing mycelial development. The discolouration was most evident on the resistant cultivar Kenya Farmer. The technique would not be applicable to screening T. durum, H. vulgare and A. sativa due to the failure to obtain infection. Differences in degree of pathogenicity between the two isolates of C. purpurea were demonstrated; the isolate from T. aestivum cv. Manitou being more virulent than the isolate from X. Triticosecale cv. Rosner.

The location of the resistance genes of T. aestivum cv. Kenya Farmer and T. durum cv. Carleton was studied utilizing a monosomic substitution series of Kenya Farmer into Chinese Spring and F1 monosomics

of Chinese Spring x Carleton A & B genome. Resistance was found to be on the 6B chromosome of Kenya Farmer and the 1B chromosome of Carleton. The resistance of the substitution lines was not as high as the fully constituted parent suggesting that there are genes on additional chromosomes in Kenya Farmer and Carleton which modify the reaction.

Studies on the inheritance of the resistant reaction of Kenya Farmer and Carleton indicate that there is more than one gene controlling the disease reaction. The results also suggest that frequency of sclerotia, size of sclerotia and honeydew production are controlled by separate genes and that the resistant genes in Carleton are linked whereas in Kenya Farmer they are independent.

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GENERAL INTRODUCTION

Ergot caused by Claviceps purpurea (Fr.) Tul., attacks all cultivated cereals and most grass species. Differences in susceptibility amongst cultivated cereals have been noted previously with rye generally being regarded as the most susceptible cereal species, and oats the least susceptible (Weniger, 1924; Dillon-Weston and Taylor, 1942).

The incidence of ergot in cereals in Western Canada has varied widely from year to year and between different cereal species with rye usually being the most severely affected (Conners, 1953). The effect of ergot is to cause off-grading of grain shipments (Seaman and Harper, 1974). Past surveys have usually indicated that the percentage of fields of all cereals, in the prairie provinces found to have ergot was usually highest in Manitoba (Campbell, 1954; Conners, 1955; Shoemaker, 1956). More recently ergot susceptibility has been a problem in the triticale and hybrid wheat breeding programs carried on by the Plant Science Department of the University of Manitoba.

Differences in susceptibility between the cereal species has been ascribed to differences in flowering habit and floret morphology which allow certain species like Avena sativa to escape infection (Weniger, 1924). On the basis of field observations Willis (1953), reported differences in levels of susceptibility amongst different cultivars of spring wheat. Kawatani (1955), ascribed differences between the reactions of cultivars of Triticum aestivum to C. purpurea that he observed in an inoculation study,

to be due to different levels of physiological resistance. There appeared to be confusion if other cereal species than spring wheat possessed physiological resistance.

The major objective of this study was to gain a better understanding of the reaction of cultivated cereals to C. purpurea prior to undertaking a major screening of cultivars for sources of resistance to C. purpurea: the latter to be used in the hybrid wheat and triticale breeding programs.

General Review of Literature

Causal Organism:

Ergot, the disease caused by Claviceps purpurea (Fr.) Tul., was first observed and recorded by the Greeks and Romans (Walker, 1969). The early and historically very interesting papers were reviewed by Barger (1931), and are summarized as follows: ergot of rye was attributed by Thallius in 1588 to excessive formation of sap within the rye plant which caused the rye kernel to grow out beyond the glumes. The ergot sclerotia were first recognized to be of fungal origin by De Candolle in 1815, who classified it as Sclerotium clavus. Fries in 1823, observed the fructifications of germinating ergot sclerotia, which he classified as Cordyceps purpurea, but he regarded these fructifications as fungi parasitic on the ergot sclerotia. The honeydew stage of the ergot disease was regarded by Leveille in 1827, as being caused by a fungus which he classified as Sphacelia segetum. It was finally established by Tulasne (1853), that the fungus giving rise to the honeydew and the fungus which forms the ergot sclerotia were one and the same: this he classified as Claviceps purpurea. Walker (1969), reviewed the life cycle of C. purpurea and reported that Kuhn in 1863, established the full life cycle of C. purpurea.

The sexual life cycle of C. purpurea was established by Killain (1919). While it is agreed by modern taxonomic authorities that C. purpurea is an ascomycete belonging to the group Pyrenomycetes there is dispute as to what particular order it belongs. Dennis (1968)

places the family Clavicipitaceae under the separate order Clavicipitales, but Müller and Von Arx (1973) regard the family Clavicipitaceae as a family in the order Sphaeriales, as originally proposed by Miller (1949).

Life Cycle of Claviceps purpurea:

Under natural conditions C. purpurea sclerotia fall from infected grass and cereal flowers during the fall and remain at the soil surface or are partially covered with soil. During the late fall and winter the sclerotia are subjected to the cool temperatures which are required to break dormacy. Recently, a comprehensive investigation by Mitchell and Cooke (1968) indicated that temperatures over the range of 0°C to 5°C over six weeks, resulted in over 80% germination, while at 10°C at least 12 weeks were required to give a percentage germination of 80%. Germination of sclerotia in nature occurs in the spring at about 11°C (Vladimirsky, 1939), with the optimum temperature range for stroma development being 12°C - 18°C (Krebs, 1936).

The peak production of ascospores in Western Canada occurs in late June (Brown, 1947), with the windborne ascospores capable of infecting a large number of different wild grasses (Sprague, 1950). One to two weeks following infection of the floret, honeydew is produced (Weniger, 1924). The honeydew, which contains millions of conidia, causes secondary infection when spread to susceptible grass and cereal florets by insects (Barger, 1931). More than 40 different insects are attracted by honeydew (Atanasoff, 1920).

Following the honeydew stage sclerotia are formed, and their formation completes the disease cycle.

Symptoms of the Disease:

The first symptom of the disease, which occurs one to two weeks after infection, is the production of honeydew. Produced by the florets, this slimy, sticky, sweetish exudate contains millions of conidia and in some years may be so abundant on susceptible hosts that the entire heads and stems of infected plants are sticky (Weniger, 1924).

The most conspicuous symptom of the disease is the formation of sclerotia in the florets, replacing the normal kernel (Walker, 1969). Composed of fungal pseudoparenchymatous tissue, the sclerotia are horn-shaped and range in size from 2 - 25mm. The outer surface is purple-black and the inner tissue is white (Sprague, 1950).

Because ergot infected heads were observed to produce fewer kernels it was thought that ergot caused blasting of adjacent florets (Seymour and McFarland, 1921). However, recent studies with ergot attacking sorghum indicate that the fungus does not always cause sterility of adjacent florets (Futrell and Webster, 1966).

Economic Importance of Ergot in Grain Crops:

A summary of the incidence or occurrence of ergot in cereals in Western Canada conducted in 1953, showed that from 1932 to 1953 the percentage of the harvested rye crop in Western Canada graded ergoty varied from 0.1% to 15.3% (Conners, 1953). The results of field inspection of rye, barley and wheat crops in the Prairie Provinces in 1953, revealed that slightly over 10% of the wheat and barley crops and about 65% of the rye crops were affected by ergot.

Surveys conducted in 1954, 1955 and 1956 also indicated that more rye fields than wheat or barley, had ergot, and that the percentage of fields of all cereals found to have ergot was highest in Manitoba (Campbell 1954; Conners, 1955; Shoemaker, 1956).

More recently Seaman and Harper (1974) reported that off grading of spring wheat shipments due to ergot dropped from 0.3 million bushels in 1972 to less than 0.01 million bushels in 1973. However, the amount of durum off graded due to ergot increased from 0.6 million bushels to more than 1.5 million bushels. Saskatchewan shipments accounted for 83% of the off graded spring wheat and 87% of the off graded durum samples. Only 10% of each of spring wheat and durum wheat shipments off graded due to ergot originated from Manitoba.

The foregoing shows that ergot is an important disease of cereals which is capable of causing considerable commercial loss. The effect of ergot is not on the yield but on the quality of the harvested grain. Although there is no tolerance for No. 1 hard red spring wheat, other export grades have tolerances not exceeding .25% ergot (Seaman and Harper, 1974). If Canada is to retain its export market, particularily to countries like Japan which insist on top quality, investigations must be carried out with the aim of improving the resistance of spring wheat and other cereal grains to ergot and thereby reduce the ergot content of the grain.

Medical Importance of the Disease:

Ergotism of humans has been recorded since the Middle Ages, with two types of ergotism being recognized from the literature:

a gangrenous and a convulsive form. A gangrenous ergotism was reported as occurring during the Middle Ages when it was called St. Anthony's Fire; and the last great epidemic occurred during the eighteenth century. Germany was reported to have a long history of the convulsive type of ergot epidemics. Ergotism in Russia has been recorded as occurring as recently as 1926 - 1927 when there was a very extensive outbreak (Barger, 1931).

A more recent concern with respect to ergotism has been in relation to the poisoning of livestock (Burfening, 1973). This ergot poisoning, as in humans, is due to alkaloids contained in the sclerotia and the two types of ergotism occurring in humans have also been reported in domestic animals. A single group of animals seldom exhibit both types of ergotism at the same time with the ergotism found in horses and sheep being nearly always convulsive in form while in cattle the gangrenous form is most common. In pigs, ergot poisoning retards growth and causes a condition known as Agalactia. This manifests itself as a failure of the farrowing sow to develop an udder and lactate which results in the piglets dying shortly after birth. Feed containing 1% ergot has been shown to cause the above problem in farrowing sows.

The ergot disease is undoubtedly important in both human and veterinary medicine. Great suffering has been caused in past ages to human populations and ergot continues to cause toxicity problems with animals. Investigations with the aim of reducing the incidence of ergot in cereal grains could be of great benefit

to the agriculture industry.

Culture of Claviceps purpurea:

The first attempts at culturing C. purpurea were made in Germany. An inorganic medium for C. purpurea was developed by Engelke (1902). The carbohydrate source he used was 5% dextrose and the nitrogen source was ammonium nitrate. Utilizing an organic medium, Fron (1926) found that C. purpurea produced abundant conidia on a liquid rye medium incubated at 15° to 25°C for four days.

McCrae (1931) conducted extensive studies to determine the growth requirements of C. purpurea. She established that the best carbohydrate source was maltose at a concentration of 2 or 3 percent and, as a nitrogen source, peptone was preferable to nitrates. The temperature range for growth was a minimum of 10°C, an optimum of between 20° to 30°C and a maximum of 37°C.

The effect of the pH of the medium on growth and sporulation of C. purpurea was reported by Schweizer (1941). Using a medium based on gas sterilized barley or wheat seeds, he found that for conidial production to occur the medium had to have an acid pH but when the medium was alkaline he observed the production of microsclerotia.

Techniques enabling the mass production of C. purpurea conidia for use in field production of ergot sclerotia were developed by several workers. The mass production of conidia in liquid shake culture was reported by Glaz (1955) and Lewis (1959). Glaz (1955) developed a medium containing 2.5% sorbitol and 1.5% corn steep solids while the medium developed by Lewis (1959) consisted of a potato

extract and utilized sucrose at a concentration of 40%. An earlier potato medium using sucrose had been reported as producing abundant conidia by Darpoux (1956).

Storage of conidia without appreciable loss of viability was reported by Glaz (1955) and Lewis (1959). They both found that by suspending the spores in a concentrated sugar solution and storing the spore suspensions under refrigeration, viability was maintained at a high level for several months.

The development of long term storage methods for viable spore suspensions and the development of shake culture media for mass production of ergot conidia has facilitated the production of inoculum of C. purpurea for field inoculation of cereals.

Method of Inoculation and Period of Susceptibility:

Various techniques have been employed by researchers in inoculating cereals and grasses with C. purpurea. Stager (1903) used three methods: 1) spraying heads with the inoculum; 2) prying glumes apart and spraying the inoculum into florets; 3) dipping heads into the inoculum.

Dipping heads into the inoculum was successful for only a few species such as rye. For many species with hard glumes the only effective method was hypodermic injection of inoculum or clipping off the ends of glumes and atomizing the inoculum into the florets.

Both Fron (1926) and Lewis (1945) obtained good infection by spraying rye heads with suspensions of C. purpurea conidia but

McCrae (1931) reported poor success in inoculating rye by means of such spraying.

A mechanical device utilizing hollow needles for inoculating rye on a large scale was developed by Bekesy (1938). The inoculum was injected into the floret in a manner similar to a hypodermic injection. The technique was very successful and further refinements were made in the inoculum device (Bekesy, 1956). Campbell (1957) used several methods of inoculation depending on the host. For wheat and rye, he removed the tips of the glumes when the anthers appeared and the heads were then atomized with a suspension of C. purpurea conidia. On the other hand, barley heads were inoculated as they were emerging from the leaf sheath with the awns and glume tips being cut back and the heads then sprayed with conidia as in wheat and rye.

To obtain maximum infection the host florets must be inoculated during the period when they are most susceptible. It has been reported that the best results are obtained if the florets are inoculated before the external anthers appear (Fron, 1926; Bekesy, 1956). Best infection results with barley were obtained when it was inoculated before heading (Campbell, 1957).

Assessment of the Disease:

In some previous studies concerned with the host specificity of the ergot fungus, the presence or absence of sclerotia was the sole method of assessing the host reaction (Stager, 1923; Mastenbroek and Oort, 1941; Campbell, 1957).

The frequency of sclerotia has been used to evaluate results

of inoculation studies with cereal and grass hosts (Muhle and Frauenstein, 1959), and relative susceptibility of Triticum aestivum cultivars (Kawatani, 1955). In cross inoculation studies with ergot from sorghum, Futrell and Webster (1966), expressed their results as percentage florets infected and they observed a wide variation in percentage infection between hosts.

The criteria of sclerotial size and amount of honeydew produced have not been utilized extensively in comparing the reaction of different hosts to C. purpurea. Rapilly (1968), observed that different species of grasses and cereals produce different sizes of sclerotia and varying amounts of honeydew and presence or absence of honeydew was utilized in assessing isolate performance in a series of hosts, by Mastenbroek and Oort (1941); they did not attempt to rate the amount of honeydew produced.

While it appears that the symptoms of ergot on cereal can be divided into several components namely number of sclerotia, size of sclerotia and amount of honeydew produced, there is a need to determine the usefulness of these criteria in assessing the disease reaction of cultivated cereals.

Control of Ergot:

Control of ergot can be achieved by use of seed free from viable ergot sclerotia with sclerotia being separated from the seed by a salt flotation method (Dillon-Weston and Taylor, 1942). Because sclerotia are viable for only one season, as shown by Rostowzeff (1902), a one year rotation between susceptible crops is sufficient to reduce the danger of ergot carry-over. The short period of viability of the

sclerotia allows safe usage of seed containing sclerotia after storage of more than one year.

Sixty-five species of grass, representing twenty-five genera, have been recorded as being naturally infected with C. purpurea in Canada (Conners, 1967). Such grasses are an important reservoir of ergot infection for the cereals and keeping grass borders mowed will reduce the amount of secondary inoculum and subsequent spread of ergot infection to cereal crops (Weniger, 1924). In the absence of resistant cereal cultivars, ergot can be reduced by following good cropping practices.

Resistance:

There have been a number of reports regarding the relative susceptibility of the cereals to C. purpurea. From field observation it has been found that the order of susceptibility of the cereals was rye, wheat, barley and oats with rye being the most susceptible and oats rarely becoming infected (Weniger, 1924; Dillon-Weston and Taylor, 1942).

Sugar cane, Saccharum spontaneum cv. Turkmenistan was found to be susceptible to C. purpurea while other cultivars and species of cane were invariably resistant (Robinson, 1960). Hybrids between resistant lines and the susceptible Turkmenistan line were susceptible leading to the conclusion that resistance to C. purpurea in sugar cane is a recessively inherited trait. It is interesting to note that the Turkmenistan line was first discovered and collected as a cold resistant variant of Saccharum spontaneum. In Turkmenistan, cold resistance was also found to be recessively inherited.

Reference to cultivars of spring wheat and durum wheat being susceptible to, or rarely infected by, ergot in the field was made by Weniger (1924). Whereas Weniger merely made reference to the existence of less susceptible varieties, Willis (1953) quantified his observations of ergot infection occurring in wheat cultivars in English field trials. He presented his results as the mean number of sclerotia per each 10 kilo sample of harvested wheat tested. The sclerotial content of the samples ranged from 128 to 12 ergot sclerotia per sample. Although Willis (1953), reported differences between wheat cultivars in susceptibility to C. purpurea, his results are based on field observation only and were not substantiated by inoculation experiments.

On the basis of inoculation experiments, spring wheat has been shown to have greater resistance to C. purpurea than rye but differences were also observed in the reaction of various spring wheat cultivars to C. purpurea (Kawatani, 1955). He attributed the greater resistance and the variation in reaction of spring wheat cultivars not only to differences in the mode of flowering but also to differences in physiological resistance.

In a study of the ergot susceptibility of some wheats, their hybrids, and certain other Gramineae, resistance in some of the F-1 hybrids was found to be recessive, but in others it was dominant (Galstjan-Avanesjan, 1967). It was concluded that resistance in wheat was an unstable quantitive character controlled by more than one gene and resistance was recessive. Although certain levels of resistance to C. purpurea in wheat and other cereals have been reported, the

nature of this resistance and its mode of inheritance have never been fully investigated.

Host Specialization of Claviceps purpurea:

The existence of physiologic races of C. purpurea was first proposed by Stager who, in the period 1903 - 1923, tested both a large number of host species and isolates of C. purpurea (Barger, 1931). There were three distinct races distinguished by Stager and these were later designated P1, P2 and P3 by Barger (1931). A fourth race designated P4 was distinguished by Mastenbroek and Oort (1941) and Baldacci and Forlani (1948) distinguished race P5. These early studies which were conducted in Europe distinguished races of ergot primarily on the basis of their differential ability to attack Secale cereale and Lolium perenne. An investigation on the possible existence of races of C. purpurea in Canada was conducted by Campbell (1957). Based on the successful inoculation of 421 isolates of C. purpurea from 38 different host species onto rye, wheat and barley cultivars in the greenhouse, he found no conclusive evidence for the existence of physiologic races. Only one of three isolates obtained from Glyceria borealis did not infect these three hosts, and cultures obtained from rye infected all of the grass species tested. There is no mention in the report of this extensive study by Campbell, of any differences in levels of virulence amongst isolates of C. purpurea.

Based on the percentage of sclerotia formed per inoculated floret, Muhle and Frauenstein (1962) showed that an isolate of C. purpurea obtained from Lolium perenne was able to infect both Lolium and Festuca species to a much greater extent than S. cereale. It is conceivable that the previous findings of physiologic races by some

workers and failure of others to show their existence may have been due to virulence of the isolate not being considered. Another area that has been generally disregarded is the effect of inoculum density on levels of infection of a host by an isolate of C. purpurea.

Coleoptile Test:

In an attempt to learn more about the host-parasite relationship, Lewis (1956) perfected a method of inoculating the shoots of rye and wheat seedlings with C. purpurea while Stoll and Brack (1944) had earlier found that the meristem above the nodes in rye could be inoculated with C. purpurea with resulting sclerotial formation. It had also been concluded by Cherewick (1953) that the ergot fungus could develop not only on young ovaries but on any physiologically young tissue of wheat or barley.

Lewis (1956) found that rye seedlings were much more readily infected than wheat seedlings and the production of small microsclerotia on some of the infected rye seedlings was obtained.

There is a possibility that this technique of inoculating seedlings might be useful in screening cultivars of the cereal species for reaction to C. purpurea.

RESULTS OF RESEARCH

The results of research are presented in the form of separate publications, the first two of which have already been published. The results of a study of the reaction of cultivated cereals to Claviceps purpurea was published in the Canadian Journal of Plant Science 56: 51-58 (1976), and the findings of a study on the resistance to Claviceps purpurea in spring and durum wheat was published in Nature 226:770 (1970). The other three papers dealing with: "Coleoptile reaction of cultivated cereals to Claviceps purpurea"; "Chromosome location of genes conditioning resistance to Claviceps purpurea in spring and durum wheat"; and "Inheritance of resistance to Claviceps purpurea in spring and durum wheat", have been prepared for submission to Canadian Journal of Plant Science.

1. Reaction of Cultivated Cereals to Claviceps Purpurea

ABSTRACT

The influence of inoculum concentration on the reaction induced in species and cultivars of commercial cereals by two isolates of ergot (Claviceps purpurea (Fr.) Tul.) was investigated. Differences were observed among and within species with respect to each of the three components of the disease reaction, i.e., frequency of sclerotia, size of sclerotia, and amount of honeydew produced. Spring wheat (Triticum aestivum L.) was less susceptible than the other cereal species tested. Significantly fewer sclerotia were produced on spring wheat except at high and low inoculum concentrations and the sclerotia were smaller and the honeydew less abundant. Inoculation of selected cultivars within each of the cereal species showed that only cultivars of spring wheat and durum wheat (T. durum Desf.) differed in the expression of the disease reaction. The spring wheat cultivar Kenya Farmer, and durum wheat cultivar Carleton were less susceptible than the commercial cultivars. Inoculum concentration affected sclerotial frequency in the species and cultivars tested but did not affect sclerotial size and honeydew development. The two isolates used in this study differed in their ability to induce sclerotia and also affected sclerotial size and honeydew production.

INTRODUCTION

The order of susceptibility of cultivated cereals to the ergot fungus Claviceps purpurea (Fr.) Tul. would appear on the basis of field observations to be rye (Secale cereale L.), wheat (Triticum aestivum L.), barley (Hordeum vulgare L.), and oats (Avena sativa L.), with rye being most susceptible and oats rarely infected (Weniger, 1924; Dillon-Weston and Taylor, 1942). Triticale (X Triticosecale Wittmack) is generally as susceptible as rye in Manitoba (Platford, unpublished data). Differences in the incidence of C. purpurea among wheat cultivars have also been observed (Willis, 1953). It has never been clearly established, however, whether the observed differences in natural infection are due to differences in physiological resistance or to an escape mechanism based on floret morphology and flowering habit.

The greater resistance of wheat has been ascribed by Stager (1923) to the brief and irregular opening of the glumes. Further, host susceptibility to C. purpurea appears to decrease following anthesis and subsequent fertilization (Abe and Kono, 1957; Campbell and Tyner, 1959; Rapilly, 1968). On the other hand, results obtained by Kawatani (1955), who inoculated wheat cultivars by spraying or injecting florets, indicate that some wheat cultivars differ in physiological resistance.

In previous studies concerned with the host specificity of the ergot fungus, the presence or absence of sclerotia was the main criterion of susceptibility or resistance (Stager, 1923; Mastenbroek and Oort, 1941; Campbell, 1957). The frequency of sclerotia was also used to evaluate the results of inoculation studies with cereal and grass hosts (Muhle and Frauenstein, 1962), and relative susceptibility of T. aestivum cultivars

(Kawantani, 1955). Considerable variation in frequency of sclerotia was observed in both cases.

The criteria of sclerotial size and amount of honeydew produced have not been extensively utilized in comparing the cultivated cereals in their reaction to C. purpurea. Rapilly (1968) observed differences between cereal and grass species in amount of honeydew produced and size of sclerotia but did not utilize these criteria in comparing a series of inoculated hosts. The importance of sclerotial size in the epidemiology of the disease has been shown (Cooke and Mitchell, 1966; Rapilly, 1968). A linear relationship was found between sclerotial size and number of clavae, the number of clavae being related to the quantity of ascospores released.

The effect of inoculum concentration on host reaction has been generally disregarded in past studies with C. purpurea. Recently, however, it was found that sclerotia were produced at a much lower inoculum concentration on male sterile barley than on wheat (Puranik and Mathre, 1971).

As part of a continuing project to find sources of resistance to ergot for triticale and wheat breeding programs, this study was initiated to determine the influence of inoculum concentration and of fungal isolates on the disease reaction of cereal species and selected commercial cultivars.

MATERIALS AND METHODS

Selection of Isolates and Preparation of Inoculum:

Two ergot isolates which appeared to differ in virulence on the basis of preliminary tests were selected for this study. Isolates from naturally infected spring wheat (M-1) and triticale (T-1) were obtained by plating surface-sterilized sclerotial pieces from each on to acidified malt agar. Conidia were increased as required on a modified potato sucrose broth (Lewis, 1959). The mycelium was removed by filtration through a coarse sintered glass filter and the conidia were concentrated by low speed centrifugation. Concentrated conidial suspensions were stored in 60% sucrose at $4^{o}C$, and used within 2 months. Inoculum of various spore concentrations was prepared by diluting the conidial suspension with sterile distilled water.

Plant Materials and Method of Inoculation:

In the first series of experiments, the disease reactions of commercial cereals were evaluated by inoculating each of the following species with each of the two isolates of C. purpurea at seven inoculum concentrations ranging from 50 to 10^6 conidia/ml: Triticum aestivum L. cv. Manitou, Triticum durum Desf. cv. Stewart 63, X Triticosecale Wittmack cv. Rosner, Avena sativa L. cv. Harmon, Hordeum vulgare L. cv. Conquest, Secale cereals L. cv. Prolific.

Subsequently, the disease reaction of selected cultivars within each cereal species was evaluated by inoculating with each of the two isolates at an inoculum concentration of approximately 10^4 conidia/ml. The cultivars and accessions of each species tested were as follows:

triticale cv. Rosner, accessions 6531, 6450, 6A189, 6A190, 8A94, and 8A95; oats cv. Harmon, Rodney and Garry; barley cv. Conquest, Montcalm and Herta; rye cv. Prolific, Petkus and Cougar. The spring and durum wheat cultivars are listed in Tables 4 and 5.

The spring wheat cultivars, Kenya Farmer and Chinese Spring and the durum wheat cultivar Carleton were further evaluated by inoculating with each of the two fungal isolates M-1 and T-1 at the seven inoculum concentrations used in the first study.

Plants were grown and tested in the greenhouse during all seasons. Artificial light was supplied as necessary to give a minimum intensity of (7,532 lx = 700 ft-c) and constant day length of 16 h. Inoculations were made approximately 2 days before anthesis on an individual head basis, in order to allow the expression of any possible genetic resistance of the host, without interference from potential affects of fertilization. The outer glumes were removed and 10 florets per head were inoculated by injection of approximately 0.02 ml. of the conidial suspension into each floret. Unless otherwise stated, 10 heads were inoculated and immediately covered with glassine envelopes. Experiments were not replicated.

Assessment of the Disease Reaction:

After about 30 days, the disease reaction of each isolate-host combination was assessed. The number of sclerotia per head was recorded and the sclerotial size was rated and averaged as follows: 1, sclerotia smaller than normal kernel; 2, sclerotia approximately the size of normal kernel; 3, sclerotia larger than kernel, extending beyond the lemma and palea. Honeydew production was also rated visually and averaged as follows: 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. The significance of the difference between the average number of sclerotia induced on each host was determined by Duncan's multiple range test. The significance of the difference between the average number of sclerotia induced by each isolate on each host at a given inoculum concentration was determined by the paired t-test.

RESULTS

Reaction of the Cereal Species to Two Ergot Isolates:

There were no significant differences in the percentage of sclerotia produced between any of the species inoculated with the M-1 isolate at the highest and lowest inoculum concentrations of 10^6 and 50 conidia/ml, respectively (Table 1). Spring wheat was significantly different from the other five cereal species tested at the other inoculum concentrations with two exceptions: that of 10^4 conidia/ml where it was the same as durum wheat, and that of 100 conidia/ml where it differed only from rye. The differences between the other species were more clearly expressed at an inoculum concentration of 500 conidia/ml; significantly fewer sclerotia were induced in durum wheat than in rye and barley.

The results of sclerotial production with the T-1 isolate are shown in Table 2. There were significant differences between the species tested except at the highest and lowest inoculum concentrations. Spring wheat was significantly different from the other species at the other inoculum concentrations except that of 100 conidia/ml where it differed only from rye and oats. The differences between the other cereal species were more clearly expressed at inoculum concentrations of 500 and 250 conidia/ml; significantly fewer sclerotia were produced by the T-1 isolate on durum wheat than on rye and barley.

There were no differences between the species with respect to sclerotial size and amount of honeydew produced with the exception of spring wheat. Both ergot isolates induced sclerotia of size 2 in spring wheat, whereas sclerotia of size 3 were induced in the other species. The amount of honeydew produced by the M-1 and T-1 isolates

received a rating of 2 on spring wheat and 3 on the other species. Inoculum concentration did not appear to influence either sclerotial size or amount of honeydew produced.

Differences in Percentage of Sclerotia Produced in Cereal Species by M-1 and T-1 Isolates:

The isolates were significantly different at a greater number of inoculum concentrations on Manitou and Stewart 63 than on the other cultivars (Table 3). The differences between the two isolates were significant on all the cultivars at inoculum concentrations of 500 and 250 conidia/ml, with the exception of the cultivar Conquest at an inoculum concentration of 250 conidia/ml.

Reaction of Cereal Cultivars:

A wide range of disease reactions was observed among the spring wheat cultivars inoculated with either isolate of C. purpurea at an inoculum concentration of 10^4 conidia/ml (Table 4). The percentage of sclerotia induced by the M-1 isolate in Kenya Farmer was significantly less than that in the other cultivars with the exception of CT 244. With the T-1 isolate, the lowest number of sclerotia was again observed on Kenya Farmer; however, the value did not differ significantly from that of several other cultivars. Considerable variation in sclerotial size and amount of honeydew produced was also observed among the cultivars. Sclerotial size ranged from a 3 to a 1 rating, with both isolates rated 1 on Kenya Farmer, Lee and CT 244. Honeydew production received a rating of 1 with both isolates on the cultivar Kenya Farmer. In addition, the T-1 isolate rated 1 on

CT 244. The number of sclerotia induced in the cultivars Wells and Carleton by both isolates, differed significantly from that of the other cultivars (Table5). Both isolates produced sclerotia of size 1 and 2 in Carleton and Wells, respectively. Lower amounts of honeydew developed on the cultivars Carleton and Wells than on the other cultivars and lesser quantities were induced by the T-1 than by the M-1 isolate.

There were no significant differences in the disease reaction among the inoculated cultivars of triticale, barley, oats and rye. The range of the percentage of sclerotia induced in cultivars of each species by the M-1 and T-1 isolates, respectively, was as follows: triticale, 69-61% and 66-53%; barley, 74-69% and 65-60%; oats, 71-64% and 66-58% and rye, 75-71% and 70-60%. Both isolates produced sclerotia of size 3 in all cultivars. Honeydew production was high (4 rating) on the cultivars of barley and rye. The M-1 isolate induced a honeydew rating of 4 on all the oat and triticale cultivars, whereas the T-1 isolate induced a 3 rating.

Effect of Inoculum Concentration on Sclerotial Production in the Wheat Cultivars Kenya Farmer, Carleton and Chinese Spring:

At inoculum concentrations of 10^6, 10^4, and 10^3 conidia/ml, the three cultivars separate into three groups on the basis of percentage sclerotia produced by the M-1 isolate (Table 6). At inoculum concentrations lower than 500 conidia/m., this isolate did not produce sclerotia in either Kenya Farmer or Carleton but in Chinese Spring, sclerotia were produced at an inoculum concentration as low as 100

conidia/ml. With the T-1 isolate, the percentages of sclerotia produced in each cultivar were significantly different from one another only at the highest inoculum concentration of 10^6. At inoculum concentrations of 10^4 and 10^3 there were no significant differences between Kenya Farmer and Carleton; however, both cultivars were significantly different from Chinese Spring.

Differences in Percentage of Sclerotia Produced by M-1 and T-1 Isolates in Wheat Cultivars, Kenya Farmer, Carleton and Chinese Spring:

The isolates differed significantly ($P = 0.05$) from each other at inoculum concentrations of 10^6, 10^4 and 10^3 conidia/ml on all three cultivars. In addition, they were significantly different at inoculum concentrations as low as 500 conidia/ml on Carleton, and as low as 100 conidia/ml on Chinese Spring.

DISCUSSION

Evidence presented here establishes that some cereal species and cultivars differ significantly in their reaction to C. purpurea. Differences were observed among and within species with respect to each of the three components of the disease reaction, i.e., frequency of sclerotial production, size of sclerotia and amount of honeydew produced.

The results of the first series of experiments indicated that spring wheat and durum wheat are less susceptible than the other cereal species. The results agree reasonably well with the relative incidence of ergot in the field in the case of spring wheat, durum wheat, triticale and rye. On the other hand, barley and oats were highly susceptible in our tests, whereas in the field the incidence of ergot in both hosts is very low. The field resistance of barley and oats may be due to escape of infection. In both species the majority of florets reach anthesis before the head has fully emerged. Furthermore, susceptibility has been shown to decrease following anthesis (Abe and Kono, 1957; Campbell and Tyner, 1959; Rapilly, 1968). It is noteworthy that the reaction of triticale was found to resemble that of the more susceptible rye parent rather than spring or durum wheat.

Results of the second series of inoculations indicate that only cultivars of spring and durum wheat showed differences in the expression of the disease reaction. On the basis of this study the spring wheat cultivar Kenya Farmer and the durum wheat cultivar Carleton were selected for further evaluation, and more detailed results concerning their reaction are presented elsewhere (Thesis , section 1).

Inoculum concentration had no apparent effect on size of sclerotia and amount of honeydew produced. Sclerotial frequency, on the other hand, was markedly affected by inoculum concentration. High inoculum concentrations were found to obscure differences in number of sclerotia induced to a greater extent in triticale, oats, barley and rye than in spring and durum wheat. A concentration of 10^4 and 10^3 conidia/ml would appear to be most suitable for detecting differences between wheat cultivars, whereas a concentration of 500 conidia/ml would appear adequate for comparing cultivars within the other species. At the highest inoculum concentrations, the differences between the number of sclerotia induced in each of the cultivars Kenya Farmer, Carleton and Chinese Spring remained significant. Sclerotial formation in Kenya Farmer and Carleton also required a higher inoculum concentration than Chinese Spring.

Differences in size of sclerotia and amount of honeydew produced were observed both among and within species. Smaller sclerotia and less abundant honeydew were generally associated with low frequency of sclerotial formation. In view of the relationship between sclerotial size and number of ascospores released (Cooke and Mitchell, 1966; Rapilly, 1968) and in view of the importance of honeydew in the epidemiology of the disease, it would seem worthwhile to consider these criteria as well as sclerotial frequency, when assessing the reaction of cereal cultivars to ergot.

The method of inoculation used in this study, that is, injection of inoculum into individual florets 2 days prior to anthesis, allows the full expression of disease reaction, and the selection of more ergot-resistant types. While somewhat more tedious than spraying heads, it gives more accurate control over inoculum concentration and

eliminates the effect of differences in flowering habit on frequency of infection.

The two isolates differed markedly in ability to induce sclerotia among and within species. Size of sclerotia and amount of honeydew produced were also affected. The differences were particularly evident on the spring wheat cultivar Kenya Farmer and the durum wheat cultivar Carleton. Our results suggest that further studies to determine the range of pathogenic variability within C. purpurea, using cultivars such as Kenya Farmer and Carelton, would be worthwhile. This would also establish the stability and potential usefulness of the type of resistance possessed by these cultivars.

Table 1. Sclerotial development in six cereal species inoculated with the M-1 isolate of *C. purpurea*

		% inoculated florets with sclerotia						
		Inoculum concentration (conidia/ml)						
Species	Cultivar	10^6	10^4	10^3	500	250	100	50
T. aestivum	Manitou†	86a*	70a	46a	24a	10a	3a	0a
T. durum	Stewart 63†	91a	81ab	54b	39b	25b	7a	0a
S. cereale	Prolific†	94a	86b	72b	49c	34b	18b	6a
X *Triticosecale*	Rosner‡	91a	83b	65b	41bc	29b	9ab	0a
H. vulgare	Conquest§	95a	86b	72b	52c	33b	16ab	6a
A. sativum	Harmon§	94a	85b	69b	47bc	31b	12ab	5a

†, ‡, and § - 20, 15 and 10 heads at 10 florets per head inoculated per inoculum concentration respectively.

* Duncan's multiple range test; values within each column followed by the same letter are not significantly different (P = 0.05).

Table 2. Sclerotial development in six cereal species inoculated with the T-1 isolate of *C. purpurea*

		% inoculated florets with sclerotia Inoculum concentration (conidia/ml)						
Species	Cultivar	10^6	10^4	10^3	500	250	100	50
T. aestivum	Manitou†	79a*	58a	23a	15a	4a	0a	0a
T. durum	Stewart 63†	84a	73b	58b	31b	19b	4ab	0a
S. cereale	Prolific†	88a	83bc	69c	41c	28c	18c	3a
X *Triticosecale*	Rosner‡	97a	77b	59b	35bc	23bc	5ab	0a
H. vulgare	Conquest§	91a	89bc	61b	43c	30c	8ab	2a
A. sativum	Harmon§	93a	78b	56b	39bc	20bc	10b	3a

†, ‡, and § - 20, 15 and 10 heads at 10 florets per head inoculated per inoculum concentration respectively.

* Duncan's multiple range test; values within each column followed by the same letter are not significantly different (P = 0.05).

Table 3. Significance of the difference in sclerotial production by M-1 and T-1 isolates of *C. purpurea* on six cereal species at seven inoculum concentrations

Species	Cultivar	Inoculum concentration (conidia/ml)						
		10^6	10^4	10^3	500	250	100	50
T. aestivum	Manitou	S*	S	S	S	S	NS*	NS
T. durum	Stewart 63	S	S	S	S	S	NS	NS
S. cereale	Prolific	S	NS	NS	S	S	NS	NS
X *Triticosecale*	Rosner	NS	S	NS	S	S	NS	NS
H. vulgare	Conquest	NS	NS	S	S	NS	S	NS
A. sativum	Harmon	NS	NS	S	S	S	NS	NS

* S and NS = significant and not significant at the 5% level as analyzed by the paired t-test.

Table 4. Host reactions of spring wheat cultivars inoculated with M-1 and T-1 isolates of *C. purpurea*

Cultivar	% inoculated florets with sclerotia†		Sclerotia size‡		Amount of honeydew§	
	M-1	T-1	M-1	T-1	M-1	T-1
Prelude	51 a*	30 a	3	3	3	3
Chinese Spring	51 a	31 a	3	3	4	3
Waldron	51 a	26 a	3	3	3	3
Thatcher	49 a	25 a	2	2	3	2
Neepawa	47 ab	24 a	2	2	3	2
Rescue	47 ab	14 ab	2	2	2	2
Manitou	48 ab	22 a	2	2	3	2
Pembina	45 ab	24 a	2	2	3	2
Selkirk	42 abc	19 ab	2	2	3	2
Pitic 62	42 abc	23 a	2	2	2	2
Red Bobs	41 abc	17 ab	2	1	2	2
Lee	31 abc	16 ab	1	1	2	2
CT-244	27 cd	14 ab	1	1	2	1
Kenya Farmer	11 d	6 b	1	1	1	1

Table 4 continued

† Ten heads per cultivar inoculated at ten florets per head at an inoculum concentration of 10^4 conidia/ml.

‡ 1 = sclerotia smaller than normal kernel; 2 = sclerotia approximately the size of a normal kernel; 3 = sclerotia larger than kernel extending beyond the lemma and palea.

§ 1 = no visible honeydew; 2 = honeydew confined with glumes; 3 = honeydew exuding from florets in small drops; 4 = honeydew exuding from florets in large drops and running down the head.

* Duncan's multiple range test; values within columns followed by the same letter are not significantly different (P = 0.05).

Table 5. Host reactions of durum wheat cultivars inoculated with M-1 and T-1 isolates of *C. purpurea*

Cultivar	% inoculated florets with sclerotia†		Sclerotia size ‡		Amount of honeydew§	
	M-1	T-1	M-1	T-1	M-1	T-1
Mindum	71a	67a	3	3	4	4
ST - 464	68a	61ab	3	3	4	4
Stewart	67a	59ab	3	3	4	4
Hercules	66a	55ab	3	3	4	4
Oumillo	65a	58ab	3	3	4	4
Stewart 63	64a	56ab	3	3	3	4
Ghiza	55a	45b	3	3	4	3
Wells	34a	25c	2	2	3	2
Carleton	21b	9c	1	1	2	1

† Ten heads per cultivar inoculated at ten florets per head at an inoculum concentration of 10^4 conidia/ml.

‡ 1 = sclerotia smaller than normal kernel; 2 = sclerotia approximately the size of a normal kernel; 3 = sclerotia larger than kernel extending beyond the lemma and palea.

§ 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.

* Duncan's multiple range test; values within columns followed by the same letter are significantly different (P = 0.05).

Table 6. Sclerotial development in three wheat cultivars inoculated with M-1 and T-1 isolates of *C. purpurea*

	% inoculated florets with sclerotia						
	Inoculum concentrations (conidia/ml)						
Cultivar	10^6	10^4	10^3	500	250	100	50
Isolate M-1							
Kenya Farmer†	43a	24a	12a	0a	0a	0a	0a
Carleton‡	55b	41b	21b	6a	0a	0a	0a
Chinese Spring‡	87c	77c	51c	33b	15b	5a	0a
Isolate T-1							
Kenya Farmer†	24a	15a	4a	0a	0a	0a	0a
Carleton‡	31b	27a	9a	0a	0a	0a	0a
Chinese Spring‡	81c	67b	33b	17b	7b	0a	0a

† and ‡ = 20 & 15 heads at 10 florets per head inoculated per inoculum concentration, respectively.

* Duncan's multiple range test; for each isolate values within each column followed by the same letter are not significantly different (P = 0.05).

2. Resistance to Claviceps Purpurea in Spring and Durum Wheat:

ABSTRACT

Resistance to C. purpurea was found in Triticum aestivum cv. Kenya Farmer and T. durum cv. Carleton. This resistance was expressed as a decrease in the number of sclerotia formed and a reduction in sclerotial size and honeydew produced. The resistant reaction was maintained against four isolates of C. purpurea. The resistance possessed by these two wheat cultivars is not of the complete immune type but it is superior to that presently found in commercial cultivars of T. aestivum and T. durum.

INTRODUCTION

The ergot fungus, Claviceps purpurea (Fr.) Tul., is common on cereals and grasses throughout the temperate regions of North America (Campbell, 1957; Conners, 1953). It is often severe on rye (Secale cereale L.) and durum wheat (Triticum durum Desf.) and in recent years it has also been troublesome on Triticale (Triticum sp. x S. cereale) and on male sterile cultivars and lines used in the development of hybrid spring wheat (Triticum aestivum L.) at the University of Manitoba. Field observations in the past have indicated that some cultivars of either spring or durum wheat are more susceptible than others (Weniger, 1924), but experimental evidence of resistance has not been reported. We therefore investigated the variability of the infection reaction to C. purpurea in the cultivated cereals, aiming to find possible sources of resistance for use in the Triticale and hybrid wheat breeding programmes (Thesis, section 1).

During these investigations two cultivars, one of spring wheat and one of durum wheat, were found to possess a higher resistance to ergot. These two cultivars were tested more extensively, along with two susceptible ones, and the results of the tests are the subject of this study. A more detailed account of our studies, dealing with the selection and virulence of isolates and the testing procedures as well as the variability of the infection reaction in cultivated cereals is presented in thesis section 1.

MATERIALS AND METHODS

Ergot sclerotia were collected from the spring wheat cultivar "Manitou" and from the Triticale cultivar "Rosner". Two isolates from each cultivar were obtained by culturing surface-sterilized sclerotial pieces on acid malt agar. Conidia were increased as required in liquid shake culture (Lewis, 1959). The mycelium was removed by filtration and the spore density was adjusted to approximately 10^4 conidia/ml. with sterile distilled water. Plants of the wheat cultivars listed on Table 7 were grown in the greenhouse and inoculated two days before anthesis. Ten florets per head were inoculated by hypodermic syringe, injecting 0.02 ml. of the spore suspension into each floret. At least five heads of each cultivar were inoculated with each of the four ergot isolates and immediately covered with a small glassine envelope. The experiments were repeated four times for all cultivars, except "Carleton", which was tested twice. The inoculated florets were rated for the amount of honeydew produced ten days after inoculations. After twenty-one days, the number of sclerotia formed was recorded and the sclerotia were rated for size.

RESULTS AND DISCUSSION

The response of the four cultivars to each of the four ergot isolates was identical to that obtained in our preliminary experiments and only the results of inoculations with an isolate from the Manitou cultivar are presented in Table 7. Kenya Farmer and Carleton were clearly more resistant than Manitou and Stewart 63. Resistance is expressed by a decrease in the number of sclerotia formed, and a reduction in the amount of honeydew produced, and in the size of the sclerotia. In the case of Manitou and Stewart 63, the number of sclerotia produced per head is a fairly accurate index of the amount of infection. In the absence of sclerotia, normal kernels were found in the majority of the inoculated florets. In the case of Kenya Farmer and Carleton, however, no kernels were found in florets where sclerotia were absent. Such florets contained small, discoloured and shrivelled ovaries or undeveloped kernels. It seems that the florets were infected and that further development of the fungus was inhibited. The nature of this reaction is still being investigated.

In the summer of 1969, Kenya Farmer was tested further at a disease nursery in conditions of natural infection and adjacent to a susceptible cultivar. In these conditions, a considerable number of sclerotia were observed in the susceptible cultivars. In Kenya Farmer however, only a few small sclerotia, of the size obtained in the greenhouse experiments, were recovered from a 20 foot row. Subsequently, an isolate was obtained from one of the Kenya

Farmer sclerotia and used to inoculate this cultivar in the greenhouse. This isolate induced the same reactions as the previous isolates tested.

The resistance of Kenya Farmer and Carleton is expected to be adequate. Although infection can occur, 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 resistance of Kenya Farmer was not influenced by the range of conditions in the greenhouse, and was maintained when plants were inoculated with some common biotypes of the fungus in our area. Carleton was not tested as extensively, but its resistance seems to be similar to that of Kenya Farmer.

TABLE 7 Response of wheat cultivars inoculated with an isolate of C. purpurea from Manitou wheat[1]

Cultivar	Percentage of florets with sclerotia 2/	Size of sclerotia 3/	Amount of Honeydew produced 4/
Manitou 5/	70	2	3
Kenya Farmer 5/	26	1	1
Stewart 63 6/	78	3	4
Carleton 6/	42	1	2

1/ Inoculum concentration 10^4 conidia/ml

2/ Based on a total of five heads with ten inoculated florets/head.

3/ Visual rating of sclerotia size measured twenty-one days after inoculation. (1) Sclerotia very small.
(2) Sclerotia approximately the size of normal kernel.
(3) Sclerotia larger than kernel, extending beyond glumes.

4/ Visual rating of honeydew produced measured ten days after inoculation. (1) No visible honeydew.
(2) Honeydew confined within glumes.
(3) Honeydew exuding from infected florets in small drops.
(4) Honeydew exuding from infected florets in large drops and running down the head.

5/ Triticum aestivum L. cv.

6/ Triticum durum Desf. cv.

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3. Coleoptile Reaction of Cultivated Cereals to Claviceps Purpurea:

ABSTRACT

The disease reaction of cultivars of Triticum aestivum, T. durum, X Triticosecale, Secale cereale, Hordeum vulgare and Avena sativum was evaluated by inoculating coleoptiles with each of two isolates of Claviceps purpurea (Fr.) Tul. The coleoptiles of T. durum, H. vulgare and A. sativum could not be infected. The T. aestivum cultivars showed varying levels of resistance to C. purpurea as evidenced by sparse mycelial development and stunted, discoloured coleoptiles. S. cereale and X Triticosecale were more readily infected than the other cereal species, and infection increased with increasing inoculum density. On rye and triticale an isolate of C. purpurea from spring wheat caused a greater percentage of infection than an isolate from triticale. Only the isolate of C. purpurea from spring wheat caused infection in the spring wheat cultivars.

INTRODUCTION

The normal site of attack by Claviceps purpurea (Fr.) Tul. is the young undeveloped ovary, however, Stoll and Brack (1944) found that the internodes and nodes as well as the ovary were susceptible to infection by artificial inoculation. This was confirmed by Cherewick (1953). Lewis (1956), utilized a seedling (=coleoptile) inoculation test to study reaction of rye, spring wheat and barley to C. purpurea. Rye coleoptiles, on the basis of a higher percentage of infection and the formation of microsclerotia, were judged to be more susceptible to C. purpurea than spring wheat or barley. However, microsclerotia were obtained on inoculated wheat coleoptiles by Rapilly (1968).

This study was initiated to determine if cereal species differ in their reaction to C. purpurea in the coleoptile stage and whether this technique might prove useful for screening wheat and triticale cultivars for resistance.

MATERIALS AND METHODS

Plant Material and Method of Inoculation:

The disease reaction of cereal cultivars was evaluated by individually inoculating coleoptiles with one of two isolates of *C. purpurea* at three inoculum concentrations ranging from 10^6 to 10^8 conidia/ml.

The cereal cultivars utilized are listed in Table 8. All the cultivars tested had been previously evaluated in the adult stage of floret inoculation.

The method of inoculation was modified from the original method utilized by Lewis (1956, 1962). Instead of using metal bake pans and glass plates covered with filter paper, aluminum foil pans 230mm x 230mm x 45mm, were used and the inoculated seedlings placed directly onto moist sterilized vermiculite in the bottom pan. Seeds were germinated in clear plastic sandwich boxes on sterilized moist filter paper. Seedlings with a coleoptile 7 to 10mm long were selected for inoculation. The tip of the coleoptile was cut off and .001 ml of inoculum was placed aseptically on the cut end by means of a Hamilton micro syringe normally utilized in gas chromatography. The inoculated coleoptiles were placed on the vermiculite layer in the bottom pan, covered with another foil pan and the two were taped together with masking tape. The pans were placed in an incubator at 28°C. The percentage of infected coleoptiles was determined after 7 - 10 days.

Assessment of Disease:

Seedlings were observed for presence of mycelium on the outer surface of the coleoptile. The percentage inoculated coleoptiles showing visible mycelium development was recorded. Inoculated coleoptiles were also observed for microsclerotia formation, abnormal growth and discolouration of the coleoptiles.

Selection of Isolate and Preparation of Inoculum:

Two ergot isolates used previously in adult plant floret inoculation were selected for this study (Thesis, section 1). The M-1 isolate was obtained from naturally infected Manitou spring wheat and the T-1 isolate was from naturally infected Rosner Triticale. Conidia were increased as required on a modified potato sucrose broth (Lewis, 1956) (Appendix). The conidia were separated from the mycelium by filtration, concentrated by centrifugation, and stored in 60% sucrose at 4°C until utilized (Thesis, section 1). Inoculum of various spore concentrations was prepared by diluting the conidial suspension with a 60% sterile distilled water sucrose solution.

RESULTS

The percentage inoculated coleoptiles showing visible mycelium was highest on rye and triticale cultivars for both isolates of C. purpurea, and increased with increasing inoculum concentration (Table 8). In addition to showing evidence of mycelial developement some of the infected rye and triticale coleoptiles showed pronounced twisting and curling. Occasionally small microsclerotia were produced on the infected rye and triticale coleoptiles (Figure 1). Only the M-1 isolate at the highest inoculum concentration produced mycelium on the spring wheat coleoptiles. The inoculated coleoptiles of the spring wheat cultivar Kenya Farmer did not show any mycelial development with either isolate at any of the inoculum concentrations employed. No twisting or curling of the coleoptiles of the spring wheat cultivars was observed but there was a definite brown discolouration extending down the coleoptile from the point of inoculation. This brown discolouration was most pronounced on the coleoptiles of Kenya Farmer especially at the highest inoculum concentration with the M-1 isolate. The primary leaf did not emerge from the discoloured coleoptiles of Kenya Farmer at the highest inoculum concentration, however, in other spring wheat cultivars the primary leaf emerged from the discoloured coleoptile sheath. The inoculated coleoptiles of the durum wheat cultivars showed no mycelial development but were discoloured to the same extent as the other spring wheat cultivars with the exception of Kenya Farmer. The primary leaf of the inoculated durum wheat coleoptiles emerged normally from the coleoptile sheath. The

inoculated coleoptiles of barley and oats did not show any mycelial development, irregular growth or discolouration.

DISCUSSION

The results indicate that rye and triticale seedlings are more susceptible to C. purpurea than spring wheat, durum wheat, oats and barley. This confirms observations made on rye, triticale and spring wheat in the adult floret test where rye and triticale were more susceptible than spring wheat (Thesis, section 1). However, the resistance of durum wheat, and in particular of oats and barley in the coleoptile test is in contrast to their susceptible reaction in the adult floret inoculations. The spring wheat cultivar Kenya Farmer shown to have the highest level of resistance in the floret test (Thesis, section 1), was also the most resistant in the coleoptile test.

The discolouration of inoculated coleoptiles appears to be associated with a resistant host reaction since it was most pronounced on the coleoptiles of the resistant spring wheat cultivar Kenya Farmer and absent on rye and triticale cultivars which had previously been shown to be more susceptible than spring wheat cultivars in the floret test (Thesis, section 1).

The T-1 isolate was less virulent than the M-1 isolate as evidenced by its inability to infect the spring wheat cultivars and by its lower level of virulence on the coleoptiles of rye and triticale. This supports results obtained in the floret test where the T-1 isolate produced fewer sclerotia than the M-1 isolate on cultivars of all three cereal species.

On the basis of this study the level of infection attained in inoculated coleoptiles appears to be adequate for this technique to be further evaluated in screening for resistance to ergot in rye and triticale, especially if other aspects of the resistant coleoptile reaction, as in wheat, develop. If the degree of discolouration of the coleoptiles and cessation of further growth in spring wheat cultivars are produced as a result of a hypersensitive resistance mechanism, then irrespective of the low frequency of mycelial growth on inoculated coleoptiles the reaction of spring wheat cultivars can be evaluated. The coleoptile test may be valuable in screening wheat cultivars for resistance to C. purpurea.

Since a coleoptile test can be conducted in a relatively short period of time and would afford a saving in greenhouse space as compared to the floret test this technique is worthy of further evaluation.

TABLE 8 - Percentage coleoptiles of selected cereal cultivars inoculated with M-1 and T-1 isolate of *C. purpurea* showing mycelial development.

Species	Cultivar	Inoculum concentration conidia/ml					
		10^8		10^7		10^6	
		Isolate		Isolate		Isolate	
		M-1	T-1	M-1	T-1	M-1	T-1
Triticum aestivum[1]	Chinese Spring	17	0	0	0	0	0
	Manitou	11	0	0	0	0	0
	Prelude	6	0	0	0	0	0
	Kenya Farmer	0	0	0	0	0	0
Triticum durum	Stewart 63	0	0	0	0	0	0
	Carleton	0	0	0	0	0	0
X Triticosecale	Rosner	51	42	30	24	17	19
	6531	62	59	45	21	26	10
Hordeum vulgare	Conquest	0	0	0	0	0	0
Avena sativum	Harmon	0	0	0	0	0	0
Secale cereale	Prolific	71	51	33	25	23	16
	Cougar	63	58	41	35	27	11

[1] 100 coleoptiles inoculated/isolate

Figure 1 Microsclerotial development on coleoptile of X Triticosecale cv. Rosner inoculated with an isolate of C. purpurea from X Triticosecale cv. Rosner

4. Chromosome Location of Genes Conditioning Resistance to *Claviceps purpurea* in Spring and Durum Wheat:

ABSTRACT

The chromosome location of the gene or genes conferring resistance to *C. purpurea* in *Triticum aestivum* cv. Kenya Farmer was determined by using a substitution series of individual chromosomes of Kenya Farmer into *T. aestivum* cv. Chinese Spring. Kenya Farmer and Chinese Spring were found to be resistant and susceptible to *C. purpurea* respectively in previous studies. The chromosome 6B of Kenya Farmer appeared to carry a resistant gene or genes to *C. purpurea*. A similar study was conducted using the F1 plants of *T. durum* cv. Carleton crossed with Chinese Spring monosomics of the A and B genomes. Carleton was found to be resistant to *C. purpurea* in a previous study. The 1B chromosome of Carleton appears to carry a gene or genes conferring resistance to *C. purpurea*. In both the Kenya Farmer and Carleton series the monosomic lines were not as resistant as the resistant parents. This may indicate that there are genes for resistance in Kenya Farmer and Carleton on other chromosomes which contribute to the reaction. Furthermore, several lines in both the Kenya Farmer and Carleton series showed resistance for one of the components i.e., frequency of sclerotia, size of sclerotia and honeydew production, but were susceptible for the other components.

INTRODUCTION

Resistance to ergot was found in Triticum aestivum L. cv. Kenya Farmer and T. durum Desf. cv. Carleton (Thesis, section 1). Resistance in both is expressed by a decrease in number and size of sclerotia and a reduction in amount of honeydew produced. The degree of resistance appeared to be greater in Kenya Farmer than in Carleton.

The use of intervarietal wheat chromosome substitution series for the location of disease resistance genes has been employed frequently, particularly in the location of genes for stem rust resistance (Knott, 1957, 1959; Sears et al. 1957, 1960; Sheen and Snyder, 1964). Chromosome substitution lines have also been used to identify chromosomes carrying resistance to leaf rust (Heyne and Livers, 1953) and bunt (Sears et al. 1960).

This study was initiated to identify the chromosome or chromosomes carrying the gene or genes for resistance to C. purpurea in Kenya Farmer and Carleton.

MATERIALS AND METHODS

Choice of Isolate and Preparation of Inoculum:

An isolate of C. purpurea designated M-1 used in previous tests on the reaction of Kenya Farmer and Carleton was chosen (Thesis, section 1). An inoculum density of 10^4 conidia/ml which was previously found to be adequate to produce infection in both Kenya Farmer and Carleton was used (Thesis, section 1).

Plant Material and Method of Inoculation:

The series of disomic substitutions of Kenya Farmer chromosomes into Chinese Spring previously utilized by Sheen and Snyder (1965) was obtained from W. J. R. Boyd of the University of Western Australia.

The monosomic series of Chinese Spring was obtained from E. R. Sears, of the United States Department of Agriculture, Columbia, Missouri, and F1 monosomics of the A and B genomes of Carleton were produced. Both series were subsequently inoculated with a conidial suspension of the M-1 isolate of C. purpurea. The conidial suspension was adjusted to a density of 10^4 conidial/ml in sterile water and 0.02 ml of the suspension hypodermically injected into 10 florets of each inoculated spike.

Assessment of the Disease:

The percentage of sclerotia produced was recorded and size of sclerotia and amount of honeydew produced were rated as described previously (Thesis, section 1).

Confirmation of Chromosome Identity:

To confirm the identity of the 6B chromosome a seedling

reaction of race C35 of stem rust, Puccinia gramini Pers. f. sp. tritici Erikss. & Henn. was utilized on the Kenya Farmer by Chinese Spring disomic substitution lines. Race C35 is avirulent towards the stem rust gene Sr 11, (Green, 1969). The location of the Sr 11 gene on chromosome 6B of Kenya Farmer was determined by Knott (1957, 1959).

RESULTS AND DISCUSSION

The percentage of florets with sclerotia on the Kenya Farmer (KF) - Chinese Spring (Csp) substitution lines and the Carleton (Ctn) - Chinese Spring (CSp) F-1 monosomics ranged from 40 to 97% (Table 9) and 37 to 93% (Table 10) respectively. The 6B line of the KF-CSp substitution series and 1B line of the Ctn - CSp F1 monosomics both showed a reduction in percentage sclerotia, size of sclerotia and amount of honeydew produced but not to the same extent as the resistant parents. The fact that several lines in both series showed a reduction in sclerotia size and in amount of honeydew produced without a marked reduction in percentage sclerotia indicates that there are other genes for resistance in Kenya Farmer and Carleton in addition to those present on the 6B and 1B chromosomes. These additional genes which appear to control only part of the resistant reaction might account for the fact that the 6B line and the 1B F-1 monosomic were not as resistant as Kenya Farmer and Carleton respectively.

The validity of these conclusions is dependent on the assumption that the chromosomes carrying resistance genes are correctly identified. The identity of chromosome 6B in Kenya Farmer was confirmed by a resistant reaction in the seedling stage to race C35 of stem rust.

Advances in the improvement of ergot resistance of commercial spring and durum wheat cultivars could be achieved by incorporating resistance genes from Kenya Farmer and Carleton.

Table 9 Response of Chinese Spring, Kenya Farmer and single chromosome substitution lines of Kenya Farmer into Chinese Spring background inoculated with an isolate of C. purpurea from Manitou wheat

Cultivar	Percentage of Florets with sclerotia	Size of sclerotia	Amount of Honeydew Produced
Kenya Farmer (KF)[1]	17	1	1
Chinese Spring (CSp)	87	3	4
CSp-KF Substitution Lines:			
1A	90	3	4
2A	93	3	4
3A	77	2	3
4A	90	3	3
5A	83	2	2
6A	93	3	2
7A	77	2	3
1B	87	3	4
2B	97	2	3
3B	73	3	3
4B	97	2	2
5B	97	3	4
6B	40	1	2
7B	87	3	2
1D	70	2	2
2D	87	3	3
3D	87	3	2
4D	87	3	2
5D	93	2	3
6D	77	3	2
7D	97	3	4

[1] 3 heads inoculated, 10 florets/head; inoculum concentration 10^4 conidia/ml.

Table 10 Response of Carleton, Chinese Spring and Carleton crossed to Chinese Spring monosomics inoculated with an isolate of C. purpurea from Manitou wheat

Cultivar	Percentage of Florets with sclerotia	Size of sclerotia	Amount of Honeydew Produced
Carleton (Ctn)[1]	27	1	2
Chinese Spring (CSp)	77	3	4
Ctn X CSp Monosomics:			
1A	67	3	3
2A	80	3	4
3A	77	3	4
4A	93	3	4
5A	87	3	4
6A	90	3	4
7A	73	3	4
1B	33	2	2
2B	60	3	4
3B	53	3	2
4B	73	3	2
5B	77	3	2
6B	77	2	4
7B	67	3	4

[1] 3 heads inoculated, 10 florets/head; inoculum concentration 10^4 conidia/ml.

5. Inheritance of resistance to Claviceps purpurea in Spring and Durum Wheat:

ABSTRACT

The inheritance of the disease reaction of Triticum aestivum L. cv. Kenya Farmer and T. durum Desf. cv. Carleton previously found to be resistant to Claviceps purpurea was investigated. The two wheat cultivars were crossed to the susceptible T. aestivum cv. Chinese Spring and T. durum cv. Stewart 63 respectively. The reaction of the two crosses was investigated in the F1, F2 and F3 populations. The overall disease reaction of Kenya Farmer appears to be controlled by two recessive genes. In Carleton the overall disease reaction is controlled by two dominant genes. The inheritance of the individual components of the disease reaction was also investigated, i.e., frequency of sclerotia, size of sclerotia and amount of honeydew produced. The results of the F2 analyses suggest that there are separate genes controlling each of the components of the disease reaction in Kenya Farmer and Carleton. The analysis of the reaction of F3 lines indicates that the resistance genes in Carleton are likely linked whereas in Kenya Farmer they are independent. Fully resistant F3 lines were recovered more frequently within the Carleton x Stewart 63 cross than Kenya Farmer x Chinese Spring cross.

INTRODUCTION

Previous studies on inheritance of resistance to *Claviceps purpurea* (Fr.) Tul. in *Triticum* species revealed that resistance was a quantitative character controlled by more than one gene which was in some cases dominant and in others recessive (Galstjan-Avenesjan, 1967). Resistance to *C. purpurea* in sugar cane was shown to be recessive (Robinson, 1960).

The location of gene(s) for resistance to *C. purpurea* was shown to be on chromosome 6B of Kenya Farmer and 1B of Carleton (Thesis, section 3).

The purpose of this study was to determine the mode of inheritance of the resistance expressed by *T. aestivum* cv. Kenya Farmer and *T. durum* cv. Carleton (Thesis, section 1).

MATERIALS AND METHOD

Source of isolate and preparation of inoculum:

The M-1 isolate of C. purpurea used in previous studies on Kenya Farmer and Carleton (Thesis, section 1) was chosen for this study. The methods of culturing the fungus and preparing the inoculum were as described previously (Thesis, section 1).

Plant material and method of inoculation:

The intervarietal crosses Kenya Farmer x Chinese Spring and Carleton x Stewart 63 were evaluated as F1, F2 and F3 populations. The reaction of the parental cultivars to C. purpurea was also recorded.

Three individual spikes from each of the crosses were inoculated with C. purpurea in the F1. A single spike from each of 165 and 143, F2 plants from the crosses Kenya Farmer x Chinese Spring and Carleton x Stewart 63 respectively, was inoculated using a hypodermic needle and an inoculum density of 1×10^4 conidia/ml as in previous studies (Thesis, section 1).

Because of the relatively tedious method of inoculation and the limitations of greenhouse space only 100 lines from a total of 165 F3 lines of Kenya Farmer x Chinese Spring and 50 lines from a total of 143 lines of Carleton x Stewart 63 were inoculated. For each F3 line only one individual spike from each of 10 plants was usually inoculated. Twenty individual spikes of each parental cultivar were inoculated with C. purpurea.

Assessment of the Disease:

The three components of the disease reaction were recorded 30 days after inoculation for each inoculated spike of the crosses and parental cultivars. The number of sclerotia was expressed as percent inoculated florets producing sclerotia on the basis of 10 inoculated florets per head. The size of the sclerotia was assessed by the numerical rating system used previously (Thesis, section 1): 1) sclerotia smaller than normal kernel; 2) sclerotia approximately the size of normal kernel; 3) sclerotia larger than kernel, extending beyond the lemma and palea. However, instead of averaging the size of the sclerotia per spike each sclerotium was given a rating. Honeydew production was also rated visually and averaged for each individual spike as in previous studies (Thesis, section 1). The rating system is as follows: 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.

The reaction based on the individual disease components was classified as susceptible or resistant on the basis of the reaction of the resistant parental cultivars Kenya Farmer and Carleton and the susceptible parental cultivars Chinese Spring and Stewart 63. The segregation of the resistant and susceptible lines in the F2 was tested against standard one and two gene ratios by means of a chi-square analysis.

RESULTS AND DISCUSSION

The reaction of the parental cultivars (Table 1) was as in previous studies (Thesis, section 1 and 2). T. aestivum cv. Kenya Farmer showed a higher level of resistance than T. durum cv. Carleton. The reaction of the F1 plants of both crosses approached the reaction of the resistant parental cultivars for sclerotia size and honeydew production but the average number of sclerotia produced per spike was closer to that of the susceptible parent (Table 11).

The disease reaction of the progeny from each cross was evaluated on the basis of the standard deviation of the average reaction of the resistant parental cultivars, and individual plants were classified as resistant or susceptible as follows; for Kenya Farmer x Chinese Spring, Resistant = maximum of four scloerota per spike; sclerotia size rated 1 and 2; no size 3 sclerotia; honeydew production rated 1 or 2. Susceptible = greater than four sclerotia per spike; majority of sclerotia rated 2 and 3; honeydew rated 3 or 4. Because Carleton was not as resistant as Kenya Farmer higher limits for sclerotia frequency and sclerotia size were set. For Carleton x Stewart 63, resistant = maximum of six sclerotia per spike; majority of sclerotia rated 1 and 2 with a maximum of one size 3 sclerotia; honeydew rated 1 or 2. Susceptible = greater than six sclerotia per spike; majority of sclerotia rated 2 and 3; honeydew rated 3 or 4.

The overall disease reaction of the 165 Kenya Farmer x Chinese Spring F2 plants was as follows: 97 susceptible to 68 resistant. The

reaction did not differ significantly (P=.70 - .50) from a 9:7 ratio of susceptible to resistant plants, indicating that the overall resistance of Kenya Farmer to C. purpurea may be controlled by two recessive gene pairs which have complementary action.

The overall disease reaction of the 143 Carleton x Stewart 63 F2 plants was as follows: 50 susceptible to 93 resistant. The reaction did not differ significantly (P=.05 - .01) from a 9:7 ratio resistant to susceptible plants. While the results are not as highly significant as for the Kenya Farmer x Chinese Spring F2 plants, they indicate that the overall resistance of Carleton to C. purpurea is controlled by two gene pairs, dominant at both loci and with complementary action.

Although the overall disease reaction of Kenya Farmer and Carleton fits a two gene inheritance, many plants showed resistance for one or two components of the disease reaction, i.e., frequency of sclerotia, size of sclerotia and amount of honeydew production, only, indicating that the individual components of disease reaction might be controlled by separate genes in both cultivars. Each component of the disease reaction was rated individually and classified as resistant or susceptible. For Kenya Farmer x Chinese Spring sclerotial size and frequency both fit a 9:7 ratio indicating that resistance is controlled by two gene pairs and that the resistance is dominant at both loci with complementary action (Table 12). Honeydew production fitted a 3:1 ratio, indicating that there is one dominant gene pair for resistance to honeydew production.

For Carleton x Stewart 63, frequency of sclerotia was not significantly different from a 15:1 ratio of resistant to susceptible plants indicating that resistance is controlled by two gene pairs dominant at both loci with duplicate action. For sclerotia size and honeydew production the ratios were not significantly different from a 3:1 ratio of resistant to susceptible plants indicating a single gene pair for each with resistance dominant (Table 12).

The reaction of the Kenya Farmer x Chinese Spring and Carleton x Stewart 63 total F2 population was compared with the reaction of the 100 randomly selected Kenya Farmer x Chinese Spring F2's and the 48 randomly selected Carleton x Stewart 63 F2's (50 were originally selected but two failed to produce enough heads in the F3 to allow inoculation). On the basis of chi-square analysis the reaction of the selected F2 population was not significantly different from the reaction of the total F2 population of both crosses. These selected F2 were used to produce an F3 which was representative of the overall F2 population.

Inoculation of the 100 F3 lines of Kenya Farmer x Chinese Spring showed that five families were homozygous resistant for frequency of sclerotia, seven families were homzygous resistant for size of sclerotia and 22 F3 families were homozygous resistant for honeydew production. Seventy-two families were homozygous susceptible for all three disease components. Furthermore, 20 families showed resistance for one component of the reaction, six for two components of the reaction and two only showed a complete homozygous resistant reaction.

With the 48 Carleton x Stewart 63 cross, 18 F3 families were homozygous resistant for frequency of sclerotia, 16 homozygous resistant for size of sclerotia and 19 homozygous resistant for honeydew production. In contrast to the Kenya Farmer x Chinese Spring cross, eleven families were homozygous resistant for all three components of the disease reaction. Seven families were resistant for two components, and four resistant for one component. Twenty-six of the 48 F3 families were homozygous susceptible for all three disease components.

It appears that frequency of sclerotia and size of sclerotia in Kenya Farmer are each controlled by more than one gene and that the genes are not completely dominant in action as indicated by the low frequency of recovery of homozygous resistant F3 families. Families showing resistance to honeydew production were readily recovered in the F3 (22 out of 100), confirming observations made on the F2 which indicated that honeydew production was controlled by one gene pair. The very low incidence of F3 families homozygous resistant for all three components of the disease reaction (two out of 100 families) indicates that the resistance genes for frequency of sclerotia, size of sclerotia and honeydew production are independent and not linked.

The Carleton x Stewart 63 cross differed from the Kenya Farmer x Chinese Spring cross in that there was a greater recovery of homozygous resistant families (11 out of 48). The greater percentage of families having resistance to all components of the disease indicates that there are relatively few genes involved in the re-

sistance of Carleton to C. purpurea and that these genes are probably linked.

For the purpose of determining inheritance ratios and of selecting within F2 and F3 families it appears preferable to treat the individual components of the disease reaction as separate entities. This is not completely satisfactory as the components may be independent but are not mutually exclusive because in a line resistant to infection and sclerotia production, genes for sclerotia size and honeydew production cannot be expressed. Because the individual disease components cannot be studied in complete isolation to each other it was not possible to establish precise inheritance ratios. However, the results presented do give some indication of the mode of inheritance of the resistance of Kenya Farmer and Carleton to C. purpurea.

Results indicate progress could be made in improving the resistance to C. purpurea of spring and durum wheat by selecting for all components of the disease reaction, however, each component of the disease reaction was shown to be individually inherited and selection for each component is also possible. Although the results indicate that the inheritance of resistance in Carleton is less complex than Kenya Farmer in the long run it may be less suitable.

Table 11 Disease reaction of T. aestivum cvs. Kenya Farmer and Chinese Spring, and T. durum cvs. Carleton and Stewart 63 and F1 progeny to C. purpurea

	Parental Cultivars				F1 Plants	
	Kenya Farmer	Chinese Spring	Carleton	Stewart 63	Kenya Farmer x Chinese Spring	Carleton x Stewart 63
	DISEASE REACTION					
Ave. Sclerotia No./spike	2.2[1]	8.5	4.5	7.8	6.7[2]	5.3
Sd.[3]	± .5	± 1.4	± 1.9	± .5	± .9	± 1.7
Ave. Sclerotia size	1.4	2.2	1.7	2.3	1.6	1.9
Sd.	± .5	± .8	± .6	± .8	± .6	± .6
Ave. Honeydew production	1.1	3.4	1.3	3.7	1.7	2
Sd.	± .2	± .5	± .4	± .5	± .5	± 0

1 20 individual spikes inoculated at 10 florets/spike
2 three individual spikes inoculated at 10 florets/spike
3 Sd. = Standard deviation

Table 12 Inheritance of resistance to C. purpurea of individual components of the disease reaction in F2 populations of Kenya Farmer x Chinese Spring and Carleton x Stewart 63

Test Ratio[1]		DISEASE REACTION					
		Sclerotia Frequency		Sclerotia Size		Honeydew Production	
R	S	R	S	R	S	R	S
Kenya Farmer x Chinese Spring							
		91	74	96	69	117	48
3 : 1		NS		NS		*	
9 : 7		*		*		NS	
Carleton x Stewart 63							
		130	13	111	32	109	34
3 : 1		NS		*		*	
15 : 1		*		NS		NS	

[1] R = resistant S = susceptible

* Significance between P = .05 and .10 on basis of chi-square analysis

NS Not significant at P - .05 - .10 level

GENERAL DISCUSSION

This study established that some cereal species and cultivars differ significantly in their reaction to C. purpurea. There appears to be three components to the disease reaction, i.e., frequency of sclerotial production, size of sclerotia and amount of honeydew produced. Frequency of sclerotial production varies within cultivars of Triticum aestivum and T. durum, inoculated with isolates of C. purpurea at a standardized inoculum concentration. Frequency of sclerotial production showed a significant variation with all cereal species when inoculated with varying inoculum concentrations. High inoculum concentrations tended to obscure slight differences amongst cereal species and between cultivars. In a previous study where inoculum concentration was not considered, Campbell (1953) reported that all cereal and grass species grown in Western Canada were susceptible to Claviceps purpurea.

Sclerotial size and amount of honeydew produced were not affected by differences in inoculum concentration. Rapilly (1968) had reported differences between cereal and grass species, in size of sclerotia and amount of honeydew produced after infection with C. purpurea. This is the first report of the successful use of differences in size of sclerotia and amount of honeydew production in determining levels of resistance amongst cereal species and cultivars. It would appear that these two components of the disease reaction should be considered in future studies regarding reaction of cereal or grass species to Claviceps purpurea.

The method of inoculation is very important when comparing the reaction of different cereal species to C. purpurea. Previous studies had established that susceptibility decreased following anthesis (Abe and Kono, 1957; Campbell and Tyner, 1959; Rapilly, 1968). In this study the effects of anthesis and floret morphology were eliminated by inoculating the florets of the different cereals 2 days prior to anthesis by injecting a known volume of inoculum at a specified inoculum density into individual florets. Thus any differences observed between the cereal species could be attributed to different levels of physiological resistances and not to an escape mechanism based on 1) flowering habit and 2) floret morphology.

Triticum aestivum cv. Kenya Farmer and T. durum cv. Carleton were found in this study to have a higher level of resistance than presently grown commercial cultivars of spring and durum wheat. This resistance was expressed as a decrease in the number of sclerotia formed, and a reduction in the amount of honeydew produced and in the size of sclerotia.

The location of the resistance to C. purpurea discovered in T. aestivum and T. durum was further investigated by means of a substitution series of Kenya Farmer into Chinese Spring, and by means of F1 monosomics of the A & B genomes of Carleton crossed to Chinese Spring monosomics. Resistant genes were located on Chromosome (6B) of Kenya Farmer and Chromosome 1B of Carleton. Studies on the inheritance of resistance to C. purpurea in T. aestivum cv. Kenya Farmer and T. durum cv. Carleton revealed that the resistance is controlled by

more than one dominant gene in each of the cultivars and that there are separate genes governing the three components of the disease reaction.

Lewis (1956), reported differences between rye, spring wheat and barley in reaction to C. purpurea when inoculated at the seedling stage. The seedling (=coleoptile) test was evaluated as a means of rapid screening for resistance to C. purpurea. As in previous studies by Lewis (1956), this study also indicates that rye is more susceptible to C. purpurea than wheat or barley. The coleoptile test confirmed the greater susceptibility of triticale and rye than spring wheat to C. purpurea as also observed in adult plant floret inoculation.

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APPENDIXES

APPENDIX 1

METHOD FOR ISOLATING CLAVICEPS PURPUREA

(A) Sclerotial isolates:

Sound sclerotia were selected for use in isolating the fungus. Selected sclerotia were peeled to remove the outer rind. The peeled sclerotia were immersed in a 2% sodium hypochlorite solution and after one minute the sclerotia were removed from the hypochlorite solution and rinsed several times in sterile distilled water. Following the surface sterilizing treatment the sclerotia were aseptically cut into several pieces and plated onto 4% malt agar that has been acidified with 1 drop of 25% lactic acid. The plates were incubated at 24°C and the developing mycelium from sclerotia pieces was transferred and maintained on 4% malt agar slants. Slants of the fungus were sealed with parafilm and stored at 4°C. Cultures stored for four years were still viable.

(B) Single ascospore isolation:

Sound sclerotia less than 1 year old were selected for germination. Unsterilized sclerotia were imbedded in sterilized moist vermiculite in covered plastic containers, and stored at 4°C for approximately six weeks. After the cold storage treatment the containers with sclerotia were incubated at 24°C to obtain stromata development. Stromata were allowed to grow until they reached their maximum development (approximately four weeks). Sclerotia with fully extended stromata were attached to the interior bottom of an anaerobic culture

dish (95mm x 62mm) one sclerotia per dish, using a contact cement. The sclerotia were inverted over a plate of 4% acidified malt agar. The plate was checked periodically to observe onset of ascospore release. When the sclerotia were observed to be releasing ascospores new plates of 4% acidified malt agar were placed under the sclerotia and changed at intervals of several minutes. Intervals of between 2 and 10 minutes were sufficient to give a sparse deposit of ascospores on the plate. Plates with ascospores were incubated at 24°C for 12 to 18 hours to allow the ascospores to germinate. Single germinated ascospores were removed from the plate with a bent dissecting needle under a sterioscopic microscope set at 100X magnification. The pieces of agar with germinated ascospores were transferred to two day old 4% malt agar plates with dry surfaces and incubated at 24°C. When the ascospore had developed mycelium so as to form a visible colony, mycelial transfers were made to tubes of 4% malt agar. If the germinated ascospores in any way come into contact with water on an agar slant or plate further development of the ascospore mycelium ceases. The germinated ascospore must be transferred to dry agar plates and placed in such a way that the spore is uppermost on the transferred agar piece.

Once the fungus had made sufficient growth on the agar slant following transfer from the plate the tube was sealed with parafilm and stored at 3-4°C.

APPENDIX 2

CULTURE MEDIA USED FOR CLAVICEPS PURPUREA

(A) Modified malt agar:

Ingredients: 10 g maltose
15 g malt extract
40 g agar
1 litre distilled water

Preparation: Melt agar in water, add maltose and malt extract. Pour into bottles, cap and sterilize 15 minutes at 121°C and 15# steam pressure.

(B) Liquid potato sucrose broth for conidia production:

Ingredients: 400 g peeled sliced potato tubers
200 g commercial sucrose
600 ml distilled water

Preparation: Peeled and sliced potato tubers were steamed for one hour in 600 ml of distilled water. The extract is poured off, to 500 ml of the strained extract 200 g of commercial sucrose is added. The broth is dispensed into 500 ml erlenmyer flasks, one hundred and twenty-five ml per flask, stoppered with foam plugs and autoclaved for 15 minutes at 121°C and 15 lb/sq. in. steam pressure.

(C) Production of conidia:

Mycelium and spores from selected agar slants of C. purpurea were transferred to flasks of sterile potato sucrose broth by flooding the slant with sterile distilled water and scraping off the layer of mycelium and spores with a flame sterilized transfer loop.

Inoculated potato sucrose flasks were placed on a gyratory shaker and agitated for 10 to 20 days at a speed of 150 rpm. Following the shaking period, conidia were harvested from the flasks by filtering the extract through a coarse sintered glass filter. The conidia were concentrated by low speed centrifugation. The conidial pellet was then re-suspended in sterile distilled water and re-centrifuged.

The concentrated conidia were then suspended in a 60% sterile sucrose solution and stored at $3^{o}C$ in small screw cap sterile vials.

Not all C. purpurea isolates produced conidia, many isolates from grasses produced mycelium only or mycelium with very few spores.

The isolates from cereal species usually produced abundant conidia in the liquid medium.

APPENDIX 3

(a) Analysis of variance

Six cereal species inoculated with two isolates of C. purpurea and seven inoculum densities:

Source of Variation	D.F.	S.S.	M.S.	F.
Host	5	646	129	106**
Isolate	1	113	113	93**
Treatment	6	13025	2171	1788**
H x I	5	9	1.8	1.49 NS
H x T	30	195	6.5	5.35**
I x T	6	12	1.97	1.63 NS
H x I x T	30	44	1.47	1.21 NS
Error	1246	1513	1.21	
Total	1329	15557		

** Significant at the 1% level

NS Not significant

(b) Analysis of variance

Three selected wheat cultivars inoculated with two isolates of C. purpurea and seven inoculum densities:

Source of Variation	D.F.	S.S.	M.S.	F.
Host	2	824.4	412.2	475.4**
Isolate	1	94.3	94.4	108.8**
Treatment	6	2480.6	413.4	476.9**
H x I	2	5.7	2.8	3.3*
H x T	12	516.7	43.1	49.7 NS
I x T	16	61.9	10.3	11.9 NS
H x I x T	12	31.3	2.6	3.0 NS
Error	658	570.1	0.9	
Total	699	4585		

** Significant at the 1% level
* Significant at the 5% level
NS Not significant

APPENDIX 4

METHOD OF INOCULATION OF RYE SEEDLINGS WITH CLAVICEPS PURPUREA AS UTILIZED BY R. W. LEWIS

Seeds of rye were soaked ten minutes in 1:1000 $HgCl_2$ decanted and soaked overnight in a 5% suspension of 50% wettable Orthocide. The seeds were then decanted and washed three times in sterile distilled water. The surface sterilized seeds were placed embryo side up on wet paper towels in aluminum pans with aluminum covers. The paper towels were previously moistened with distilled water and the pans covered and autoclaved before using. The seeds were germinated at 27 - 28°C for 28 - 32 hrs. Seeds with shoots from 3 - 7mm long were chosen for inoculation. The top half of the rye seedling shoot was cut off with a sterile knife and approximately 40,000 ± 5000 conidia in a sucrose solution (2 g sucrose/3 ml 1:1000 $KH_2 PO_4$) was applied to the cut end of the shoot by touching it to a droplet of the spore suspension at the end of a No. 26 hypodermic needle. The inoculated seedling was placed shoot uppermost on moist filter paper in an incubation pan, twenty-five seedlings per pan.

The inoculated seedlings were incubated for 10 days at 28°C in the aluminum boxes. A seedling was recorded as being infected only if visible evidence of mycelium was present on the inoculated shoot.