THE UNIVERSITY OF MANITOBA

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

by

R. GARY PLATFORD

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

DOCTOR OF PHILOSOPHY

DEPARTMENT OF PLANT SCIENCE

WINNIPEG, MANITOBA

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ABSTRACT

Platford, Robert Gary. Ph.D., The University of Manitoba, October, 1976.

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.

ACKNOWLEDGEMENTS

The author is very appreciative of the assistance given by Dr. C. C. Bernier, during the duration of this study. The author is grateful for Technical Assistance given by Mr. R. Smith, Mrs. J. Tucker, Mrs. K. Wilkinson and Miss C. Rex. Plant materials supplied by Dr. L. E. Evans, Dr. E. N. Larter, and Dr. W. J. R. Boyd were invaluable in carrying out this study. Advice on statistical analysis by Drs. A. Storgaard and D. Wood and Professor J. Menzies was appreciated. The author is very grateful for the continuing encouragement to complete this study offered by his wife Norma and Mr. F. J. Weir, Provincial Horticulturalist, Manitoba Department of Agriculture. Finally the author wishes to thank Mrs. R. Collins who typed this manuscript.

Financial assistance from the University of Manitoba Plant Science Department and Faculty of Graduate Studies is gratefully acknowledged.

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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