AETIOLOGY OF ERGOT DISEASE OF MALE STERILE WHEAT

A Thesis Presented By

Steven Shaw, M.Sc.

In part fulfilment of the requirements for the degree of Doctor of Philosophy in the Faculty of Science of the University of London

April, 1975

From the Department of Biochemistry,
Imperial College of Science and Technology,
London S.W.7.

ABSTRACT.

During the commercial development and seed production of F.1. hybrid wheats in this country, it will be necessary to cultivate large crops of male-sterile, open-flowering wheats in which ear-borne diseases, including ergot (Claviceps purpurea), might proliferate. When cross-pollination was prevented in ears of cms Capitole a high percent of florets retained susceptibility to ergot for 6 - 10 days after the onset of flowering. Thereafter, susceptibility declined until, by 10 - 20 days (depending on the ambient temperature), male-sterile florets rarely supported an infection. Spore-bearing honeydew first exuded from infected florets 7-13 days after inoculation and provided a source of inoculum for secondary infection, the extent of which was influenced by environmental conditions, the amount of seed set, and the stage of decline of susceptibility of unfertilized male-sterile florets.

Five winter, and twelve spring male-sterile wheats, together with two fertile wheats (Kenya Farmer and Carleton - previously reported as being resistant to ergot), were screened for resistance to a strain of wheat ergot collected in England. Although some differences in the degree of varietal susceptibility were measured, a commercially acceptable level of resistance was not encountered.

Experimental cross-infection on wheat of 47 strains of ergot collected from 25 gramineous host species has shown that isolates could be segregated into highly and weakly infective groups. The marked pathogenicity of strains of ergot that occur on some grass species has been correlated with distinctive patterns of alkaloids within the sclerotia.

Analyses of the alkaloid content of 241 samples of ergot, collected throughout Great Britain from 20 gramineous hosts, have demonstrated the

existence of host restricted strains of ergot characterised by their particular spectra of alkaloids. Alkaloid analyses have also provided a means of tracing the aetiology of ergot disease in several localities.

Similarity of the alkaloid spectra of blackgrass and wheat ergots, ease of cross-infection of strains of ergot from blackgrass to wheat and an association between blackgrass infestation and ergot in surveyed wheat crops have confirmed the hypothesis that the presence of this early flowering weed grass in wheat crops increases the risk of high levels of ergot infection.

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INTRODUCTION

1. Ergot - an old disease with new significance

During the 20th century, the development of monoculture and latterly, the trend towards minimal cultivation of arable crops has presented Plant Pathologists with many new disease problems. It seems to be inevitable that whenever man imposes major changes in the micro-environment of his crops, some pathogens find themselves better able to exploit the new situation and thus assume greater prominence. It is not altogether surprising, therefore, that the changes in floral morphology and gametogenesis, associated with the introduction of male-sterility into hitherto self-pollinated wheats, have contributed to a potential resurgence of ergot as an important pathogen of cereals.

Ergot has long been of interest to man, though most prominently in the medical sciences for the contrasting toxic and medicinal properties of the alkaloid components of ergot sclerotia. Alkaloid poisoning from ergot-contaminated bread is reputed to have been the cause of widespread human misery in the form of gangrenous and convulsive ergotism during the middle ages, especially in the rye-growing areas of France and Northern Europe (Barger, 1931) and occasionally in Britain (Henslow, 1841).

Similarly, some of the medicinal properties of ergots are said to have been known to ancient Greeks, Romans, Egyptians and Chinese for possibly 2-3000 years and have been used by European midwives since the 16th century. (Bové, 1970).

Ergot alkaloids are still extensively used in modern medicine, especially during childbirth and for the treatment of migraine (Hofmann, 1961. Bové, 1970). Consequently much of the literature concerning the growth and parasitism of the ergot fungus is based on experimentation designed to investigate ways to improve the production of ergot alkaloids both *in vitro* and *in vivo*.

Geoffroy (1711) and De Candolle (1815) recognised ergot as a fungus growing on the ovaries of cereals, but it was not until the work of Tulasne (1853) that the role and aetiology of the ergot fungus as a plant pathogen was at all understood. Reviews of subsequent investigations of the plant pathology of ergot are given by Weniger (1924), Barger (1931) and Bové (1970). Records of a wide phyllogenetic and geographic distribution of parasitism by *Claviceps* sp. have been collated by Brady (1962).

During the late 19th and early 20th centuries, concern that ergot presented a hazard to animal husbandry led to the expansion of knowledge relating to ergot of grasses; especially of host specificity and the alkaloid content of grass ergots (section 5).

The occurrence of ergot disease of rye is ubiquitous (Brady, 1962), but ergot has seldom been regarded as a serious disease of other temperate cereals. Marshall (1960) reported that, in Britain, samples of commercial oat seed stocks examined by the Official Seed Testing Station (O.S.T.S.) from 1918 to 1957 contained no oat-ergots. Elsewhere, however, there have been occasional reports of ergot-infected oats, particularly during wet summers (Warburton, 1911; Ducellier, 1922). Marshall (1962) also examined O.S.T.S. records from 1926 to 1957 and found that the frequency of contamination of samples of barley and wheat by ergot was generally less than 1% and 2%, respectively. More recent records of the purity of seed samples received at the O.S.T.S. during the period 1967 to 1972 (Hewett, Personal Communication) show that a small percent of samples contained either grass or cereal ergot sclerotia. 0.9% and 1.8% of barley and wheat samples were contaminated respectively. However, it should be noted that these observations were based on samples of seed after they had passed through seed cleaning processes. Increased efficiency of extraction of ergots from seed over the past decade may, therefore, have masked any major increase in the incidence of the disease. Rapilly (1966) reported that between 1961 and 1966 ergot of wheat became an increasingly common disease in France.

Many workers have expressed the view that the frequency of occurence of ergot disease is greatest when periods of wet weather occur during the flowering period of the host (Vladmirsky, 1939; Stoll, 1943; Hersheno and Plant, 1947; Marshall, 1962; Rapilly, 1968). In Britain, where wet, warm summers are not uncommon, it is, therefore, perhaps surprising that ergot has so far failed to become established as a very important pathogen of wheat or barley. One explanation may be that self-pollination, in association with the short period of open flowering of modern, commercial wheats and some barleys, imparts some degree of field resistance to infection of florets at anthesis. However, commercial Research and Development of F.1. hybrid cereals requires the use of male-sterile cultivars which rely on cross-pollination for fertilization. It may, therefore, be expected that, as male-sterile florets gape for some considerable time to receive pollen, they will be at increased risk of infection by spores of Claviceps purpurea (Done & Macer, 1972). In 1970, British Hybrid Cereals Ltd., whilst stating their intent to develop F.1 hybrid wheats and barleys for British conditions, acknowledged that ear-borne diseases, including ergot, might proliferate in large crops of male sterile plants, and planned to support research into ways of minimising this disease problem. The present study stems from this, and has, to some degree, been integrated with the F.1 hybrid wheat breeding programme of the above company.

2. F.1 Hybrid Cereals

The current development of first generation (F.1) hybrid wheat was stimulated by the marked success, in cereals, of hybrid maize and hybrid sorghum (Purseglove, 1972), particularly in the United States where hybrid seed is today used to sow virtually the entire acreage of these two crops. An account of the techniques, problems, advantages and successes encountered in the development of F.1 hybrid wheat is given by Johnson and Schmidt (1968), whilst early investigations of hybrid vigour (heterosis) have been reviewed by Briggle (1963). Curtis and Johnson (1969) provided a lucid account of hybrid wheat.

2.1 The object and principle of F.1 hybrid wheat breeding

It is well known that crossing two dissimilar plant varieties gives rise to first generation (F.1) plants which commonly have, by comparison with either of the parent lines, increased vigour (East and Hayes, 1912; Shull, 1948). This hybrid vigour, or heterosis, is expressed in many biochemical, physiologic and morphologic ways (Gowan, 1952; Briggle, 1963; Walton, 1971), some of which contribute to increased yield of the F.1 generation of plants. In a conventional breeding system, the first stage in the production of a new, inbreeding wheat variety involves emasculation and cross-pollination, by hand, of wheat ears of two parent varieties to obtain a small quantity of "hybrid" seed. This initial intervarietal cross is then followed by a

programme of selfing and selection through more than 10 generations before a pure-breeding line is suitable for multiplication and subsequent commercial use. Thus, the yield advantage of hybrid vigour, which declines markedly after the first filial generation (Shull, 1948; Hayes, 1969), may not be exploited. The object of F.1 hybrid wheat breeding is to produce first generation hybrid seed in large enough quantity to enable F.1 hybrid wheats to be grown as commercial crops.

Among 23 reports of heterosis in wheat surveyed by Briggle (1963) increases in yield of F.1 wheat plants over their best parent ranged from 0 to over 100%. However, in view of the use of experimental designs incorporating widely spaced planting of non-commercially productive varieties, Johnson & Schmidt (1968) regarded these studies as being of limited application. In their own survey of subsequent reports of heterosis in F.1 hybrid winter wheats, Johnson & Schmidt (1968) concluded that although heterosis for yield of limited space-planted seedlings of hand-crossed plants had been demonstrated, it was still not clear whether this would be equally demonstrable on a commercial scale.

Wells and Lay (1970) and Walton (1971), whilst stressing the importance of the combining ability of the parent lines, have reported that some F.1 hybrid spring wheats also produced substantially greater yields than their higher yielding parent .

Despite the continued world-wide development of F.1 hybrid cereals and, in particular, their recent commercial usage in the United States, well documented evidence of yield benefits resulting from the introduction of F.1 hybrid wheat cultivars into non-experimental, large-scale arable situations is not yet available.

In addition to yield advantages which may accrue from the introduction of F.1 hybrid cereals, it has also been suggested that this system of breeding cereals would alleviate some of the current problems of disease control (Done, A.C., 1973). As mentioned above, new, conventional wheat cultivars need to pass through a period of multiplication before they are ready for commercial use. During this time, a resistant variety is at risk of infection by new, virulent physiologic races of pathogens. Thus, resistance of a new cultivar to a pathogen may be broken down even before it is available on a commercial scale. In an F.1 breeding system, where there is no multiplication phase after the hybrid cross, this problem would not exist. Furthermore, it may be possible to introduce a system for the planned cycling of resistance gene combinations to hinder the build-up of physiologic races of foliar pathogens.

2.2 The F.1 hybrid wheat breeding system

To produce F.1 seed in bulk it is necessary to engineer a field situation in which only cross-pollination between two

parent wheat cultivars can occur. Since conventional wheats have hermaphrodite florets, within which self pollination generally takes place before the anthers are exserted (Peterson, 1965), the first requirement is to separate and maintain male-sterile parents (female) and pollen donating parents (male) - see below. Having achieved this, a "crossing block" system is employed in which the male-sterile and pollen donating cultivars are sown in alternate strips and harvested separately. Assuming that the female parent is fully male-sterile, seed set in the male-sterile strips must result from cross-pollination by the adjacent male parent, and is thus first generation (F.1) hybrid seed.

A number of heritable sources of male sterility have been employed in the production of male-sterile cereal cultivars (Johnson and Schmidt, 1968). In addition, male sterility can be induced by the application of chemical gametocides (Bennett and Hughes, 1972; Stoskopf and Law, 1972). For the current development of F.1 hybrid wheats in the United Kingdom, only one source of male-sterility is in general use. This is inherited maternally from the interaction of Triticum timopheevi Zhuk. cytoplasm with the nucleus of Triticum aestivum L.emend. Thell. subsp. vulgare Mackay (Wilson and Ross, 1962). Cytoplasmic male sterility is introduced into a normal fertile parent by a) a cross

between a parent bearing a source of male sterility (as above) and the fertile parent, and b) maintenance and multiplication of the segregated cytoplasmic male-sterile progeny. The latter being achieved by recurrent back crossing with the fertile parent until the final progeny is identical to the fertile parent in most characteristics except for the retention of male sterility. In the following account, cytoplasmic male-sterile cultivars are distinguished from fertile cultivars by the prefix cms.

To produce fully restored F.1 plants, the pollen donor parent used in a cross block must possess the ability to overcome the effect of cytoplasmic male-sterility. Sources of dominant genes for restoration of fertility of T.timopheevi - derived male sterile wheats were first discovered in the early 1960's (Wilson and Ross, 1961; Schmidt et al, 1962). Since then, many sources of genetic restoration have been found (approximately 1% of wheat varieties may have good restoring qualities - D.W.Joyce, personal communication) and, in addition, genes for restoration offertility have been introduced into a large selection of suitable pollen parents. In selecting the best restorer parent to pollinate any particular male-sterile line, many factors, including suitable earliness of anthesis in relation to flowering of the cms parent, combining ability and disease resistance have to be considered (Done, A.C., 1973).

3. The problem of ergot in F.1 hybrid cereals

Suneson and Houston (1942), having studied floral infection of male-sterile barley by loose smut (Ustilago nuda) and by barley leaf stripe (Pyrenophora graminea), concluded that if male-sterile cultivars were grown in the field, there would probably be an increased incidence of diseases which attack floral parts. Subsequent experience of field cultivation of male-sterile cereals has supported this view, though it has been ergot disease that has presented the major problem. In Mexico, the introduction of hybrid maize was followed, in 1964, by the first record of maize ergot (Claviceps gigantea Fuentes.) in that country (Fuentes et al, 1964). Fucikovsky and Moreno (1971) surveyed the incidence of this disease in two maize cultivars and found that 46-52% of corn cobs were infected. Similarly, the introduction of hybrid Sorghum was accompanied by increased incidence of ergot, particularly where male-sterile lines had been inefficiently pollinated. (Futtrell & Webster, 1965; Chinnadurai and Govindaswamy, 1970). Partial sterility and a degree of open floweredness of the inter-generic wheat-rye cross, Triticale, has also been held responsible for the high susceptibility to ergot (Hulse, 1974), especially in Hungary where this is regarded as a serious drawback to the use of Triticale as a fodder crop (Kiss, 1974).

Whilst accepting that the open-flowering characteristic of cms cereal florets may expose the floral parts to infection by

ergot, Done (1973) was of the opinion that this open-flowering characteristic itself does not necessarily predispose a cultivar to high levels of infection. Done observed that when ms barley cultivars were grown in field plots in Lincolnshire, and were efficiently pollinated shortly after flowering, only low levels of ergot-infection were observed. In the United States, Puranik and Mathre (1971) conducted field experiments to determine whether or not fertilization of an ovary gives rise to some degree of resistance to ergot infection. Florets of ms barley were inoculated with ergot at various times after they had been hand pollinated. Results demonstrated that although fertilized ovaries remained very susceptible for two days after fertilization, susceptibility rapidly declined during the 3rd and 5th days until no ergot-infection was recorded after 9 days. This was in contrast to unfertilized ms barley florets which retained a high degree of susceptibility for 10 days after flowering.

Firm reports of natural infection of hybrid wheat and barley by ergot have so far been limited to accounts of infection of male-sterile plants in small-scale crossing blocks (Lindquist and Carranza, 1960; Johnson and Schmidt, 1968; Hayes, 1969; Puranik and Mathré, 1971). Reitz (1967) reported that, in some male sterile plots, ergot sclerotia constituted as much as 65% of the harvest. More recently, Atkins (personal communication)

recorded 10-15% infection of the cms winter wheat, Shawnee, at McGregor, Texas where he had not previously observed ergot disease in wheat crops during 43 years of crop inspection.

If, as the above reports of ergot in hybrid cereals would suggest, there is a high risk that ergot disease may be encountered wherever male sterility is employed in the field, the F.1 hybrid wheat breeding programme could be confronted by a number of ergot-associated problems, some of which could seriously limit its potential success. These are briefly listed below:-

(a) Severe ergot infection may effectively reduce seed set both at the male-sterile multiplication stage, and in F.1 hybrid seed crossing blocks;

(b) Ergot contamination of grain required for seed may:

(i) increase seed cleaning costs;

(ii) create a problem of disposal of ergot-contaminated screenings which might be sold as grain for animal feed;

(iii) prevent seed merchants from cleaning batches of heavily contaminated seed to the proposed E.E.C. and higher voluntary cleaned seed standards (see p 27);

(iv) if not successfully removed from batches of seed, lead to compensatory claims against seed merchants either for the introduction of ergots into hitherto ergot-free land, or for possible toxic effects on livestock.

(c) Partially ergot-infected seed may be difficult to separate from normal seed.

In addition, the possibility of ergot infection of the crop grown from F.1 hybrid seed must be considered. Complete restoration of fertility of the F.2 crop may greatly reduce the risk of high levels of infection (p.165). However, despite a recent report (Done et al, 1974) that, in Britain, fertility restoration in excess of 95% can regularly be obtained, it is still not clear whether absolute restoration can ever be achieved commercially.

4. The ergot fungus

4.1 Nomenclature

All forms of the ergot fungus used in this study have been regarded as Claviceps purpurea (Fr) Tul. However, it must be emphasised that this identity has, in most instances, been assigned to sclerotial accessions and isolates without examination of the sexual stage. In the literature there is some confusion regarding the existence of another Claviceps species, Claviceps microcephala (Wallr.) Tul., which is said to parasitise some of the grass hosts, notably Poa annua and Phragmites communis, that have been included in this study (Barger, 1931; Kreba, 1936; Grasso, 1952; Kawatani, 1952-53). Other, more recent researchers both in Britain (Mantle, 1969) and elsewhere (Petch, 1937; Langdon, 1949; Mastenbrock and Oort, 1941) have been unable to find any morphological character to support this distinction.

4.2 Sclerotial dormancy, vernalization and germination

During the winter months ergot sclerotia, which have fallen from infected grasses and cereals at the end of the previous summer, lie dormant on, or in, the soil. To germinate in the spring, sclerotia need to have been subjected to temperatures close to zero for at least 30-40 days (Kirchoff, 1929), but freezing is not necessary (Petch, 1937). More recently, laboratory

investigations of ergots from Phalaris arundinacea have shown that, in general, the longer and cooler the chilling period (up to an optimum of 8 weeks) the higher is the final % germination; but storage of ergots for six weeks at 0°C, or below, reduced germination. (Mitchell, 1967; Cooke and Mitchell, 1970).

Temperature requirements during the actual period of germination have consistently been reported as being within the range 9-11°C (Minimum) to 18-22°C (Optimum) (Kirchoff, 1929; Krebs, 1936; Vladmirsky, 1939; Mitchell, 1967; Rapilly, 1968), but following germination Krebs found that perithecial development was favoured by higher temperatures. A more critical factor limiting germination is relative humidity. When germinating wheat ergots were investigated, the appearance of perithecial heads (capitula) and elongation of stipes only occurred at 90-100% R.H. (Rapilly, 1968).

Krebs (1936) reported that after 1-3 months storage of ergots at -1°C, germination was more successful in field soil (80-90% germination) than in sand (5-30% germination). In the field, fruiting bodies from wheat ergots buried at a depth greater than 4 cms, did not reach the soil surface (Rapilly, 1968).

Extensive morphological studies of germination of ergots (Grasso, 1952) revealed that the formation of perithecial fruiting bodies was initiated in the outer cortical regions of sclerotia. However, other workers have claimed to have observed stroma initials

growing out from small, longitudinal splits in the cortex (Petch, 1937; Jenkinson, 1958).

The capacity for repeated stroma formation by sclerotia collected from Phalaris arundinacea was investigated by Cooke and Mitchell (1967), In the laboratory, new stroma were formed when clavae had been removed up to 56 days after germination had commenced. However, it was assumed that undisturbed sclerotia exhausted their food reserves during limited stroma production and ascospore discharge. Observing sclerotia in the field, earlier workers (McFarland, 1922; Vladmirsky, 1932) had concluded that after vernalization requirements had been satisfied during the winter, ergots which failed to germinate in the spring or summer would loose their viability before the spring of the following year.

Jenkinson (1958) observed the field germination of ryegrass ergots at Newton Abbot, Devon, where, in 1955-57, stroma (capitula) emerged above the soil surface from early June to mid July. A period of about seven days normally elapsed between the first appearance of stroma and the development of mature ascospores, and ascospore discharge then occurred at intervals for about 14 days. Observations of wheat ergots in the Versailles region of France indicated that ascospore discharge commenced towards mid April and was complete by the end of May (Rapilly, 1969).

4.3 The infection process

Engelke (1902) stated that spores (either ascospores or conidia), having been deposited on the stigmatic surface of a suceptible graminaceous host, germinate, penetrate the stigma and grow down the style and ovary wall to the base of the ovule, in a way similar to pollen tubes. This mode of entry of the fungus was disputed by Kirchoff (1929) who claimed that after spores have germinated on a stigma, hyphae grow down over the surface of the ovary to the base, where penetration occurs. Basal penetration was later confirmed by Ramstad and Gjerstad (1955)and Campbell (1958). The latter investigated the histopathology of ergot-infected barley. Following penetration, which occurred within 24 hours after inoculation, intercellular hyphae grew generally upwards through the ovary wall to surround the ovule. By 4-5 days after inoculation, hyphae were growing intracellularly, had penetrated the ovule, and began to errupt on the surface of the ovary to produce conidia (sphacelial stage). Downward growth of hyphae from the infection court was limited, and Campbell noted a definite line of demarcation between colonized and uncolonized host cells in the receptacle.

In most general accounts of the life cycle of ergot (Barger, 1931; Campbell, 1958; Rapilly, 1968; Bové, 1970), it is stated that the first outward signs of infection, inthe form of droplets

of a sweet, conidiospore-bearing "honeydew" exudate, appears on infected florets 1-2 weeks after infection. Léveillé (1827) described honeydew as a fluid containing seeds (spores) of the fungus Sphacelia segetum and, although conidia in honeydew were subsequently identified as being produced by the imperfect state of C.purpurea (Tulasne, 1853), the term sphacelial stage has been retained in general use.

The morphological changes which occur when sclerotial tissue develops from the sphacelial stage were initially described by Tulasne (1853). At the base of a sphacelium, hyphae became more numerous and interwoven, and, at the same time, show increased thickness and septation. Eventually a dense, pseudoparenchymous, mass of hyphae - a sclerotium - is formed, whilst at the distal end of the developing sclerotium, production of conidia on the surface of the remains of the sphacelial stage ceases. During an investigation of ergot infection of rye, Ramstad and Gjerstad (1955) noted that the remains of the ovary and style were often found at the tip of an ergot sclerotium. Thus, it would appear that, as a result of expansion of the sclerotial tissue, the ovary becomes detached from the receptacle. Furthermore, since Ramstad and Gjerstad observed no anatomical connection between the sclerotial tissue and the receptacle, they concluded that the sclerotial stage of the fungus obtains its nutrients from host sap (the fluid portion of honeydew) which exudes from the unhealed scar of detachment of the ovary. As the sclerotium develops to full size it may not only replace the whole of the

grain, but may also protrude from the glumes forming a long, hard, deep purplish or brown structure. Investigation of the anatomy and biochemistry of developing ergot sclerotia have shown that the youngest sclerotial tissue is at the base, near to the point of contact with the host (Nisbet, 1975).

4.4 Vectors and alternative hosts

The sweet, sticky nature of spore-bearing honeydew is attractive to insects which, after feeding, may convey spores to previously uninfected plants (Atanasoff, 1920; Weniger, 1924; Neill, 1941; Ingold, 1971; Mongolkiti et al, 1969). However, Noble (1936), commenting on the dissemination of Claviceps paspali on Paspalum grass in Australia, expressed the view that rain and wind were more important than insects as agents for dissemination of honeydew. Langdon and Champs (1954) demonstrated that the fly Pyrellia caerulea is an important insect vector of conidiospores of C.paspali; after feeding on honeydew, viable spores were found to be deposited in the fly's faeces. In the United States, Monolkiti et al (1969) observed that moths - in particular the Cabbage Looper Moth (Moreno et al, 1970) - were closely associated with the presence of ergot disease in hybrid barley.

Claviceps purpurea has a wide gramineous host range (Brady, 1962) and, although subject to some conflicting evidence (Section 5.1), is generally thought not to exhibit marked host specificity.

Weed grasses have, therefore, frequently been regarded as constituting important reservoirs of ergot inoculum for the infection of cereal crops (Campbell, 1957; Minz et al, 1960; Anon, 1967). Regretably, however, the role of individual grass species in the disease cycle of ergot of cereals has not been investigated in depth. In England, Batts (1956) conducted preliminary cross-inoculation experiments which showed that the fungus giving rise to ergots on blackgrass (Alopecurus myosuroides) could readily infect wheat. The implications of this observation have not, however, been studied further, except that Thurston (1966) observed that 18 out of 129 samples of blackgrass collected throughout England and Wales during 1966 were infected with ergot. Ergot was also found on blackgrass within beans grown as a break crop between successive cereal crops (Thurston, 1970). Commenting on the above observations, Moore and Thurston (1971) stated that, on the assumption that the fungus is the same strain as attacks wheat, Alopecurus can be a source of inoculum within cereal crops, or can perpetuate the disease in the absence of a cereal host.

5. Investigations of host specificity

5.1 Cross-infection.

The extensive work, in Switzerland, by Stäger (1903, 1904, 1905, 1907, 1908 and 1910) was, for many years, the only comprehensive source of information regarding host specialization of the ergot fungus. This work has been summarised by Weniger (1924). Using a strain of the ergot fungus isolated from typical rye ergot, Stäger successfully infected many cereal and grass hosts including barley, Alopecurus pratensis, Anthoxanthum odoratum, Arrhenatherum elatius, Dactylis glomerata, Festuca pratensis, Phalaris arundinacea, and some Poa species. Later (Stäger, 1923), isolates from rye were successfully used to inculate wheat plants. However, infection was not achieved using the rye strain on some other grasses including Bromus erectus, Glyceria fluitans, Lolium spp, Nardus stricta and Poa annua. A second biologic race*, which occurred on Brachypodium sylvaticum was found to infect Milium effusum, Poa pratensis and P. trivialis; whilst the third, collected from Lolium perenne, was able to infect other Lolium species and Bromus ereotus. Stäger did not secure infection using the third strain on rye, wheat, barley, Alopecurus pratensis, Arrhenatherum elatius, Brachypodium sylvaticum or Poa pratensis. On the basis of these results, three "biological races" of Claviceps purpurea, each with its own distinctive host range, as described above, were proposed. Stäger also noted the possibility of 4th and 5th races from Glyceria sp. and Anthoxanthum odoratum.

*Stäger's usage of this term is not synonymous with the currently accepted meaning of the term "physiologic race" as defined by a committee of the Federation of British Plant Pathologists (Anon, 1973).

This distinction between 'races' was not, however, confirmed by subsequent researchers using similar cross-infection techniques. Brown (1947) successfully used a single strain of C.purpurea from L.perenne to infect wheat, barley, oats, rye, Bromus inermis, Agropyron spp, Elymus spp and Poa pratensis. Similarly, Békésy (1956) obtained infection of both rye with ergot collected from L.perenne, and L.perenne with an isolate from rye-ergot.

During the following year, Campbell (1957) reported that rye, wheat and barley had been successfully inoculated with ergot from 38 different gramineous host species. Campbell also successfully inoculated 46 gramineous host species with ergot from rye, both in the field and in the glasshouse. Commenting on Stäger's results, Campbell suggested that failure to obtain infection may, in some instances, have been due to unsuitable and/or impersistent inoculation techniques: some of Stäger's conclusions, including the postulation of the existence of "biological races", were based on negative results obtained from unreplicated tests. Minz et al (1960) demonstrated the cross-infectability of strains of ergot between wheat and some wild grasses indigenous to Israel.

5.2 Comparison of the qualitative alkaloid content of ergot sclerotia

Ergot sclerotia have been found to contain a wide range of ergot alkaloids (Bové, 1971; Hofmann, 1972). Most commonly the

major alkaloid components of C.purpurea are one or more of the lysergic acid derivatives: ergometrine, ergotamine, ergosine and ergotoxine (a term which encompasses three alkaloids: ergocornine, ergocristine and ergokryptine). Isomeric forms of these alkaloids (ergometrinine, ergotaminine, ergosinine and ergotinine, respectively) may also be present. In addition, trace-amounts of lysergic acid, lysergic amide and a number of clavine alkaloids can commonly be extracted from ergot sclerotia.

The discovery of the first-known clavine alkaloid, agroclavine, in surface culture of an isolate of C.purpurea collected from couchgrass, Agropyron repens, stimulated the investigation, by many workers, of the alkaloid content of ergots from the whole range of gramineous hosts. Rochelmeyer (1949), Silber and Bischoff (1955) and Meinicke (1956) determined the alkaloid content of ergots collected from wild grasses and noted considerable variation in the range of alkaloids encountered, even within collections of ergots from a single host species. Meinicke (1956) also determined the alkaloid content of sclerotia resulting from the cross-infection of one host, rye, with sclerotial isolates of C.purpurea collected from a number of different wild grasses. The qualitative spectrum of alkaloids found in these sclerotia (on rye) was variable, but generally appeared to have been influenced by the fungal strain rather than by the host plant. Similarly the sclerotial alkaloid spectrum of a single rye-strain of ergot

showed little change, except in total alkaloid content, when passaged through 29 different grass species and back again to rye plants.

In the United States, Riggs et al (1967) cross-infected rye, Festuca arundinacea, and Lolium multiflorum/Festuca hybrids, with naturally occurring strains of ergot obtained from the above grasses and also from Agropyron repens. No convincing evidence of host specificity, or of host influence on the production of alkaloids, was obtained.

Bojor (1968) studied the alkaloid content of ergots collected from wild grasses in Rumania. Although his results indicated that, in terms of alkaloid production, there was much diversity among the species C.purpurea, Bojor concluded that further investigation of the natural alkaloid content of sclerotia from different grass hosts could give valuable information relating to the possible existence of "alkaloid races" of the pathogen. Kybal and Brejcha (1955) used the quantative alkaloid content of ergot sclerotia to classify strains of C.purpurea into three main biochemical races: namely those producing ergotamine, ergocornine-ergocristine and ergocornine-ergocristine-ergokryptine. However, this categorisation of the main alkaloid types was not linked with host specificity of the fungus, and thus does not appear to have been intended to support the possible existence of host restricted strains of the species. Indeed, in

a subsequent report of the alkaloid content of ergot sclerotia, Kleinerova and Kybal (1969) divided strains of two different species, C.purpurea and C.paspali, into three groups on the basis of their ability to biosynthesise alkaloids, i.e. into producers of peptide alkaloids, ergometrine and lysergic amides.

5.3 Measurement of conidia

In continuance of his evaluation of the use of the honeydew state in the identification of ergot species (Loveless, 1964), Loveless (1971) investigated the dimensions of conidia in 101 samples of honeydew collected from 31 host species of British grasses. Samples showed considerable variation in size and shape of conidia, but, after applying Bartletts test for homogeneity of variance, it was claimed that independent samples collected from different specimens of the same host could reasonably have come from the same population. Working on this assumption, Loveless demonstrated that conidiospore populations from different hosts significantly differed in both size and shape. Furthermore, Loveless showed that the most rational method of grouping the hosts of these apparently host-restricted conidial forms of C.purpurea was as follows:-

1. Lolium perenne, Arrhenatherum elatius, Secale cereale, Ammophila arenaria, Festuca arundinacea, Triticum aestivum, Agropyron pungens, A.repens, Dactylis glomerata and Holcus lanatus,
2. Alopecurus pratensis and Molinia coerulea.

3. Deschampsia caespitosa, Phalaris arundinacea, Agrostis canina, Phleum pratense.

4. Nardus stricta and Spartina townsendii.

In addition, Phragmites communis, Anthoxanthum odoratum and Glyceria fluitans may each be hosts to separate groups of conidial forms.

6. Ergot in grain

Harvested cereals may be utilized as food or as seed to sow subsequent cereal crops. In either case, ergot contamination of grain samples is only tolerated at low levels. The Cereal Seeds Regulations, 1974 (Anon., 1974) state that, to comply with minimum sample purity standards for wheat and barley, 500g. random samples should contain not more than one and three pieces of ergot in basic and F.1-2 generation seed, respectively. Similarly, to comply with the Higher Voluntary Seed Standard, not more than one piece of ergot should be found in a 2kg sample of wheat for any generation of seed.

Ergot mixed with wheat grain destined for the production of flour, or animal feed, represents a possible toxic hazard. Dixon (1932) noted that after an epidemic of ergotism in the U.S.S.R. in 1926, a limit of 0.15% by wt of ergot became the maximum permissible contamination of flour. In Germany, at this time, the limit was set at 0.1%. More recently, the standard for ergot in milling wheat in Japan has been quoted as 0.04% (Anon., 1972); whilst in the United States, wheat may contain up to 0.3% ergot before it is officially described as "ergoty wheat" (Anon., 1964).

Following experimental milling tests of ergot-contaminated grain passing through flour mills in the U.S.A., Amos (1973)

noted that although pre-milling ergot/grain separation methods based on size differences were not successful, the use of a (Kipp Kelly) Specific Gravity Separator led to the segregation of over 95% of the ergots present in wheat. Furthermore, when the grain was passed into the milling stream, only 7 to 20 percent of the remaining ergot contaminants were separated into the flour making fraction after the first break. On this basis, Amos calculated that, when considering the production of flour for human consumption, the maximum permissible percent of ergot going into the first break should be 0.05%.

In addition to the problems of contamination of seed by ergot sclerotia, the ergot fungus has been shown to be responsible for some degree of seed spoilage. Whilst investigating ergot of rye, Seymour and McFarland (1921) found that, as a result of parasitism, numerous "blasted kernels" (ergot-infected grain that had not developed into a sclerotium) were formed. Partially ergot-infected barley seed, having a water soaked appearance, was also noted by Cambell (1958).

7. Control of ergot in cereals

7.1 Control by exclusion and sanitation

Exclusion of ergots from seed prior to sowing a crop would, of course, effectively remove one possible source of inoculum. In the preceding section it was noted that efficient separation of ergots from seed may be achieved by mechanical

means, especially by machinery designed to segregate seed fractions on the basis of specific gravity. The slightly lower specific gravity of ergot, as compared with seed, was also utilized by Jaczewsky (1904) in devising a flotation method for the removal of ergot from small quantities of seed. Grain was pre-soaked for 3 hours in water and then transferred to brine (40lbs salt: 25 gallons of water) in which ergots floated.

In order to exclude susceptible F.1 Hybrid seed crops from ergot-infected land, Puranik and Mathre (1971) suggested that seed crops could be grown in areas where environmental conditions were restrictive to the pathogen; although it was acknowledged that the subsequent cost of transport of seed to normal growing areas may be prohibitive. Done (1973) suggested that it may be advantageous to move F.1 hybrid production sites from year to year to avoid the excessive build-up of inoculum of the ergot fungus.

For control of ergot by sanitation it has been recommended (Weniger, 1924: Anon, 1967) that stubble should be deep ploughed following an epidemic of ergot. Furthermore, in view of the possibility that ergot disease of cereals may have a reservoir of inoculum in wild grasses (section 5.1) it has commonly been recommended that sanitary measures be taken

against grass ergots in headlands and in arable weed grasses (Kuhn, 1863; Weniger, 1924; Campbell and Friesen, 1954; Anon, 1967). Campbell and Friesen successfully controlled ergot in headlands by spraying grasses with maleic hydrazide to suppress heading.

7.2 Chemical Control

Puranik and Mathre (1971) suggested that although protective fungicides have generally been ineffective in controlling ergot, the use of a systemic fungicide that is translocated to the site of infection at the base of an ovary (p.17) could offer some hope of control. When the systemic fungicide benomyl was applied to seed beds at the rate of 210 lbs/acre, no control of ergot was observed. On the other hand, some control of ergot in male-sterile barley was obtained when the heads were sprayed with benomyl at the rate of 1-2lbs/acre in 100 gallons of water. Three applications over a period of 18 days at about the flowering time decreased the infection of florets by 56%. However, further tests showed that the maximum fungicidal effect of benomyl had not been achieved since there appeared to be only limited penetration of the fungicide to the ovary tissues.

Hardison, (1972) evaluated the effect of 27 systemic (including benomyl) and 9 protectant fungicides on germination of ergots from Lolium perenne. Although some suppression of ascocarp formation was obtained in two trials using benomyl,

a third test, in which ergot sclerotia with an apparently greater capacity for repeated ascocarp production were used, adequate suppression by benomyl was not obtained. Of the remaining fungicides tested, only cadmium chloride had the combined attributes of adequate suppression of ascospores and relatively low cost.

Following an attempt to control ergot by spraying the insecticide Diazinon against insect vectors of the disease, Puranik and Mathre (1971) concluded that although some reduction in the rate of infection did occur, this method of control did not seem to be worthwhile; especially considering that insect control over a wide area would have to be accomplished.

7.3 Control by the use of resistant varieties

Although some field observations had indicated that certain wheat cultivars may be more susceptible to ergot than others (Weniger, 1924: Willis, 1953) experimental evidence of resistance to ergot had not been reported prior to 1970. On the basis of a few (not statistically analysed) results of inoculations of the wheat cultivars Manitou, Stewart 63, Kenya Farmer and Carleton with two isolates of C.purpurea., Platford and Bernier (1970) claimed that cultivars Kenya Farmer and Carleton were more resistant to ergot than the other two varieties. Resistance was expressed as a decrease

in the number and size of sclerotia formed, and as a reduction of the amount of honeydew produced. In a subsequent interim report of an investigation of the inheritance of the resistance of Kenya Farmer and Carleton, Platford et al (1971) described preliminary results which suggested that these cultivars carried different (but probably not more than two) genes for resistance. However, most recent publications from this research group (Ratnopas and Bernier, 1972) indicate that the importance of Kenya Farmer and Carleton as sources of resistance may be in dispute. Following inoculations of these cultivars with 59 different isolates of C.purpurea, Ratnopas and Bernier reported that in view of the high virulence of 11 of the isolates to cv Carleton, this variety could no longer be considered resistant to the disease. Furthermore, Kenya Farmer was only rated as being moderately resistant, though its resistance was still regarded as "acceptable".

Cunfer et al (1974) investigated the diversity of reaction to ergot among male-sterile barleys. The susceptibility to ergot of 86 two-rowed and 55 six-rowed male-sterile barleys was tested both by artificial inoculation and by natural infection. Although most cultivars were susceptible, various lines of the cvs Betzes, Compana, Atlas x Kindred, C.I.4894 and Midwest showed low susceptibility. However, it was emphasised that partial fertility may have been responsible for reducing the susceptibility of some of the above lines.

8. Abbreviations and Definitions

8.1 Definitions

The following definitions were prepared solely for use in the present studies and are not intended to accurately define the meanings of the terms sphacelium, sclerotium and germination as used in other contexts.

Since the visual appearance of both the sphacelial and the sclerotial stages of ergot-infection were somewhat variable, the following definitions were used as guidelines when recording the results of experimental infection and when observing the disease in the field:

Sphacelium: The first stage of ergot-infection resulting in the colonization of at least half of the ovary tissue by soft, white mycelium, producing many conidia.

Sclerotium (Ergot): The second stage of ergot-infection characterised by the presence of compact, purplish mycelium and attaining a size at least twice that of a normal, unfertilized ovary.

The very early stages of formation of a fruiting body on a germinating ergot are often obscured by the overlying rind of the sclerotium (Figure 13). Using the following definition, ergots were not regarded as having germinated until fruiting bodies were well developed and thus more easily observed.

Germinated ergot sclerotium: A sclerotium bearing at least one fruiting body in which a clearly defined stipe was present between the sclerotial body and the capitulum.

8.2 Abbreviations

Acc. No.	Accession number of ergot sample or isolate (See table 2).
A.S.C.	Alkaloid Spectrum Code.
C.M.F.	Chequers Manor Farm.
cms.	Cytoplasmic male-sterile.
C.P.G.	Chelsea Physic Garden.
C.V.	Cultivar.
d.f.	Degrees of freedom. (statistics)
L.E.F.	Lane End Farm.
L.R.R.C.	Lord Rank Research Centre.
L.S.D.	Least significant difference. (statistics)
P.D.A.B.	Para dimethyl amino benzaldehyde.
R.H.M.	Ranks Hovis McDougall (Research) Ltd.
T.L.C.	Thin layer chromatography.
U/V	Ultra-violet.
X	Unidentified alkaloid. (see p.135)

MATERIALS and METHODS

9. The Pathogen

9.1 Isolation from sclerotia and maintenance of cultures

After the removal of all sphacelial remains and adhering host tissue, air-dried sclerotia were rinsed in a wetting agent, Tween 80, surface sterilized in mercuric chloride solution (0.1%) for 2-5 minutes and washed five times in sterile distilled water. Sclerotia were then aseptically cut into fragments and incubated at 24°C on medium "T" agar (see below) to which Aureomycin (0.25% w/v) had been added. After two weeks, pure isolates were obtained from the mycelium growing out from the sclerotial fragments, transferred to standard medium "T" slopes and maintained at 24°C.

9.2 Culture medium (medium "T")

The standard culture medium was modified from Stoll et al (1957) by Castagnoli and Mantle (1966). The formulation of one litre of medium was as follows (gms/l):
$Ca(NO_3)_2 . 4H_2O$, 1.0; $Mg\ SO_4 . 7H_2O$, 0.25; KH_2PO_4, 0.25; KCl, 0.125; $Fe\ SO_4 . 7H_2O$, 0.033; $Zn\ SO_4 . 7H_2O$, 0.027; Sucrose, 100; L.Asparagine, 10; Cysteine hydrochloride, 0.01; yeast extract, 0.1; Oxoid No.3 agar, 15; and distilled water to volume. The pH was adjusted to 5.2 with sodium hydroxide and the medium was sterilized for 20 minutes at 5 p.s.i.

9.3 Preparation and storage of spore suspensions

With the exception of a few isolates used in early cross-infection experiments, all inoculum for field and glass-house experiments was prepared from fresh honeydew obtained *in vivo* from host plants, which had initially been inoculated with an aqueous suspension of spores from the surface of cultures of the sclerotial isolates.

Honeydew, collected from the infected plants, was diluted with a sterile solution of 10% glycerol in distilled water, to which a drop of Tween 80 had been added, and the spore concentration of a sample of the resultant suspension was measured by means of a haemocytometer. It was found essential to add Tween 80 to ensure that all conidia settled on the grid of the counting chamber. This additive also aided the re-suspension of spores after storage.

Three methods of storing honeydew conidia were evaluated. Two of these, freeze drying and storage in sucrose were discontinued after the initial investigation.

Lyophilization of conidia was achieved using a multiport, continuous freeze dryer. Since the success of this storage technique is influenced by the nature of the suspending medium (Khan and Boyd, 1968), honeydew, which normally contains 20 to 50% sugars, was diluted four times with distilled water to give a medium similar to the 10% sucrose solution which Khan and Boyd found to be suitable. Storage of the lyophilized spores was in sealed tubes at 5°C.

Honeydew, containing spores to be stored by the "sucrose" method, was diluted with distilled water and the spores were centifuged out of suspension. After a further wash in sterile, distilled water, the spores were re-suspended in a 50% solution of sucrose in which germination of the spores was completely inhibited (Lewis, 1945). Storage was in sealed tubes at 5°C.

A number of variations of the technique (Hwang, 1968) for storage of conidia at ultra-low temperatures, under liquid nitrogen, were investigated (table 1) and the following modified technique was selected. Suspensions of conidia in 10% of glycerol (Polge et al, 1949) were sealed in glass ampoules and slowly pre-cooled by wrapping the ampoules in cotton wool before placing them inside a polystyrene box in a deep freeze at -50°C. After 24 hours the ampoules were unwrapped and immediately immersed in liquid nitrogen where they were maintained at -196°C. When required for inoculation, rapid thawing of the ampoules was achieved by removing them from liquid nitrogen and plunging into warm water (25-30°C).

Table 1.

Modifications of the techniques for storage of conidia of Claviceps purpurea in liquid nitrogen.

Treatment.	Glycerol additive. 0%	5%	10%	15%	20%	Pre-cooling: Rate of cooling.	Method of insulation.	Post-storage treatment: Intermediate storage in cardice.	Ampoules warmed at: -10/2°C	2°C	30°C.
A			+			Slow.	Ampoules wrapped in cotton wool and placed within one polystyrene box.				+
B		+				"	"				+
C	+					"	"				+
D				+		"	"				+
E					+	"	"				+
F			+			Very slow.	As above, but placed inside two polystyrene boxes.				+
G			+			Rapid.	Wrapped in cotton wool only.				+
H			+			Slow	Ampoules wrapped in cotton wool and placed within one polystyrene box.			+	
K			+			"	"				+
L			+			"	"	+		+	
M			+			"	"	+			+

P(Control). Fresh honeydew.

The success of the various storage techniques was assessed by a spore germination test before and after storage. Dilute suspensions of conidia were streaked on to thin, medium "T" agar plates, and the percentage germination recorded by direct observation under a microscope after incubation at 24°C for 18 hours (up to 36 hours for conidia taken from culture). After selection of the best technique, used in all subsequent experiments, male-sterile wheat ears were inoculated with both fresh and stored honeydew conidia to determine whether pathogenicity of the isolate had been impaired during storage.

9.4 Inoculation of test plants

Normally, both male-sterile and fertile wheat florets were inoculated at the growth stage 10.1 to 10.5 (Large, 1954) between emergence of the head from boot and the date of flower opening (equivalent to anthesis). A spore suspension of approx. 10^6 spores per ml was injected, at a median level, into the two basal florets of each spikelet by means of a hypodermic syringe. Care was taken to avoid damage to the developing ovaries at the base of the floral cavities.

Grasses were inoculated by one, or a combination, of three methods: 1) by injection into the florets as described above: 2) by spraying a spore suspension onto the

flowering head at anthesis, using an atomizer spray gun (Shandon Scientific Co. Ltd., London) and: 3) by complete immersion of the inflorescence in the spore suspension. Following inoculation, grass heads were enclosed in glassine bags to prevent cross-infection.

9.5 Collection and storage of ergot sclerotia

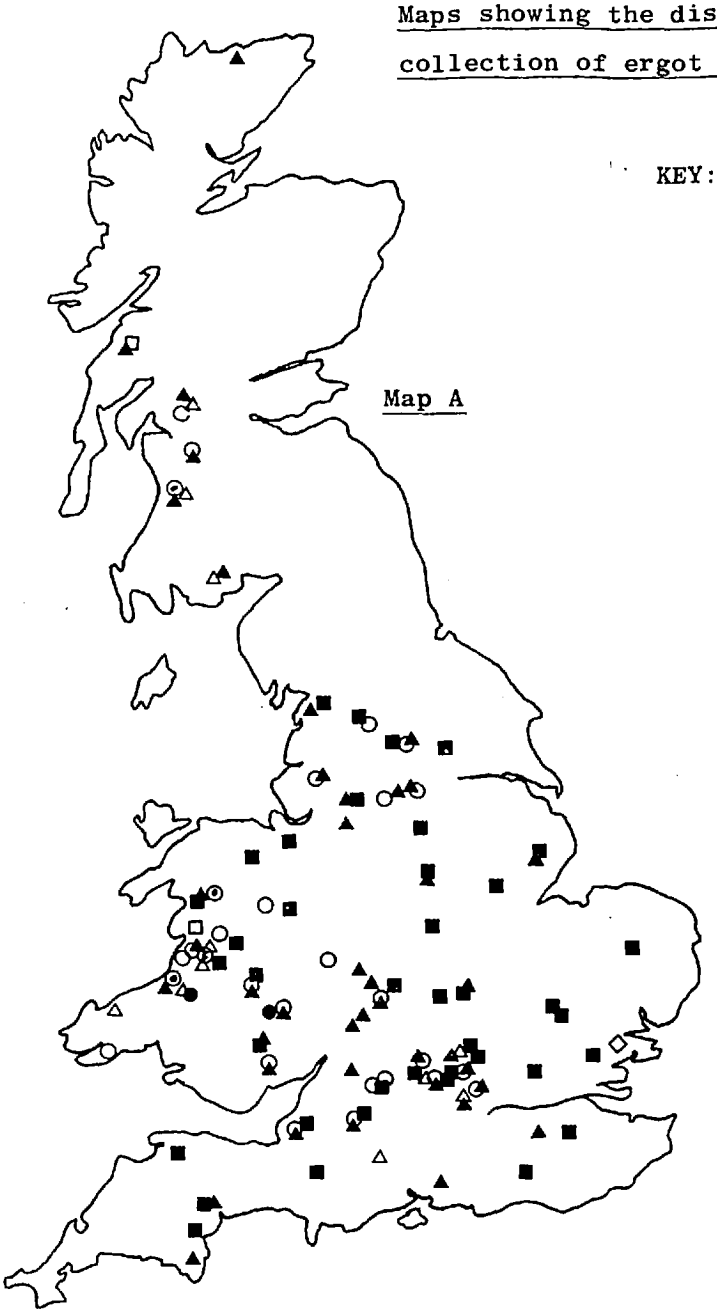
Ergot sclerotia were collected from cereals and grasses from a wide variety of habitats throughout the British Isles, though mainly from England and Wales. Distribution maps of these collections are shown in figure 1.

On receipt of each ergot sample it was given an accession number which was used to identify the strain in analysis, in culture and in any subsequent inoculations. A list of accessions is given in table 2.

All sclerotial samples were air-dried at room temperature and then placed in manilla envelopes before storage in the laboratory at room temperature. To collect sclerotia from large samples of wheat, a small threshing machine (Garvie and Sons, Aberdeen) was used. When threshing hussocks of wheat from experimental plots it was possible to clean out all the residual ergot and seed between successive samples.

Figure 1.

Maps showing the distribution of sites for collection of ergot samples listed in table 2.



KEY:

Map A.

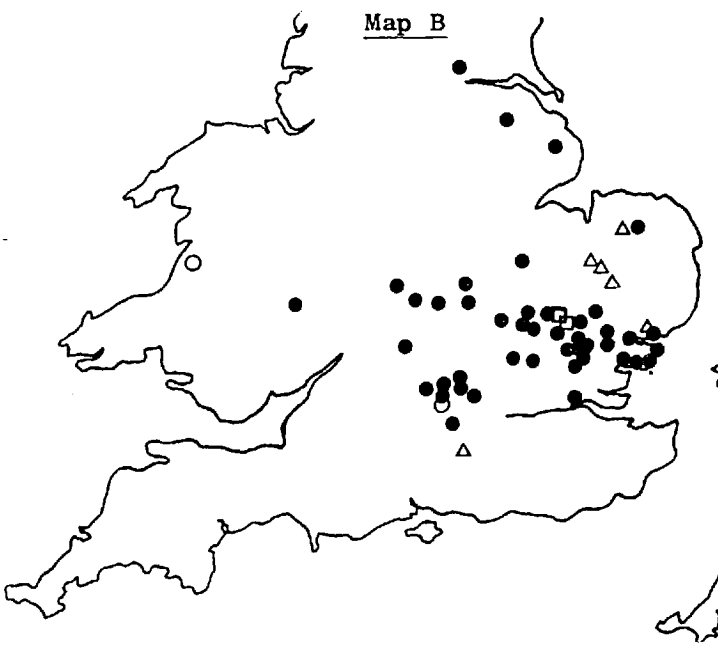
- ■ Lolium perenne.
- ● L. multiflorum.
- ◇ L. temulentum.
- □ Anthoxanthum odoratum.
- ▲ Dactylis glomerata.
- △ Arrhenatherum elatius.
- ○ Holcus lanatus.
- ⊙ H. mollis.

Map B.

- ● Wheat.
- ○ Barley.
- □ Triticale
- △ Rye

Map C.

- ▲ Agropyron sp.
- ● Alopecurus myosuroides.
- □ A. geniculatus.
- △ A. pratensis.
- ○ Festuca arundinacea.
- ◇ Glyceria sp.
- ■ Phleum spp.



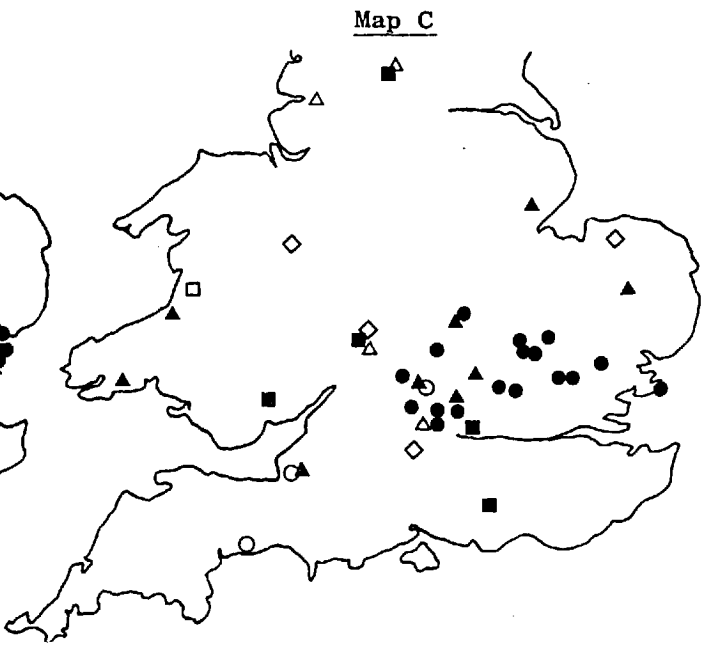


Table 2

List of ergot samples used in experiments or described in text

	Host	Accession number	Locality	Year collected	Collected by
(W = Winter) (S = Spring)					
Cms.	W.Wheat	16	Rothwell,Lincs	1971	A.C.Done
	W.Wheat	19	Essex	1971	RHM
	"	22	Selby,Yorks	1971	"
	S.Wheat	25	Castle Camps,Cambs	1971	"
	W.Wheat	28	Essex	1971	"
	S.Wheat	39	Orsett,Essex	1971	"
Cms.	Wheat	40	Stokenchurch,Bucks	1971	D.A.Doling
	Wheat	53	Essex	1971	RHM
	"	88	Warwickshire	1971	G.Wingfield
	"	89	Stokenchurch,Bucks	1971	D.A.Doling
Cms.	"	90	Berkshire	1971	"
	S.Wheat	93	N.I.A.B. Cambridge	1971	N.I.A.B.
	"	94	Warwickshire	1972	-
	W. "	95	Cranfield,Bedford	1972	-
	W. "	100	Dunmow,Essex	1971	RHM
	"	101	Roystoh, Herts	1971	P.D.Hewitt
	"	108	Norfolk	1971	"
	W. "	123	Churchill,Oxon	1972	S.Shaw
	W. "	124	Warborough,Oxon(LEF)	1972	"
	W. "	145	Essex	1972	RHM
	S. "	146	Kingston Lyle,Oxon	1972	S.Shaw
	W. "	147	Sutton Manor,Hereford	1972	D.Moore
	"	148	White Colne,Essex	1972	RHM
	"	223	P.B.I. Cambridge	1972	T.E.Miller
	"	235	Haslingfield,Cambs	1972	"
	W. "	323	Rothamsted,Herts	1972	J.M.Thurston
	"	325	Spain	1972	A.C.Done
	S. "	327	S.E.England	1972	RHM
	W. "	344	E.Haddon,Northants	1973	S.Shaw
	W. "	348	Towcester,Northants	1973	"
	S. "	360	Dunmow,Essex	1973	"
	"	370	Huntingdonshire	1973	D.Stormant
	W. "	375	Tempsford,Beds	1973	S.Shaw
	W. "	376	Sandy,Beds	1973	"
Cms.	S. "	381	High Wycombe,Bucks	1973	D.Joyce
	W. "	383	Stebbing,Essex	1973	RHM
	W. "	384	Felsted,Essex	1973	"
	W. "	391	Duton Hill,Essex	1973	"
	"	393	Walton-on-the-Naze, Essex	1973	P.G.Mantle
	W. "	404	Lindsell,Essex	1973	RHM
	W. "	435	Essex	1973	"
	W. "	436	Saffron Walden,Essex	1973	"
	W. "	440	Takeley,Essex	1973	"

W. Wheat	441	Takeley,Essex	1973	Farm Seeds Ltd.
W. "	442	Manningtree,Essex	1973	RHM
W. "	443	Colchester,Essex	1973	"
"	444	Colchester,Essex	1973	"
W. "	445	Harwich,Essex	1973	"
S. "	450	Hatfield,Herts	1973	A.Hall
"	451	Alford,Lincs	1973	S.Shaw
W. "	453	Quinton Green, Northants	1973	C.Gibson
W. "	454	Garford,Oxon	1973	S.Shaw
ms. Barley	17	Rothwell,Lincs	1971	A.C.Done
"	55	Essex	1971	RHM
"	126	Warborough,Oxon(LSF)	1972	S.Shaw
ms. Barley		W.P.B.S.Aberystwyth	1972	C.A.Foster
ms. "		"	1972	"
ms. "		"	1972	"
ms. "		"	1972	"
Rye	27	East Anglia	1971	RHM
"	107	Norfolk	1972	P.D.Hewitt
"	134	Fettwell,Suffolk	1972	S.Shaw
"	140	Wheating,Suffolk	1972	"
"	452	Fleet,Hampshire	1973	D.Thompson
Triticale	47	N.I.A.B. Cambridge	1971	J.K.Doodson
"	222	P.B.I. Cambridge	1972	-
Agropyron pungens	166	Rye, Sussex	1972	R.A.Finch
A. repens	172	Chinnor,Oxon	1972	D.J.Gedye
"	174	Oxford	1972	S.Shaw
"	233	St.Florence,Pembs	1972	A.Arnold
"	243	Bunwell,Norfolk	1972	C.A.Foster
"	250	Mendips,Somerset	1972	S.Shaw
"	265	Northampton	1972	"
"	311	U.S.A.	1972	R.Clark
"	316	U.S.A.	1972	"
"	354	Chinnor,Oxon	1973	D.J.Gedye
"	363	Boston,Lincs	1973	S.Shaw
"	364	Llanrhystydd,Cards	1973	"
"	397	Leighton Buzzard	1973	L.Nisbet
"	401	Blackeney,Norfolk	1973	P.G.Mantle

Agrostis canina	198	Dolgellau, Merioneth	1972	S.Shaw
Agrostis sp.	9	-	1972	RHM
Alopecurus geniculatus	152	W.P.B.S., Aberystwyth	1972	C.A.Foster
A. myosuroides	44	Rothamsted, Herts	1971	J.M.Thurston
"	102	-		-
"	122	Churchill, Oxon	1972	S.Shaw
"	131	Warborough (LEF)	1972	"
"	132	"	1972	"
"	143	Stokenchurch, Bucks	1972	"
"	149	White Colne, Essex	1972	"
"	150	Kingston Lyle	1972	"
"	330	Rothamsted, Herts	1973	"
"	345	E.Haddon, Northants	1973	"
"	349	Towcester, Northants	1973	"
"	352	Watlington, Oxon	1973	"
"	371	Cambridge	1973	"
"	374	Tempsford, Beds	1973	"
"	377	Sandy, Beds	1973	"
"	378	Hattley St.George, Beds	1973	"
"	379	Abingdon, Oxon	1973	"
"	388	Great Dunmow, Essex	1973	"
"	394	Walton-on-the-Naze, Essex	1973	P.G.Mantle
"	428	Rothamsted	1973	J.M.Thurston
"	429	"	1973	"
"	440	Takeley, Essex	1973	RHM
"	449	Hatfield, Herts	1973	A.Hall
A. pratensis	251	Otley, Yorks	1972	S.Shaw
"	273	Leyland, Lancs	1972	"
"	308	Evesham, Worcs.	1972	"
"	328	Warborough (LEF)	1973	"
"	329	Rothamsted, Herts	1973	"
"	385	Warborough (LEF)	1973	"
"	411	New Galloway, Dumfries	1973	D.J.Gedye
Ammophila arenaria	87	Moray, Scotland	1971	C.S.Millar
"	109	" "	1972	"
"	402	Pembroke	1973	A.Arnold
"	417	Strathy, Sutherland	1973	D.J.Gedye

Anthoxanthum odoratum	73	-		-
"	202	Corrig,Merioneth	1972	S.Shaw
"	399	Skye,Inverness	1973	L.Nisbet
"	422	Inverliever,Renfrew	1973	D.J.Gedye
Arrhenatherum elatius	34	Salisbury,Wilts	1971	C.A.Scyzerbak
"	36	Cookham,Bucks	1971	"
"	80	Cardigan	1971	A.R.Loveless
"	130	Warborough (LEF)	1972	S.Shaw
"	157	W.P.B.S.,Aberystwyth	1972	C.A.Foster
"	210	" "	1972	"
"	225	Auchincruive,Ayr	1972	Channon
"	335	Chinnor,Oxon	1973	D.J.Gedye
"	361	Chinnor,Oxon	1973	D.J.Gedye
"	366	Llanrhystyd,Cards	1973	S.Shaw
"	392	Dumbartonshire	1973	L.Nisbet
"	415	New Galloway, Dumfries	1973	D.J.Gedye
Brachypodium sylvaticum	76	-		-
Dactylis glomerata	6	Exeter,Devon	1971	S.Shaw
"	14	Spilsby,Lincs	1971	-
"	37	Slapton,Devon	1971	W.R.Carlile
"	121	Fareham,Hants	1972	S.Shaw
"	127	Warborough (LEF)	1972	"
"	159	Warwick	1972	D.H.Glover
"	160	Quernmore,Lancaster	1972	P.G.Ayres
"	171	Chinnor,Oxon	1972	D.J.Gedye
"	177	Hay,Hereford	1972	S.Shaw
"	181	Tewkesbury	1972	"
"	184	Evesham	1972	"
"	186	Stratford	1972	"
"	187	Bromsgrove	1972	"
"	193	Stroud	1972	"
"	199	Dolgellau,Merioneth	1972	"
"	206	Cwmbran,Monmouth	1972	"
"	209	W.P.B.S.Aberystwyth	1972	C.A.Foster
"	211	" "	1972	"
"	219	Glasgow	1972	P.G.Mantle
"	226	Auchincruive,Ayr	1972	Channon
"	229	Manchester	1972	M.Faulkner
"	246	Purley,Surrey	1972	P.G.Mantle
"	257	Crickhowell,Mon	1972	S.Shaw
"	258	Oxford	1972	"
"	260	New Mills,Yorks	1972	"
"	261	Otley,Yorks	1972	"
"	263	Bath,Somerset	1972	"
"	268	Nottingham	1972	"
"	274	Penistone,Yorks	1972	"

Dactylis glomerata	277	Leyland, Lancs	1972	S. Shaw
" (cont.)	279	Knutsford, Cheshire	1972	"
"	284	Mendips, Somerset	1972	"
"	301	Northampton	1972	"
"	314	U.S.A.	1972	R. Clarke
"	337	W.P.B.S. Aberystwyth	1973	S. Shaw
"	353	G.R.I. Hurley, Berks	1973	"
"	362	Llanrhystyd, Cards	1973	"
"	369	Beaconsfield, Bucks	1973	"
"	396	Dumbartonshire	1973	L. Nisbet
"	407	West Wycombe, Bucks	1973	P. Shaw
"	414	New Galloway, Dumfries	1973	D. J. Gedye
"	418	Strathy, Sutherland	1973	D. J. Gedye
"	423	Inverliever, Renfrew	1973	"
Deschampsia caespitosa	72	-		-
Festuca arundinacea	79	-	1971	A. R. Loveless
"	97	Sidmouth, Devon	1971	J. Webster
"	249	Mendips, Somerset	1972	S. Shaw
"	276	Oxford	1972	"
"	312	U.S.A.	1972	R. Clarke
"	313	U.S.A.	1972	"
Glyceria sp.	185	Evesham	1972	S. Shaw
"	247	Hungerford, Berks	1972	E. Green
G. fluitans	294	Shrewsbury	1972	S. Shaw
Holcus lanatus	62	Radnor	1971	J. Bradley-Jones
"	77	-	1971	-
"	129	Warborough (LEF)	1972	S. Shaw
"	155	"	1972	"
"	173	New Mills, Yorks	1972	"
"	178	Hay, Hereford	1972	"
"	183	Stratford	1972	"
"	188	Kidderminster	1972	"
"	189	Welshpool	1972	"
"	192	Newtown, Montgomery	1972	"
"	201	Talybont, Aberystwyth	1972	"
"	207	W.P.B.S., Aberystwyth	1972	C. A. Foster
"	213	Glasgow	1972	P. G. Mantle
"	253	Mendips, Somerset	1972	S. Shaw
"	266	Penistone, Yorks	1972	"
"	270	Lyneham, Wilts	1972	"

Holcus lanatus (cont).				
	275	Oxford	1972	S.Shaw
"	278	Leyland,Lancs	1972	"
"	280	Bath,Somerset	1972	"
"	286	Cwmbran,Mon.	1972	"
"	288	Otley,Yorks	1972	"
"	292	Swindon,Wilts	1972	"
"	293	Boltons Abbey,Yorks	1972	"
"	367	Beaconsfield,Bucks	1973	"
"	395	Dumbartonshire	1973	L.Nisbet
"	405	West Wycombe,Bucks	1973	P.Shaw
Holcus mollis	52	-	1971	A.R.Loveless
"	156	W.P.B.S. Aberystwyth	1972	C.A.Foster
"	161	Mynydd Prescelly, Pembs	1972	A.Arnold
"	196	Dolgellau,Merioneth	1972	S.Shaw
"	208	W.P.B.S.Aberystwyth	1972	C.A.Foster
"	232	Auchincruive,Ayr	1972	Channon
"	365	Llanrhystyd,Cards	1972	S.Shaw
Lolium multiflorum	2	Newton Abbot,Devon	1971	S.Shaw
"	154	W.P.B.S.Aberystwyth	1972	C.A.Foster
"	305	Hay,Hereford	1972	S.Shaw
Lolium perenne	1	Exeter,Devon	1971	S.Shaw
"	3	Newton Abbot,Devon	1971	"
"	8	West Watch,Sussex	1971	-
"	18	Cambridge	1971	P.B.I.
"	26	Essex	1971	RHM
"	33	-	1971	"
"	38	Sevenoaks,Kent	1971	S.Shaw
"	41	Castle Bytham,Lincs	1971	K.S.Malone
"	46	N.I.A.B., Cambridge	1971	J.K.Doodson
"	61	Radnor	1971	J.Bradley-Jones
"	67	Arkholm,Lancs	1971	C.D.Pigott
"	78	Drayton,Hants	1971	A.R.Loveless
"	81	Hatfield,Herts	1971	A.Hall
"	83	Warborough (LEF)	1971	S.Shaw
"	98	Shrewsbury	1972	A.D.A.S. Wolverhampton
"	103	Sweden	1972	O.S.T.S. Cambridge
"	106	Warwickshire	1972	"
"	125	Warborough (LEF)	1972	S.Shaw
"	151	W.P.B.S.Aberystwyth	1972	C.A.Foster
"	153	" "	1972	"
"	158	Barnstaple	1972	-

Lolium perenne (cont.)	170	Chinnor,Oxon	1972	D.J.Gedye
"	175	Northampton	1972	S.Shaw
"	191	Newtown,Montgomery	1972	"
"	195	Ruthin,Denbigh	1972	"
"	203	Chrickhowel,Brecon	1972	"
"	204	Dolgellau,Merioneth	1972	"
"	212	W.P.B.S.Aberystwyth	1972	C.A.Foster
"	216	Glasgow	1972	P.G.Mantle
"	220	Lacock,Wilts	1972	A.G.Walker
"	227	Auchincruive,Ayr	1972	Channon
"	231	Manchester	1972	M.Faulkner
"	239	Bunwell,Norfolk	1972	C.A.Foster
"	252	Chester	1972	S.Shaw
"	254	Mendips,Somerset	1972	"
"	255	York	1972	"
"	256	Swindon	1972	"
"	259	Otley,Yorks	1972	"
"	264	Nottingham	1972	"
"	267	Leicester	1972	"
"	281	Boltons Abbey,Yorks	1972	"
"	282	Northampton	1972	"
"	291	Spilsby,Lincs	1972	"
"	347	Towcester,Northants	1973	"
"	355	Chinnor,Oxon	1973	D.J.Gedye
"	380	Abingdon,Oxon	1973	S.Shaw
"	419	-	1973	D.J.Gedye
Lolium temulentum	12	Colchester,Essex	1971	C.A.Brooks
Molinia caerulea	70	-	1971	J.Webster
	86	Dartmoor,Devon	1971	"
Nardus stricta	74	-	1971	A.R.Loveless
Phalaris arundinacea	214	Moffat,Dumfries	1972	E.G.Mills
"	412	New Galloway,Dumfries	1973	D.J.Gedgye
Phleum paniculatum	85	Reading	1973	S.Shaw
Phleum pratense	7	Sussex	1971	-
"	179	Cwmbran,Mon.	1972	S.Shaw
"	180	Evesham	1972	"
"	224	Auchincruive,Ayr	1972	Channon
"	262	Otley,Yorks	1972	S.Shaw
"	368	Beaconsfield,Bucks	1973	"

Phragmites communis	56	Boston, Lincs	1971	A.Done
Poa annua	5	Newton Abbot, Devon	1971	S.Shaw
"	104	-	1971	RHM
Spartina anglica	68	Sidmouth, Devon	1971	J.Webster

10. The cultivars

10.1 Wheat cultivars

Cytoplasmic male-sterile Capitole ((Cappelle Desprez x S6) x M/S), a soft, winter wheat, was the variety grown for most glasshouse and field experiments. Other varieties, screened for resistance to ergot infection, are listed in section 16.5.

A restorer line, Maris Beacon, and the F.1 hybrid of Maris Beacon x cms Capitole were also used.

Wheat cultivars were sown in field plots at R.H.M. trial sites near High Wycombe, Bucks and at Great Dunmow, Essex (p53). Two types of plot units formed the basis of experimental designs. Except where otherwise stated, small, standard hussocks, approximately 12" diameter, were hand-sown with approximately 4.5 gm of seed. Larger, standard plots, measuring 5ft x 48ft, were sown with a small seed drill at the rate of 400 gm/plot (approx. 1¼ cwts/acre), each plot having eight drill coulters 6" apart. Herbicides and fertilizers (normally a spring top dressing of 50 units of nitrogen) were applied as necessary by personnel of the L.R.R.C. No fungicides or seed dressings were used.

Prior to cultivation in the glasshouse, winter wheats were vernalized by the method described by Purvis and Gregory (1952). Plants were grown in $4\frac{1}{2}$" plastic pots, in potting soil consisting of sterilized loam/sand/peat, 7:2:3, plus John Innes base (No.2) at the rate of $\frac{1}{2}$lb per bushel. Capillary beds provided continuous watering, and 700W MBRR/U mercury vapour lamps (Phillips) were controlled by a time switch to maintain an appropriate daylength. Electrical heating was regulated by a cycling thermostat to give a differential day and night temperature, and very high temperatures, induced by sunlight, were reduced by a thermostatically controlled extractor fan.

At the time of this study, when F.1 hybrid cereals were in an early stage of development, a fully reliable male sterile line was not available in large quantity. Incomplete male sterility of the cultivars provided by Rothwell Plant Breeders, Ltd., created a number of recurrent problems, especially in the design of experiments. In the glasshouse, where only a limited number of wheat plants could be grown at any one time, the numbers of replications of treatments often had to be reduced during the course of an experiment as self

fertile "male sterile" plants were discarded. In the field, small amounts of pollen, from self fertile plants amongst plots of male sterile wheat presented other difficulties when the effects of male sterility on ergot infection were under investigation. Whenever possible fertile plants were rogued, but these could not be identified before the first ear of each plant had anthesed.

10.2 Grasses

Grass species were obtained from collections at the Royal Botanic Gardens, Kew; Chelsea Physic Garden; Reading University Botanic Gardens and the Grassland Research Institute, Hurley. They were grown in garden plots and in the glasshouse at the Chelsea Physic Garden, under the conditions previously described.

During 1972, observations were made of the flowering dates of established clones of many grass species both in natural collections at the sites mentioned above, and in normal field situations.

11. Location of field experiments

11.1 R.H.M. (Research) Ltd.

a) Chequers Manor Farm, Near High Wycombe, Bucks

Two sites were used at this farm; one $\frac{3}{4}$ mile NNW of Fingest (Field No. O.S.202 - Map SU.79.SE) and the other $\frac{1}{2}$ mile SSE of Stokenchurch (Field No. O.S.9 - Map SU.79.SE). The Fingest site (C.M.F. - Fingest) was situated in a valley bottom; at approximately 400ft above sea level, where the soil was flint and clay loam overlying chalk. Similar soil conditions were found at the Stokenchurch site (C.M.F. - Stokenchurch) which was in a more exposed position at approx. 600ft above sea level, though afforded some shelter from the north by a boundary wood.

b) Throws Farm (R.H.M., Agriculture), Great Dunmow, Essex.

This farm (R.H.M., Throws), which was chosen for the 1973/74 experiments involving blackgrass, already had a history of blackgrass infestation on its heavy clay soil.

11.2 Other experimental sites

a) Chelsea Physic Gardens (C.P.G.), Chelsea, London.

This garden, bounded on three sides by buildings and on the fourth by the embankment of the river

Thames, provided a sheltered, mild environment for small-scale plot experiments.

b) Lane End Farm (L.E.F.), Warborough, Nr. Oxford

This mixed arable and pig farm had a long history of ergot infestation; thought by the farmer to have been associated with reproductive failure of his pigs, and known to have been the cause of rejection of at least one crop of seed wheat. Regular observations of natural incidence of ergot disease on this farm were made during the summers of 1972 and 1973.

c) Observations and collections of ergot were also made at the Grassland Research Institute, Hurley (G.R.I.); at the Welsh Plant Breeding Station, Aberystwyth (W.P.B.S.); at a winter wheat variety observation trial at Manor Farm, Garford, Nr. Oxford; and at many other farms especially in the South Midlands, South and South East of England.

12. Weather recordings

With the exception of the wind direction recorder (see below), all instruments used were of a Meteorlogical Office-approved design.

12.1 At Chequers Manor Farm

A seven-day recording thermohydrograph provided a continuous record of ambient temperature and relative humidity. Weekly maximum and minimum, wet and dry bulb temperature recordings were taken from mercury thermometers (-20°C to +60°C) and used to calibrate and check the thermohydrograph records. The above instruments were placed in a Stevenson's screen at approximately 1.5 meters above ground level. Rainfall was measured continuously by means of a self-emptying, recording rain guage.

During 1973/74 weekly maximum and minimum soil temperatures were recorded from a mercury thermometer placed in a metal tube at a depth of approximately 2" in exposed, bare earth.

For experiments described in section 16.42 a copy of a prototype continuous wind direction recorder (under commercial development by G.Dugdale) was positioned within the crop to sample the wind direction at approximately 1.2 metres above ground level.

12.2 At the Chelsea Physic Gardens

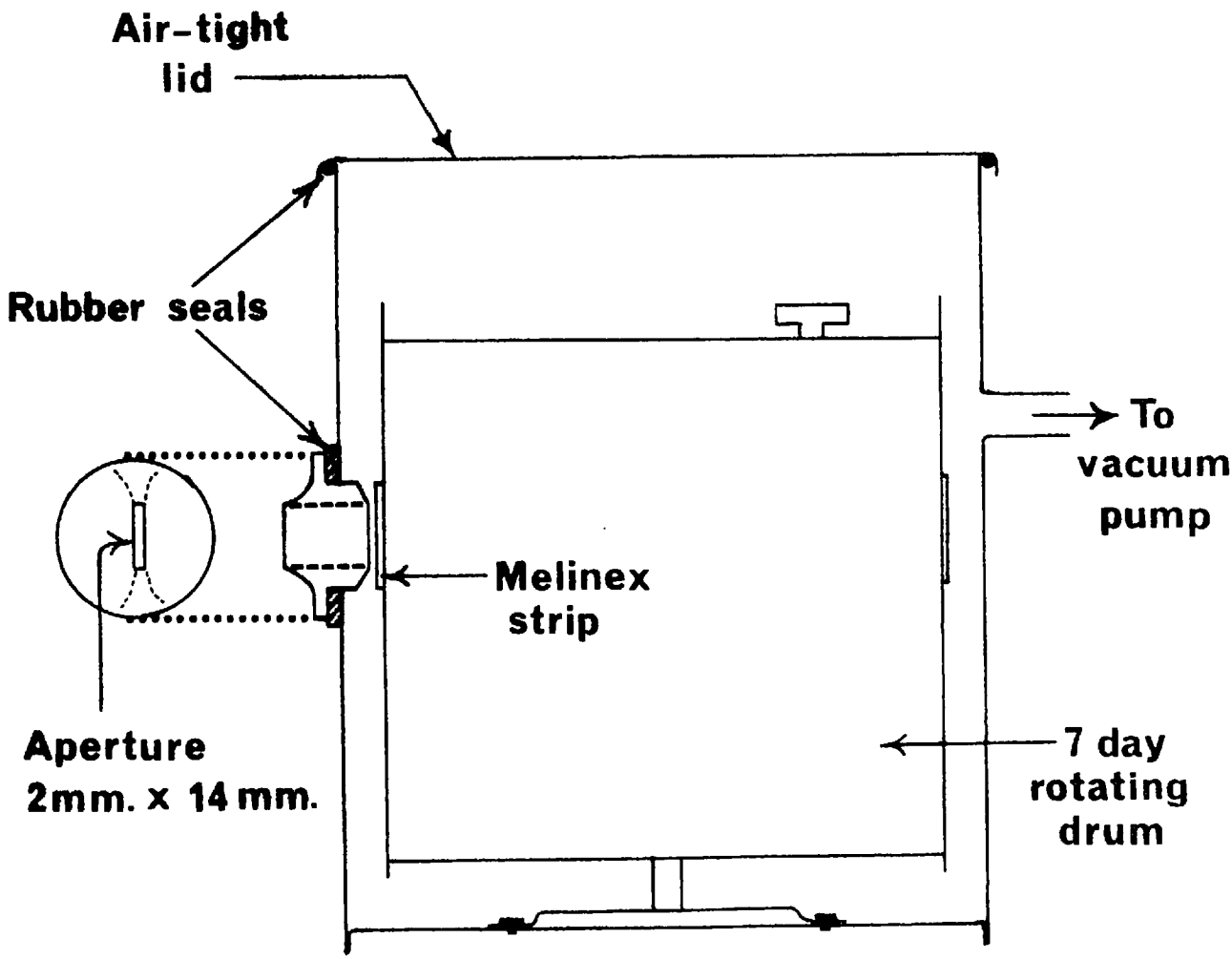
Temperature and relative humidity were recorded on a seven-day recording thermohydrograph which was calibrated by a maximum/minimum mercury thermometer.

13. Sampling of airborne ascospores and pollen

A number of devices to trap and study airborne particles were employed. A volumetric spore trap (Burkard Manufacturing Co., Rickmansworth, Herts) based on the design of Hirst (1967), was used for one week during anthesis of wheat in 1973. This was used to trap airborne ascospores of C.purpurea at 0.5 metres above the soil surface, near to dishes of germinating ergots and alongside hussocks of male-sterile wheat. During May and June of the following year, 1974, a simple volumetric trap (fig.2) was installed at ground level within a plot of male-sterile wheat and near to germinating ergots. The design of this trap is similar to that described by Rapilly (1970), except that no provision has been made for alignment of the aperture into the prevailing wind. Spores were impacted onto a melinex strip coated with a sticky mixture to which they adhered. Initially the sticky surface was prepared in two stages. A thin film of gelvatol (a water soluble plastic) was first applied and allowed to dry, and then a second thin film of a mixture of vaseline and high melting point paraffin wax was added. This technique was modified by the use of an alternative trapping surface, Araldite "hardener" (Ciba-Geigy), in an attempt to overcome the problem of melting of the trapping surface during hot weather. The melinex strip, also used in the Burkard spore trap, was removed at weekly intervals from the seven-day rotating drum and mounted on microscope slides prior to examination.

Figure 2.

Diagram of a 7 day continuous spore trap, designed for use within a crop canopy.



To determine the distribution of pollen over plots of male sterile wheat (Section 16.42), vertically orientated, vaseline smeared microscope slides were clamped to canes and held just above the tallest ears of the standing crop.

Assessments of the numbers of both ascospores and wheat pollen grains trapped by the above methods, were made by microspopic identification and counts of the spores/pollen passing through the limits of the scale of an eyepiece graticule as slides were scanned in longitudinal strips.

14. Laboratory tests for the ejection of ascospores from stromata

Germinating ergot sclerotia, collected from the experiments described in section 16.1, were subjected to an "ascospore ejection test" to determine whether or not the fruiting bodies (stromata) were still capable of ejecting ascospores. Germinating ergots were fixed with vaseline to the lid of a shallow dish (figure 3) so that the inverted ergots were suspended near to a clean glass plate on the bottom of the dish. High humidity was maintained by water soaked paper tissue inside the enclosed dish, which was then incubated at 24°C for six days. Evidence of ascospore ejection was clearly seen as a milky white deposit of the glass plate, close to a capitum.

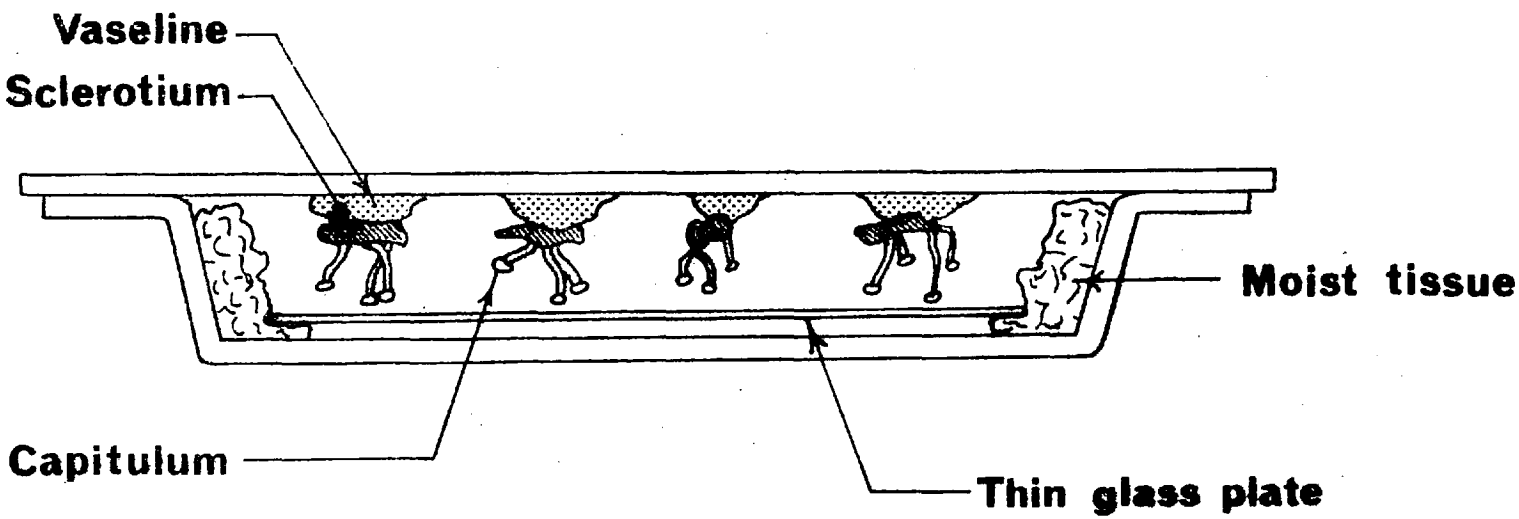


Figure 3.

Diagram of apparatus used to investigate ascospore discharge from germinating ergots

15. Analysis of the alkaloid content of ergot

15.1 Quantitative extraction of alkaloids

Extraction and colorimetric assay of alkaloids was as outlined by Mantle (1967). Up to 2.5gm samples of finely ground sclerotial tissue were weighed, mixed to a stiff paste with sodium bicarbonate solution, and extracted five times in diethyl ether A.R., over a period of four and a half hours. The total alkaloids, having been removed from the combined ethereal extracts by shaking in a separating funnel with 2% tartaric acid solution, were collected volumetrically (10ml of tartaric acid extract for 0.5 gm of sclerotial tissue assayed). After mixing with Van Urk reagent (Allport and Cocking, 1932) in the ratio 2:1 (Van Urk reagent to tartaric acid solution of alkaloid) the intensity of a blue colour which developed in the presence of alkaloid, was measured by an Eel colorimeter and compared with a standard ergotamine tartrate solution. The percent of total alkaloid in the assayed sclerotial tissue was calculated from the formula:

$$\frac{\text{Absorbance(sample)}}{\text{Absorbance of a 50 g/ml solution of ergotamine base}} \times K \times \frac{2.5}{\text{Weight of ground sclerotia(gms)}} = \%$$

Where K = 0.2 when - 50ml of tartaric acid used

- Extract diluted x 2 before mixing with Van Urk reagent

The remainder of the tartaric acid extract was adjusted to pH 8.5 with ammonium hydroxide solution and the alkaloids extracted again by shaking with an equal volume of Chloroform A.R., the volume of which was subsequently reduced in a rotary evaporator prior to further analysis by thin layer chromatography.

15.2 Qualitative extraction of alkaloids

To facilitate the analysis of a large collection of naturally occurring ergots, on a qualitative basis only, a less laborious technique for extraction of a portion of the total alkaloids was developed. Samples of ground sclerotia were mixed into a paste with sodium bicarbonate solution, as above, and alkaloids were extracted in diethyl ether A.R., in sample tubes which were slowly rotated for two hours. The extracted alkaloids were then prepared for chromatographic analysis as shown in figure 4.

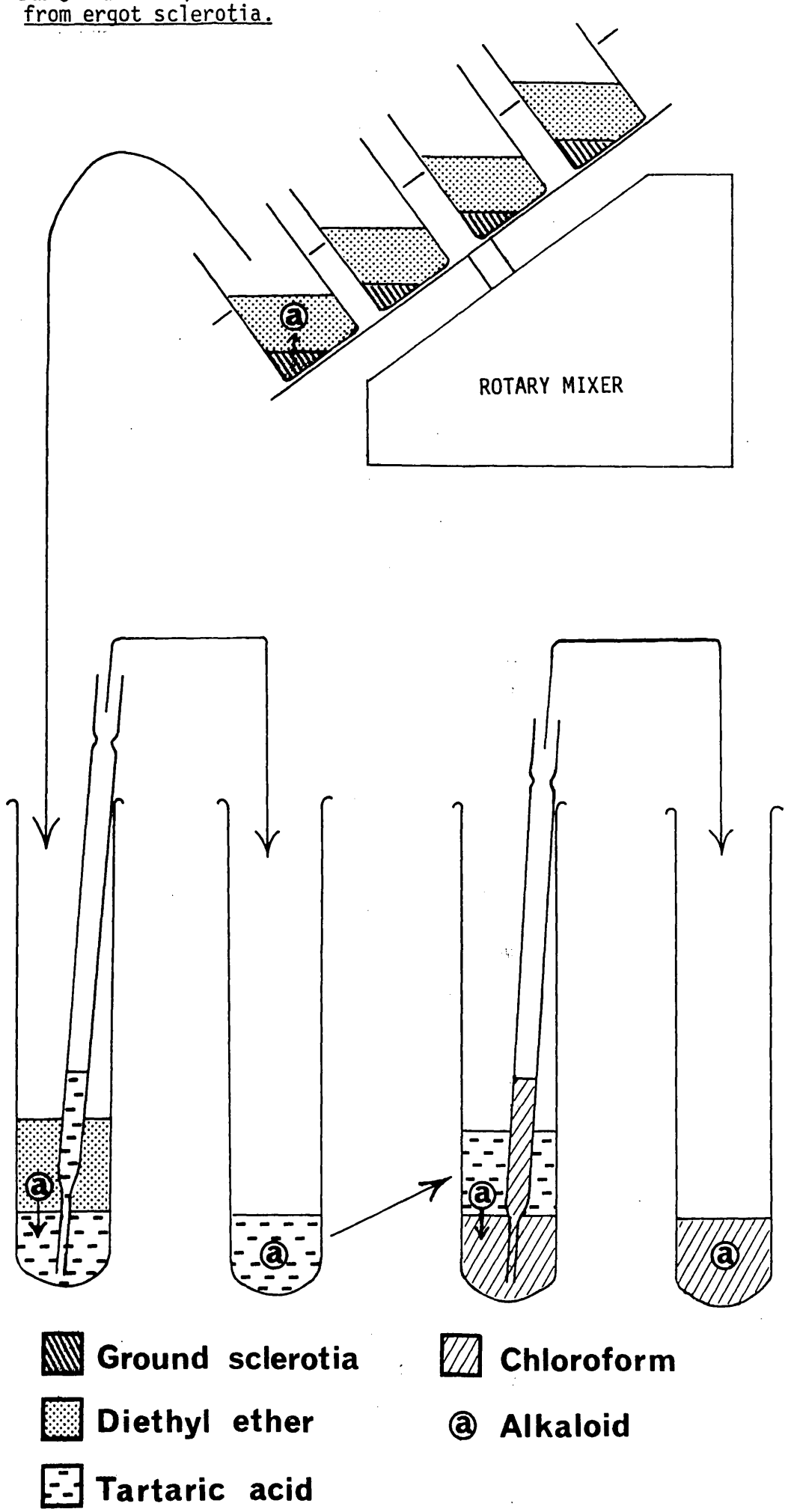
15.3 Thin layer chromatography (T.L.C.)

To separate and identify individual alkaloid components, concentrated extracts in chloroform were loaded on to thin layer chromatography plates. Initially the thin layers were prepared from silica gel G (Merck Chemicals) on glass and run in a 13:0.1:1.9 mixture

of ethyl acetate/ethanol/dimethyl formamide (McLaughlin et al, 1964). Later, commercially preprepared Silica Gel G/UV^{254} layers on plastic sheets (Camlab,Cambridge) were used with a slightly modified solvent in which the amount of ethyl acetate was increased to 200mls in 225 mls of solvent. Fluorescence of the alkaloids under near U/V light (254nm) was observed and recorded, and the chromatograms were developed by a spray of 5% paradimethyl amino benzaldehyde in concentrated hydrochloric acid. A photographic record was kept of all important chromatograms.

Figure 4.

Diagram of a qualitative method for extraction of alkaloids from ergot sclerotia.



16. Experimental Designs

16.1 Field Germination of sclerotia

a) 1972/73 Experiment. Ergot sclerotia, selected for large size by passing over a sieve with apertures measuring 2.5 x 20mm, were buried in field soil in shallow, foil dishes which had been set into the surface layer of the ground (fig. 5b) at three experimental sites: C.P.G., G.R.I. and C.M.F. (Stokenchurch). At the latter two sites the dishes were incorporated into the design of the cycle of infection experiment (fig.7a). 25 ergots, were buried in each dish on 22/11/72, 2-9/11/72 and 17/11/72 at the three sites respectively.

At weekly intervals from the first week of May, three pre-selected dishes from each site were removed and examined in the laboratory. The number of capitula visible above the soil surface were counted and, by careful washing and sieving of the contents of each dish, intact sclerotia and their fructifications were collected. A random selection of 15 germinated ergots from each site were subjected to the ascospore ejection test (p.60).

For one week, during the flowering period of cms.Capitole, a Burkard spore trap (p. 57) was installed alongside one of the rows of dishes at the C.M.F. site.

b) 1973/74 Experiment. At C.M.F. (Fingest) the above experiment was repeated with the following improvements in design:

i) Dishes were set in the ground within drilled wheat plots (fig.5a) so that shading afforded by the plants would help to maintain a moist environment, similar to that found in normal field situations.

ii) The number of dishes, each containing 20 ergots, lifted on each sampling date was increased to eight, and sampling commenced on 3/4/74.

iii) An additional spore trap (fig.2) was used to sample the air at 7 cms above the ground near to a tray of ergots, and within the leaf canopy.

iv) When available, 32 germinated sclerotia were subjected to the ascospore ejection test at each sampling date.

16.2 Experiments to investigate cross-inoculation of strains of ergot from grasses to wheat

16.2.1 1972 Field Experiment

Three replicate blocks, each consisting of 28 hussocks of cms Capitole alternating with hussocks of winter barley (cv.Senta) were sown at C.M.F. (Fingest) on 12/11/71. (Fig.6). As the male-sterile wheat ears emerged from boot, ten ears in each hussock were selected and bagged to prevent both cross-pollination and natural infection

Figure 5a.

Design of the 1973/74 experiment to investigate field germination of wheat ergots

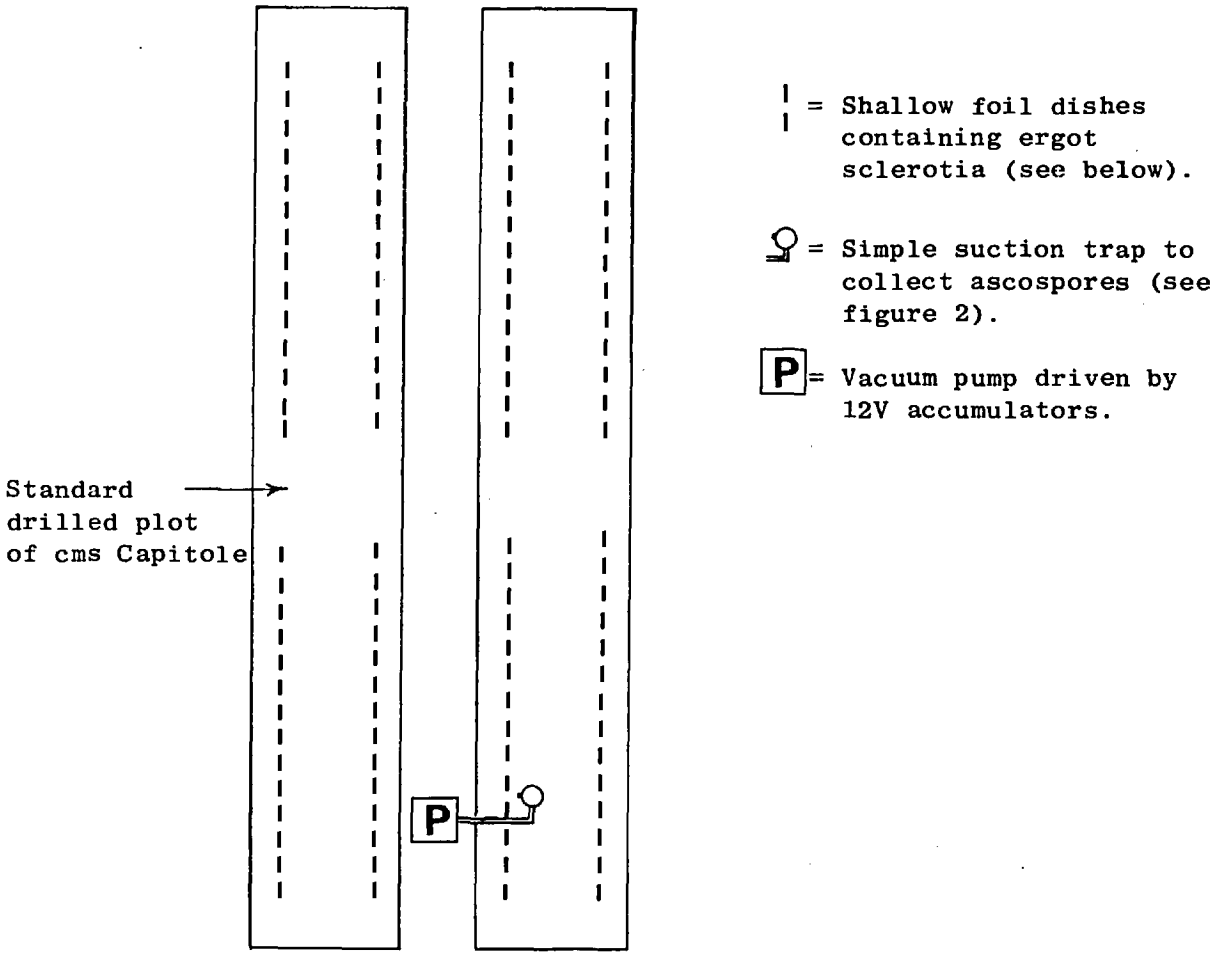
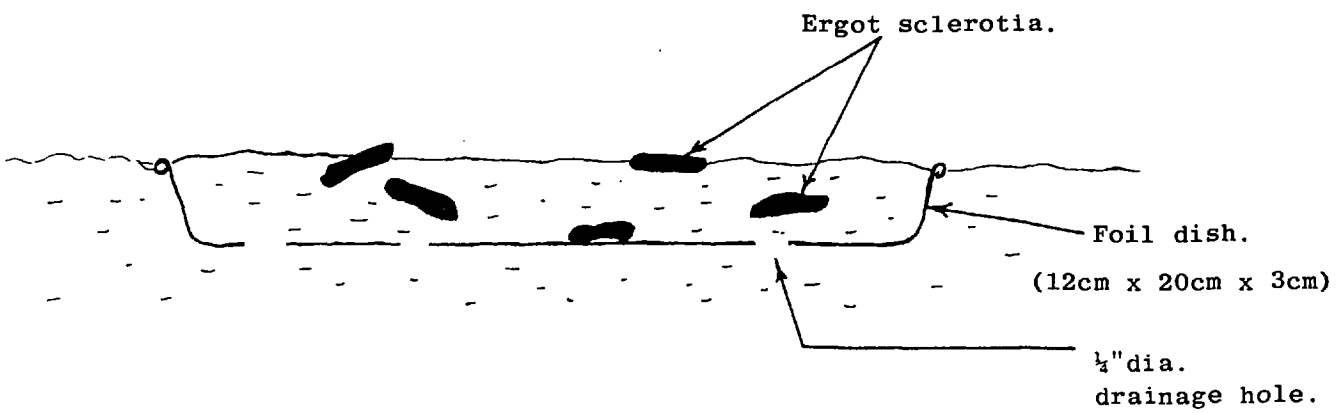


Figure 5b.

Diagram showing the distribution of ergots in the soil profile.



by ergot. Each hussock was randomly assigned to be inoculated with one of 28 strains of ergot isolated from naturally occurring ergot sclerotia that had been collected from various grass hosts in the British Isles. Except where otherwise stated (p109), each strain had been grown on a plant of its original host (or a related host species) to obtain honeydew spores for inoculation of the wheat. In each block there was a random distribution of all 28 treatments. Inoculations of blocks A, B and C were carried out on the 13th, 14th and 15th of June, respectively. Although 200 florets (20 florets per ear) were inoculated in each hussock, storm damage during the week following anthesis resulted in a number of broken straws, so the number of infected heads in each hussock was reduced to 9 (180 inoculated florets) to maintain a uniform sample size.

After four weeks, all inoculated heads were harvested, inoculated florets examined individually, and ergot sclerotia counted and weighed. These sclerotia were subsequently analysed to determine their alkaloid content.

16.2.2 1973. Field experiment

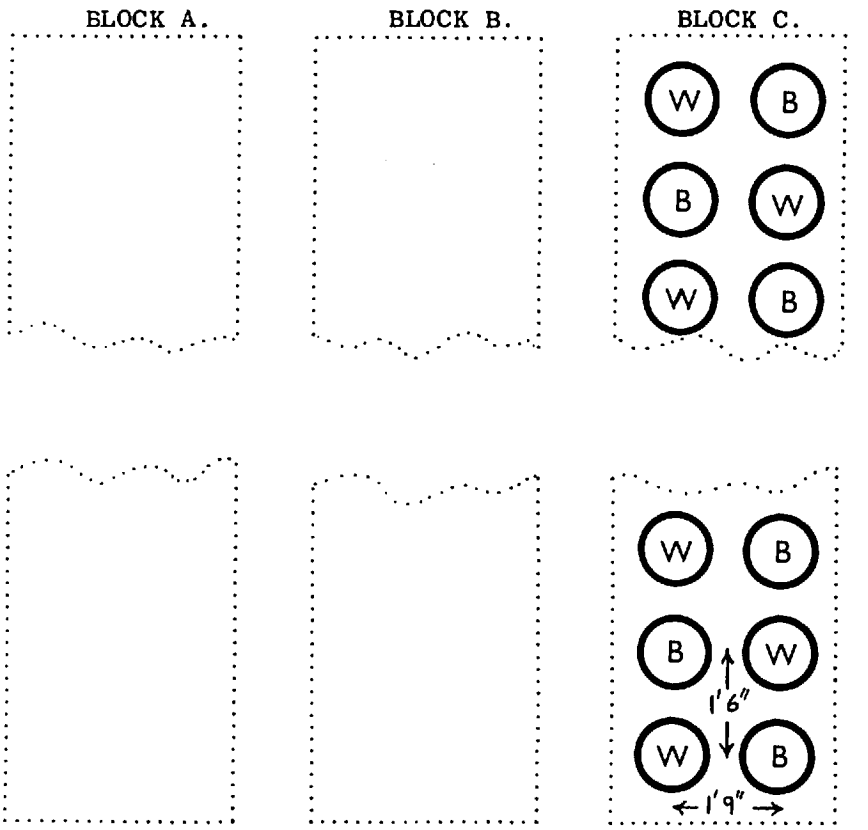
Male-sterile Capitole was inculated with spores (section 16.2.1) of 21 strains of ergot, including two strains (25B and 44) that had also been used in the previous experiment. The experimental design was as described above except that, again due to stormy weather conditions

at about anthesis, the three replicate blocks of treatments were not inoculated on consecutive days. Dates of inoculation of blocks A, B and C were 19th, 21st and 22nd June, respectively. Ten inoculated heads (200 florets) were harvested for each sample, except in treatments 232 and 187 where the total available volume of spore suspensions of the desired concentration was less than that required to inoculate ten complete heads.

Accession numbers of isolates used in the cross-inoculation experiments are listed with the results (Section 21.1).

Figure 6.

Design of the experiments to investigate cross-inoculation on wheat of strains of ergot collected from grasses(1972 & 1973)



Each block containing 28 (1972 expt.) or 21 (1973 expt.) hussocks of cms Capitole.

(W) = Hussock of cms Capitole.

(B) = Hussock of winter barley.

See pages 67-70 for an explanation of treatments.

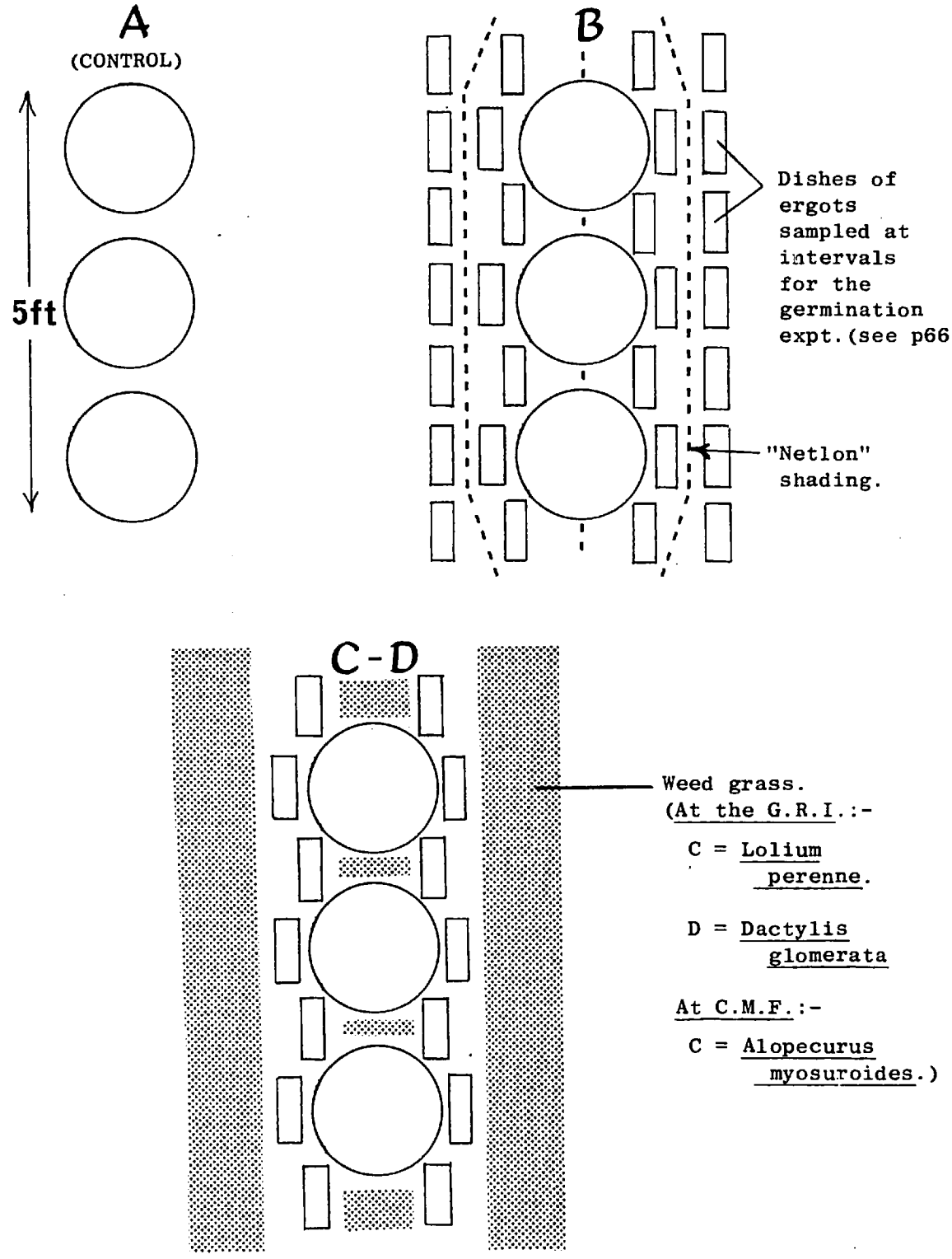
16.3 Investigation of the role of weed grasses in the cycle of infection of ergot of wheat

The role of three weed grass species in the cycle of infection of ergot of wheat was investiated by field experiments at C.M.F. (Stokenchurch) and at the G.R.I., during 1973. The basic treatments, common to both sites, were as shown in figure 7. Dishes of wheat ergot sclerotia were set in the ground as previously described (figure 5b). The grass species used in treatments C and D at the G.R.I. were Dactylis glomerata (Cocksfoot) and Lolium perenne (Ryegrass, cv. S24); and in treatment C at C.M.F. was Alopecurus myosuroides (Blackgrass). Treatments were arranged in three randomised blocks at each experimental site.

The experiment was observed at weekly intervals from the first week of May, and harvested on 30/7/73. Severe bird damage at the G.R.I. during July prevented any quantitative assessment of the amount of ergot in the cms wheat, but hussocks of wheat from C.M.F. were threshed individually to collect all contaminating ergot sclerotia.

Figure 7.

Design of an experiment to investigate the role of weed grasses in the cycle of infection of ergot of wheat.



16.4 Experiments to investigate the susceptibility of male-sterile wheat during the flowering period

16.4.1 Period of susceptibility

a) Glasshouse experiments

Wheat ears of both male-sterile and fertile cultivars were inoculated at different time intervals after "anthesis" (For convenience, the times of inoculations are referred to as anthesis ± x days, but it is acknowledged that true anthesis does not occur in male-sterile cultivars). As the plants approached growth stage 10.1, daily observations commenced at a set time each day, and individual heads were labelled as they reached anthesis. The basal florets of each of ten central spikelets of each head were inoculated by injection of spores into the floral cavities. After four weeks, individual heads were harvested and dissected, and the numbers of sclerotia, sphacelia, aborted infections, damaged seeds, unfertilised ovaries and normal seeds were recorded. Relative humidity and temperature were recorded continuously during these experiments.

The system of designation of treatments (time intervals between anthesis and inoculation) to individual wheat ears was not the same for all experiments in this section. The system used in the first experiment (D in Figure 28) was influenced by industrial action at power stations, which

precluded the use of artificial lighting in the glasshouse during the earlier stages of growth of the test plants. Although normal supply was available during flowering, restrictions of lighting had caused the daylength requirements of the plants to be lapsed during earlier growth stages. Consequently, flowering of tillers was delayed and extended over a period of three weeks. All inoculations were carried out on one date (22nd April) so that the numbers of inoculated heads in each treatment varied from 1-6, depending on the number of heads which had reached anthesis on each day prior to the inoculation date.

A more satisfactory design was used in the two remaining glasshouse experiments. As each ear reached anthesis it was given a particular inoculation date, chosen in such a way that each complete set of treatments was formed before the next replicate set was started. For example, in experiments B/C (see below) the first seven anthesing ears of fertile Capitole formed the first replicate set of treatments, the next seven ears to reach anthesis formed the second replicate, and so on. In this way, any possible effect of ageing of the whole plant on the period of susceptibility was mostly between replicates, rather than between treatments.

Greenhouse experiments were as follows:

B & C* (May/June, 1972). Cms Capitole was inoculated at daily intervals from anthesis + 1 day to anthesis + 15 days. Fertile Capitole was inoculated at daily intervals from anthesis + 1 day to anthesis + 7 days.

D (March/April, 1972). Cms Capitole was inoculated on one day, 22nd April, at which time heads were at various stages of flowering from anthesis to anthesis + 17 days.

E & F (April/May, 1973). Cms Capitole inoculated at one day intervals from anthesis + 1 day to anthesis + 9 days. The fertile hybrid of cms Capitole x Maris Beacon was inoculated at daily intervals from anthesis to anthesis + 5 days.

b) Field Experiment (A*)

At C.M.F. (Fingest) a single row of fourteen hussocks of male-sterile Capitole was sown on 12/11/71. Soon after emergence from boot, 20 ears of similar size and stage of development were selected in each hussock. These were randomly assigned inoculation dates from anthesis + 2 days to anthesis + 21 days. In addition, five heads from each of five of the largest hussocks were selected for inoculation at anthesis + 22 days to anthesis + 26 days. Heads were bagged prior to inoculation to prevent both pollination and cross-infection. The treatments were harvested at daily intervals, starting four weeks after the first inoculation date.

* A-F refer to graphs in figure 28.

16.4.2 The period of susceptibility in relation to seed set

a) 1973 Experiment (C.M.F. Stokenchurch)

The design of this experiment, to investigate the field susceptibility of cms Capitole to ergot in the presence of pollinators, is shown in Figure 8. Male-sterile plants, grown initially in Jiffy pots, were planted out during March within plots of barley which were subsequently cut back to expose the wheat plants as they came into head. The position of the experiment within the trial ground was chosen so that there was only one nearby source of wheat pollen, from a block of winter wheat variety observation plots along the eastern boundary of the experiment. The gradient of pollination and seed set in the male-sterile plots was monitored by pollen trapping devices (p.57), a wind direction recorder and, after harvest of the experiment, a count of seed set in each hussock of cms Capitole.

At the earliest flowering date of the primary tillers of the male-sterile plants, ten ears in each hussock in rows A, B and C, and four ears in each hussock of rows D, E, F and G were selected. In rows A, B and C, one ear was inoculated in each hussock on each of ten successive days after anthesis. In rows D, E, F and G, all four selected heads in each hussock were inoculated at anthesis, anthesis + 3, anthesis + 6 and anthesis + 9 days.

respectively. Selected heads were bagged prior to inoculation in rows D, E, F and G, but not in rows A, B and C, except in the control hussocks. At harvest, all inoculated heads were examined individually, whilst the remaining heads in each hussock, some of which were infected as a result of natural spread of honeydew exuding from the inoculated florets, were counted and threshed to collect all the ergots and seed.

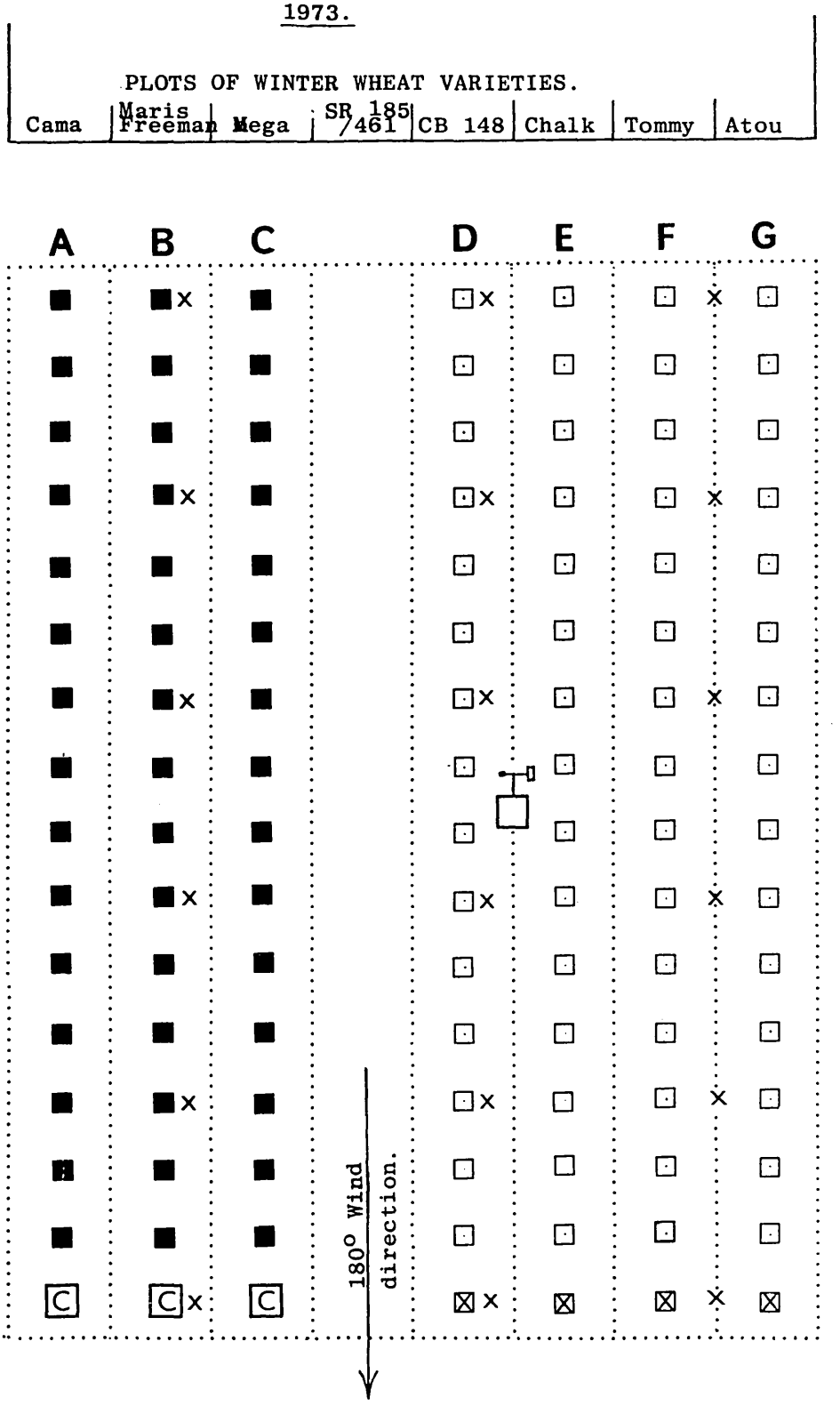
b) 1974 Experiment (C.M.F., Fingest)

Modification of the previous experiment resulted in the design as shown in figure 9. At growth stage 10.1, ten male-sterile ears, matched for size and degree of emergence from boot, were labelled in lines running laterally across plots A, C and E, at three foot intervals from the ends of the plots adjacent to the fertile wheat.

On the date when the first precocious ears within the plot began flowering, all previously labelled ears were inoculated. Six weeks later, all inoculated ears were discarded and the remaining heads, standing within 6" of the lines of inoculated ears were harvested, counted and threshed to collect seed and ergots. Samples of wheat from similar positions in the uninoculated plots B, D and F were harvested as controls. The grain was subsequently examined for signs of partial infection and other defects which might have impaired its quality as commercial seed.

Figure 8.

Design of the field experiment to investigate the effects of seed set on infection of cms Capitole by ergot. C.M.F.(Stokenchurch) 1973.



■ = Hussocks of cms Capitole within drilled plots
⊡ of barley. (See p 77 for an explanation of treatments.)

[C] = Control: heads bagged to prevent fertilization of male-sterile florets before they were inoculated.

⊠ = Control: uninoculated hussocks.

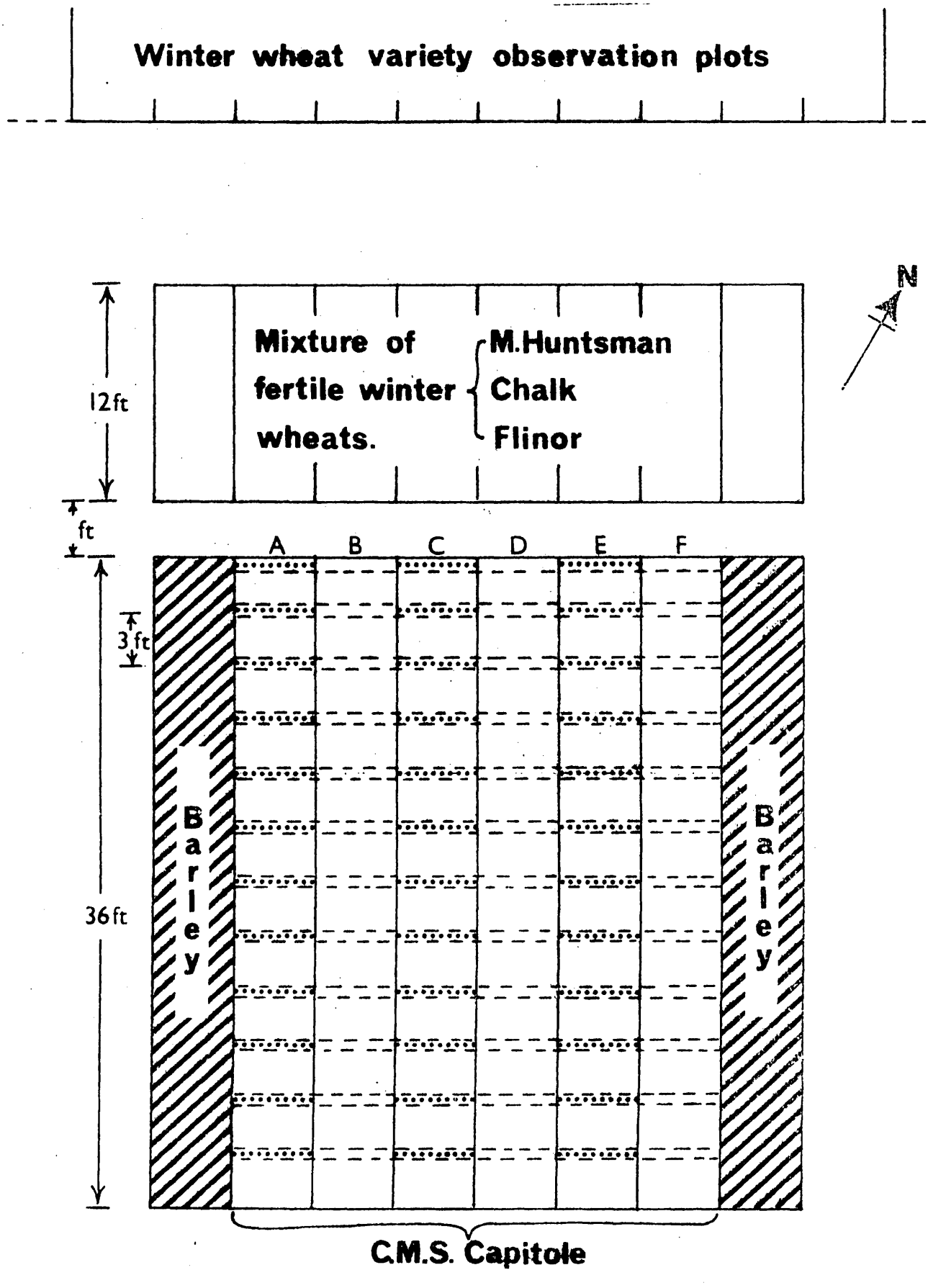
X = Position of greased slides used to trap pollen.

= Wind direction recorder.

Figure 9.

Design of the field experiment to investigate the effects of seed set on infection of cms Capitole by ergot.

C.M.F. (Fingest), 1974.



16.5 Experiments to screen wheat cultivars for resistance to ergot disease

16.5.1 In the glasshouse (C.P.G. April/May 1973)

The following wheat cultivars were grown in the glasshouse in a ramdomised block arrangement, each block consisting of one pot of each of the seven cultivars:

cms	Shawnee	(Winter wheat)
"	Capitole	"
"	Cappelle Desprez	"
"	42	"
"	46	"
Fertile	Carleton	(Spring wheat)
"	Kenya Farmer	"

During flowering of the male-sterile plants, and at anthesis of the fertile plants, four ears from each pot were inoculated by injecting a spore suspension of strain 25B into 20 florets of each ear. The total number of spores in the honeydew which subsequently exuded from one of the four inoculated ears in each pot was measured as follows. From seven days after inoculation, all droplets of honeydew were collected daily until exudation ceased, at which time traces of honeydew remaining on the ears were washed off. The combined honeydew and washings were made up to 200ml with water to which 10% phenol and a few drops of Tween 80 had been added. The suspensions were shaken for 10 minutes to ensure uniform dispersion of the spores, whose concentration was determined by the use of a haemocytometer.

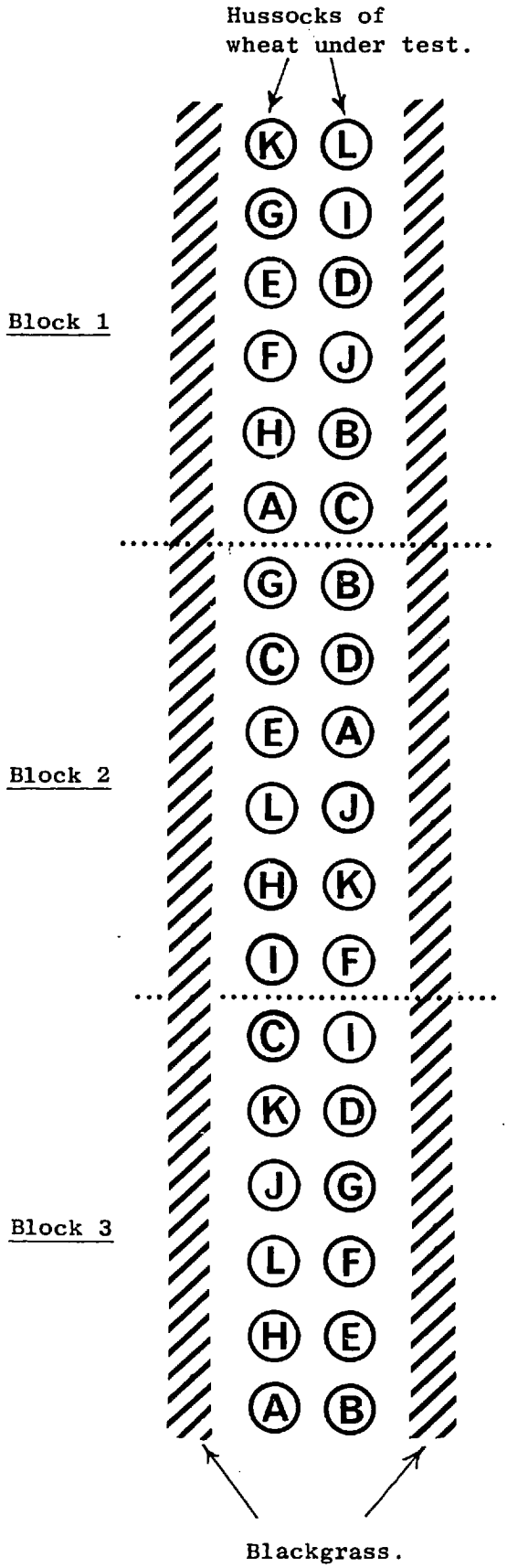
16.5.2 In the field (Throws Farm, 1974)

Screening of twelve male-sterile spring wheat cultivars for field resistance to ergot was achieved by means of a modified "spreader bed" technique (Doling, 1967). Wheat varieties under investigation were sown in rows of hussocks alongside rows of blackgrass plants (fig.10); hussocks being sown with 16 seeds in each. Blackgrass was inoculated, by spraying the flowering heads with a suspension of honeydew spores of strain 25B, on June 10th (this being 2-3 weeks before the expected date of flowering of spring wheats at Throws Farm) to ensure that infected grass heads would be exuding honeydew by the time the wheat cultivars reached anthesis.

The experiment was harvested on August 20th, and each hussock was threshed to determine the number of ergots that had resulted from natural cross-infection.

Figure 10.

Design of an experiment to investigate field resistance of male-sterile, spring wheat cultivars to ergot disease.



cms Spring Wheat Cultivars:

A. Clarion.
B. Kleiber.
C. RHM 573.
D. Rask.
E. Sirius.
F. RHM 576
G. Trident.
H. RHM 578.
I. Cardinal.
J. Rothwell Sprite.
K. Maris Dove.
L. Maris Ranger.

17. Statistical analysis

17.1 Types of distribution encountered

In most experiments binomially distributed values have been measured. Individuals in a sample have been regarded as having, or not having, a particular character, e.g. infection or non-infection of florets by ergot; germination or non-germination of spores.

17.2 Standard error

Throughout the text, the standard deviation of the mean (standard error) has been calculated as S.E. = $\frac{\sigma}{\sqrt{n}}$ (where σ = the estimated standard deviation of the population mean and n = the number of individuals constituting the sample mean). Limits for the mean at a particular level of significance (normally quoted at the 95% level) are calculated from: SE x the value of t at n-1 degrees of freedom. The sample means are thus expressed as mean $= \bar{x} \pm t\frac{\sigma}{\sqrt{n}}$ (level of significance), (Bishop, 1966).

17.3 Analysis of variance

Where applicable, the analysis of variance technique (Snedecor and Cochran, 1972) has been applied to data comparing three or more treatments in randomised and replicated experiments.

Least significant differences (L.S.D.) between treatment means have been calculated as follows:-

$$\sigma = \frac{\sqrt{2 \times \text{residual mean square}}}{\text{Number of observations per treatment}}$$

L.S.D. = σ x value of t for the degrees of freedom of the mean square

Binomially distributed data, expressed as proportions or percentages often give rise to a skewed distribution of observations about the mean. This effect is particularly marked when dealing with data representing high or low percentages, or with proportions which approach unity or zero, when the distribution of points to one side of the mean is restricted. Most data of this type has therefore been transformed by means of the arcsine transformation (Bartlett,1937; Bliss, 1937) to give values that are approximately normally distributed before proceeding with statistical analysis.

RESULTS

18. Observations on the flowering mechanisms and ecology of grasses in relation to their possible role as carriers of ergot disease of wheat

It is well known that ergot disease may spread to susceptible aereal crops from infected grasses indigenous and/or adjacent to arable land. (Section 7.1). Cross-inoculation experiments (Section 21.1) have shown that some grasses carry wheat-infective strains of ergot; but other factors including the flowering date, field susceptibility to ergot, and the ecology of individual grass species must also be taken into consideration before their possible role in the cycle of infection of ergot of wheat can be properly assessed. Since it was not within the scope of this study to undertake a comprehensive investigation of grasses, information has been drawn from the literature and supplemented with field observations which particularly relate to ergot infection.

Grasses and cereals are only susceptible to infection by ergot during their flowering period. The earliest date of flowering of grasses in relation to the period of susceptibility of wheat is thus an important factor governing the role of individual grass species as alternative hosts in the cycle of infection of wheat.

A summary of observations of dates of anthesis of grasses in 1972-74 is shown in table 3, together with a list of flowering dates described by Hubbard (1968) and Armstrong (1948).

Discrepancies between current observation and the abstracted literature, may, in part, be due to the use of the term "flowering" by both Hubbard and Armstrong, which is not clearly defined as the date of anthesis - in many grasses there is an appreciable time interval between emergence of inflorescences from the leaf sheath and anthesis, which may be as long as 2 to 3 weeks under cool or overcast weather conditions. Allowance must also be made for variation of seasonal flowering time due to the effect of environmental conditions on vernalization, photoperiodic and other inflorescence initiation requirements (Evans, 1964).

Warm, sunny weather prevailed during Spring and early summer, 1974. Although flowering of grasses was not observed in detail, it was noted that, in general, flowering of many grasses was 1-2 weeks in advance of the previous year. However, since anthesis of winter wheats was also recorded as being 7-10 days earlier in 1974 than in the previous two years, the comparison of earliest dates of anthesis of grasses with the period of anthesis of wheat (table 3) was still generally applicable.

In table 4, other factors which may influence the potential role of April-June flowering grasses in the aetiology of ergot disease of wheat have been summarised. Columns 3, 4 and 5 are based on observations during 1972-74 coupled with

descriptions of the habitat and abundance of grasses by Hubbard (1968), and accounts of flowering mechanisms by Knuth (1908), Beddows (1931) and Evans (1964). Claviceps purpurea has been recorded as a pathogen of a wide range of grass species in temperate climates (Brady 1962), but some grasses are more susceptible than others. Column 6 relates to the frequency of ergot infection of particular grass species, and is based mainly on the frequency of collection, in Britain, of ergot sclerotia from grass species during 1971-73 (table 1). Referring again to table 4, those grasses having a protogynous flowering mechanism appear to be most commonly infected by ergot. That is, where chasmogamy is encouraged by stigmas being exserted a few days before anthers from the same floret dehisce, as in Alopecurus spp., individual florets are, in effect, male-sterile for a short time and are thus more susceptible to ergot than are self-fertilizing grass species.

Table 3.

Observations of the flowering dates of common British grasses.

Grass species.	Dates of anthesis of most advanced inflorescences recorded at:		Stage of flowering on particular dates at:		Flowering dates* according to:		Relation of flowering of grass species to anthesis of winter wheats.**
	C.P.G.(1972).	Kew(1972)	Reading.(7.6.72)	Other localities.	Hubbard(1968)	Armstrong(1948)	
Agropyron repens.	-	July	-	Anthesing on 6.7.72, 2.7.73 at C.M.F.	June-Aug.	July.	C-D
Agrostis canina.	July	July	Not heading.	-	June-Aug.	June -July	C-D
A. stolonifera.	July	-	-	-	July-Aug	July-Sept.	D
A. tenuis	July	July	Not heading.	-	June-Aug	June-Sept.	D
Alopecurus geniculatus	-	-	-	Post-anthesis at: L.E.F. on 14.6.73 W.P.B.S. on 11.6.73	June-Aug	June	B
A. myosuroides	23 May (Stigmas exserted)	-	-	Post-anthesis at: Henley on 21.5.73 L.E.F. on 15.5.73 Throws Farm on 20.5.74	May-Aug.	-	A

* Not necessarily equivalent to anthesis (see p 74) /continued.

** Under normal cropping conditions of winter wheats, the first anthesing grass inflorescences could:-

A. be infected by ergot and produce honeydew before anthesis of the earliest heads of wheat.
B. " " " " " " " " " " " latest " " "
C. " " " " but seldom produce honeydew before anthesis of the latest heads of wheat.
D. not be infected by ergot before anthesis of the latest tillers of wheat.

Table 3 (continued)

Grass species.	C.P.G.(1972).	Kew(1972).	Reading. (7.6.72)	Other localities.	Hubbard(1968)	Armstrong(1948)	Relation to wheat anthesis.
A. pratensis.	19 May (Stigmas exserted)	3rd wk.May.	Post-anthesis.	First anthesis on: 30.5.73 at L.E.F. 25.5.74 at Throws.	Apr.-June	Apr.-June.	A
Anthoxanthum odoratum.	23 May (Stigmas exserted)	2nd wk.May.	Post-anthesis.	Post-anthesis at: W.P.B.S.(11.6.73)	Apr.-July.	Apr.-May.	A
Arrhenatherum elatius.	30 June.	July.	Pre-anthesis.	Pre-anthesis at: W.P.B.S.(11.6.73) L.E.F.(14.6.73)	June-Sept.	June.	C-D
Brachypodium sylvaticum.	July/Aug.	July.	-	-	July-Aug.	-	D
Bromus spp.	-	-	-	-	May-Aug.	-	A-B
Bromus sterilis.	17 June.	-	-	-	May-July.	May	B(?)
Dactylis glomerata	15 June	3rd wk.June	Pre-anthesis	Anthesing at: W.P.B.S.(11.6.73) C.M.F.,G.R.I.and L.E.F. during 2nd-4th wk.June,1973.	June-Sept.	Early June.	B-C
Deschampsia caespitosa	August.	July.	-	-	June-Aug.	-	D

/continued.

Table 3 (continued)

Grass species.	C.P.G.(1972).	Kew(1972).	Reading. (7.6.72)	Other localities.	Hubbard(1968)	Armstrong(1948)	Relation to wheat anthesis.
Festuca arundinacea	28 June.	5 July.	-	-	June-Aug.	Early July.	C-D
F. pratensis	17 June.	-	-	-	June-Aug.	-	B-C
F. rubra	21 June.	3rd wk.June.	-	-	May-July.	June-July.	B-C
Glyceria fluitans	-	-	-	Anthesed at L.E.F. on 24.5.73	Late May-August.	Late June.	A-B
Holcus lanatus	4th wk.June.	-	Pre-anthesis.	Pre-anthesis at W.P.B.S.(11.6.73) Anthesing at L.E.F. on 14.6.73.	May-August	June-July.	C
H. mollis.	Early July.	Mid July.	-	Pre-anthesis at W.P.B.S.(11.6.73) (Later than H.lanatus)	June-Aug.	June-July.	C-D
Lolium perenne cv.S23.	12 June.	-	30% of heads at anthesis.	Pre-anthesis at: W.P.B.S. on 11.6.73 L.E.F. on 14.6.73	May-August.	Early June.	B-C
cv.S24.	1 June.	1st wk.June.					A-B
L. multiflorum.	10 June.	-	-	-	June-Aug.	-	B-C
Phleum pratense.	-	3rd wk.June.	Not heading.	Anthesing at L.E.F. on 28.6.73.	June-Aug.	July.	C-D
P. bertolonii.	July.	July.	-	-	June-Aug.	-	D

/continued.

Table 3 (continued)

Grass species.	C.P.G.(1972).	Kew(1972).	Reading. (7,6.72)	Other Localities.	Hubbard(1968)	Armstrong(1948)	Relation to wheat anthesis.
Phragmites communis.	-	-	-	-	Aug-Oct.	August.	D
Poa annua.	All year.	-	-	-	All year.	-	A
P. pratensis.	-	-	Pre-anthesis.	Anthesing at: W.P.B.S.(11.6.73) Beaconsfield on 15.6.73	May-July.	Late May-June.	A-B
P. trivialis.	-	-	-	Anthesing at L.E.F. on 14.6.73	June-July.	Midsummer	C
Spartina spp.	-	-	-	-	Sept-Oct.	-	D

Table 4.

Observations on the flowering, abundance, habitats and frequency of ergot infection of some common British grasses.

Grass species.	Flowering in relation to wheat anthesis. (see table3)	Habitat.	Abundance.	Flowering mechanism.	Frequency of ergot infection.
Alopecurus geniculatus.	B	Moist areas.	Widespread, frequent.		-
A. myosuroides.	A	Arable weed; also on waste land.	Common on clay soils.		Very common.
A. pratensis	A	Field margins, low lying areas.	Common.	Markedly protogynous: Stigmas exposed and self pollination is generally excluded.	Common, but only following a wet spring.
Anthoxanthum odoratum.	A	Wide range, incl. arable.	Widespread; frequent.		Infrequent.
Arrhenatherum elatius.	C-D	Rough grass-land and hedgerows.	V. common.	Andromonoecious: Cross pollination favoured by dehiscence of anthers outside the floret.	Common.
Bromus spp.	B	Arable and waste land.	Common.	Homogamous: Mainly self-fertile. Some deistogamous.	Infrequent.
Dactylis glomerata	B-C	Meadows, hedgerows, waste land.	Abundant (cultivated)	Protogynous: Cross pollination followed by automatic self-pollination.	Very common.

/continued.

Table 4.(continued)

Grass species.	Flowering in relation to wheat anthesis(see table 3)	Habitat.	Abundance.	Flowering mechanism.	Frequency of ergot infection.
Festuca pratensis.	B-C	Low - lying grassland, old pasture, hedgerows.	Common.	Homogamous: mainly self-fertile.	Occassional.
F. rubra.	B-C	Coastal, lowland and hill pastures.	Widespread; abundant.	Homogamous: " " "	-
Glyceria spp.	A-B	Shallow water and ditches.	Frequent.	Protogynous: Self pollination is excluded.	Common.
Holcus lanatus.	C	Pastures, wasteland and sometimes arable.	V. common.	Andromonoecious: Cross-pollination and self-pollination equally favoured.	Very common.
Lolium perenne.	A-C	As above.	V. common.	Feebly protogynous.	V. common in late summer.
L. multiflorum.	B-C	As above.	V. common.		As above.
Poa annua.	A	Many habitats, often an arable weed.	V. common.	Homogamous: generally self-fertile.	Occassional,but difficult to observe ∴ may be more common than it appears.
P. pratensis.	A-B	As above.	Common.		Infrequent.

19. Results of experiments to investigate techniques for preparation and storage of inoculum

During a preliminary investigation of the germinability of a) conidia taken from cultures of isolate 25, and b) conidia collected from honeydew exuding from host plants infected by the same isolate, it was found that whilst the latter were 100% viable, only 59% of the culture spores germinated (table 5). This low rate of germination was not improved by washing the spores in sterile distilled water prior to the gemination test. Furthermore, it was noted that the time taken for germination of culture spores was more than 30 hours at 24°C; almost double the time taken for honeydew spores to germinate under the same conditions. In view of these observed differences in germination potential, considered together with reports that the pathogenicity of isolates may decline in culture (Mantle, 1967; Kirchoff, 1929) it was decided that for the experiments reported in this thesis, honeydew would, wherever possible, be used as a source of inoculum.

Preliminary investigations indicated that storage under liquid nitrogen would be the most suitable technique. Freeze drying of spores was initially rejected since it was difficult to prevent cross-contamination of samples on the multiport freeze drier. Short-term storage of conidia in neat honeydew, or in a

10% sucrose solution did not impair germination, but contaminants (especially bacteria present in the original honeydew sample) multiplied over a longer period of time to an unacceptable level. Storage of spores under liquid nitrogen eliminated the contamination problems and had an advantage over the other storage techniques in that spore suspensions of known concentrations could be prepared and stored ready for immediate use. This proved to be particularly advantageous in field experiments when it was necessary to perform a large number of inoculations during a short time at anthesis of wheat.

A number of variations of the technique for storage of spore suspensions under liquid nitrogen (p37 and table 1) were investigated (table 7). The addition of glycerol to aqueous spore suspensions clearly increased the rate of survival of spores during the freezing and thawing processes. Although 15% glycerol (w/v) in the suspension appeared to give optimum protection, the addition of 10% glycerol (w/v) was preferred on the basis of a compromise between maximum survival of spores and minimum risk of any phytotoxicity of the glycerol. Different rates of pre-cooling of ampoules to -50°C, regulated by different amounts of insulation, had little effect on the rate of survival of spores. However, treatment A (table 1) was found to be most convenient since the polystyrene box also maintained the low temperature of the ampoules during transfer to liquid nitrogen storage refrigerators. Additional insulation only served to delay the transfer procedure.

After a period of storage under liquid nitrogen; rapid warming of ampoules by transferring them immediately to a water bath at 30°C gave the best results. Insertion of an intermediate stage in the warming up process involved storage of ampoules alongside solid carbon dioxide, which was necessary on some occasions when transferring inoculum from Imperial College to High Wycombe. This did not reduce germination if it was followed by the rapid warming technique (table 1). A loss of 5% viability of spores over the control was measured even when the best available storage technique was employed. Some of this loss may be accountable to overheating of droplets of the spore suspension remaining in the neck of an ampoule as it was sealed. Samples of spores from treatments K, M and P were also tested for their pathogenicity to wheat. Results in table 7 indicate that pathogenicity was not impaired during storage, but these results should be viewed with caution. Although initially incorporated in a replicated design, test plants used for this experiment were found to be partially fertile (see p. 51) and results obtained from sterile wheat heads had to be combined to give a single, unreplicated value for each treatment.

Confirmation that pathogenicity to wheat of isolates had not diminished during storage in liquid nitrogen was eventually obtained by the high rate of infection of wheat plants inoculated with honeydew stored for 1 and 2 years (table 6). By means of

applying Students "T" test to the measurements of mean weights per ergot, and to the arcsine transformations of % infection (T score), no significant difference between treatments was found.

Table 5

Results of an experiment to compare the viability of conidia obtained from culture with natural honeydew spores.

Origin of conidia.	Mean % germination.	Mean T Score.	S.E.*
Culture	58.9%	50.1	± 1.00
Honeydew	100%	90.0	± 0

* Limits for standard error of the mean T score at 95% significance. (19 d.f.)

Table 6.

Results of inoculation of wheat with spore suspensions of isolate 25 (table 2) after storage in liquid nitrogen for 0, 1 and 2 years.

Treatment.	Mean weight per ergot (g.)	S.E.*	Mean number of ergots per 20 inoculated florets.	Mean T score.	S.E.*
Control. (Fresh honeydew)	0.048	± 0.003	18.0	71.7	±2.1
Storage for 1 year.	0.050	± 0.004	18.1	75.7	±9.2
Storage for 2 years.	0.050	± 0.003	18.4	75.8	±7.8

* Limits for standard error of the mean at 95% significance (7 d.f.)

Table 7.

Results of germination and pathogenicity tests on conidia following various techniques of storage in liquid nitrogen, as described in Table 1.

TREATMENT (See Table 1).	TESTING THE EFFECT OF:-		GERMINATION.	T.SCORE S.E.*	% INFECTION OF WHEAT
C	Glycerol	0%	77.1%	61.3 ± 4.3	
B		5%	83.7%	66.2 ± 4.3	
A		10%	89.3%	71.1 ± 6.0	
D		15%	94.1%	75.9 ± 3.0	
E		20%	90.3%	71.9 ± 1.3	
G	Rapid cooling.		84.3%	66.7 ± 4.7	
A	Slow cooling.		89.3%	71.1 ± 6.0	
F	Very slow cooling		85.0%	67.0 ± 4.3	
H	Warming at -10°C then 2°C		89.6%	71.3 ± 1.3	
A		2°C	89.3%	71.1 ± 6.0	
K		30°C	94.8%	77.1 ± 3.4	75%
L	Intermediary stage in cardice followed by warming as in :	H	82.4%	65.4 ± 1.4	
M		K	95.1%	77.4 ± 1.9	80%
P	Control (fresh conidia)		99.6%	88.1 ± 1.9	75%

*Limits for standard error of mean T scores at 95% level of significance (2 d.f.).

20. Investigations of field germination and ascospore discharge by wheat ergots

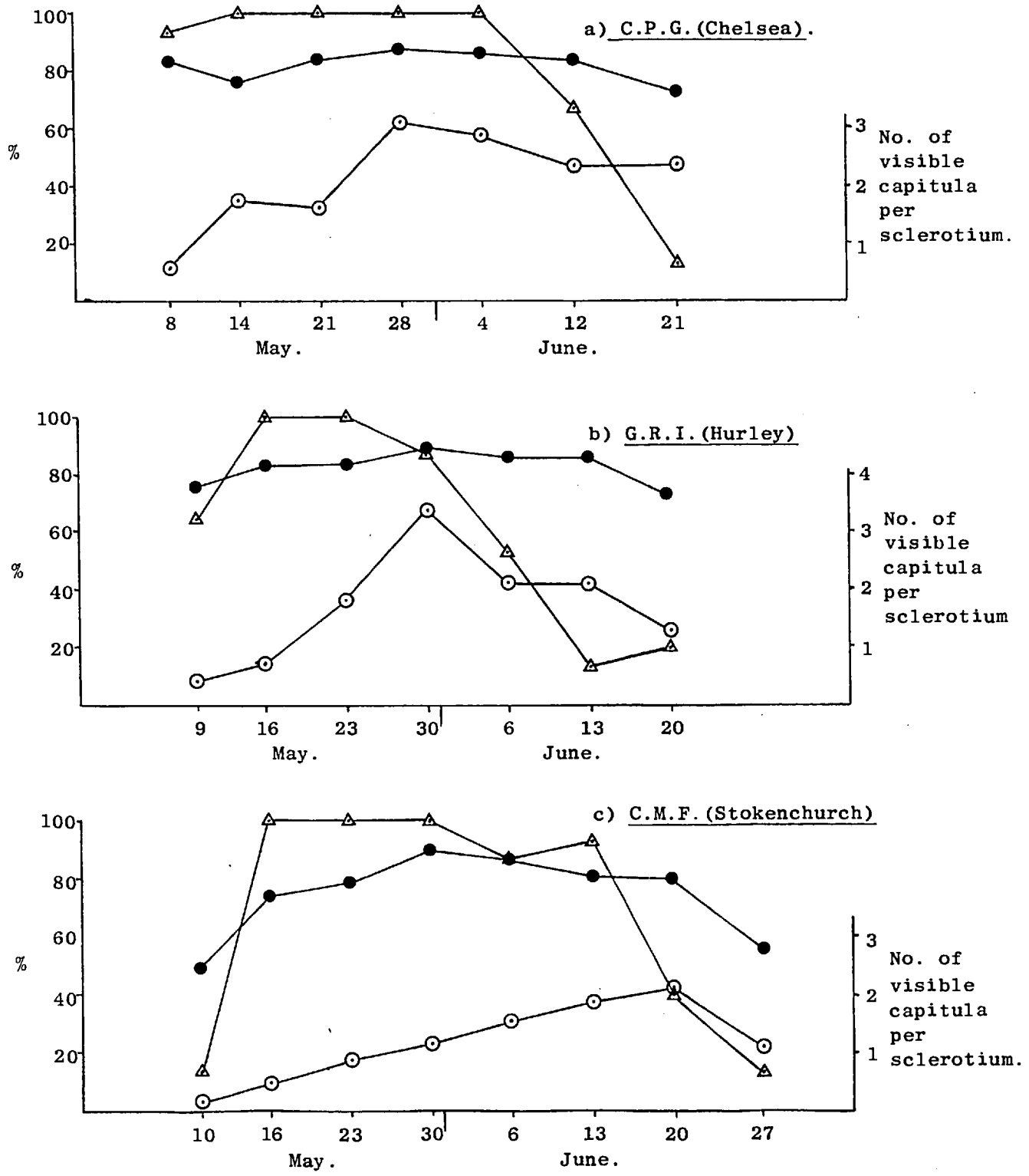
20.1 1972/73 Experiment

Field experiments incorporating samples of ergots buried in field soil were established at three sites during the autumn of 1972, as described in section 16.1 . The soil surface above the buried ergots was examined at approximately monthly intervals throughout the winter, but no sign of ergot germination was observed until 2nd May at the C.P.G., and on 9th and 10th May at the G.R.I. and C.M.F. respectively, when immature capitula (perithecial fruiting bodies) began to push through the soil surface. Results of examination of samples of ergots taken at weekly intervals from all sites are summarised in figure 11 a-c.

At all three sites, the maximum % germination was recorded by the end of May. Furthermore, at two of the experimental sites, the numbers of capitula visible above the soil surface did not increase after the beginning of the first week of June, and also reached a maximum at the third site, C.M.F.(Stokenchurch), by mid June. From graphs a-c in figure 11 it would appear that subsequently both the % germinated sclerotia and the number of visible capitula declined; though this result may be regarded as an artifact caused by the rotting of the senescent stromata.

Figure 11.

Field germination and spore discharge potential of sclerotia at three experimental sites during May/ June, 1973.



The ascospore ejection test (p 60) demonstrated that, at all sites, over 50% of the germinated sclerotia had lost their ability to discharge ascospores by the time wheat reached anthesis, presumably because the spores had already been discharged in the field. Stages of ergot germination and ascospore discharge were not co-incident at the three experimental sites (figure 11). Ergots buried at C.M.F. continued to produce fresh capitula and thus discharge ascospores 2-3 weeks later than ergots at the other sites. 17%, 37% and 34% of capitula of germinated ergots at C.P.G., G.R.I. and C.M.F., respectively, failed to reach the soil surface and were therefore prevented from discharging ascospores into free air. Such ergots were generally situated at the bottom of the foil dishes, and hence beneath 1"-1½" of soil. Occasionally fruiting bodies growing out from sclerotia near to the soil surface were obstructed by small stones, or roots of self-sown weed seedlings.

From 20th to 25th June, 1973, at about the time of anthesis of cms Capitole, daily counts of ascospores in air sampled at 0.5 metres above the soil surface were obtained by means of the Burkard spore trap. Prior to the installation of this trap, indirect

evidence that ascospores had already been discharged from the germinated ergots was obtained in two ways. a) By the ascospore ejection tests as described above, and b) by the discovery of honeydew on near-by blackgrass which indicated that infection had occurred during the fourth week of May (p.141). Since dry weather conditions prevailed from the end of May until the day before spore trapping commenced, it is unlikely that any more ascospores were discharged during this period. All capitula near to, or above, the soil surface were desiccated. However, comparison of daily spore counts with daily rainfall measurements (fig.12) showed that ascospores were discharged from the re-hydrated fruiting bodies within 52 hours after the onset of rain on the 19th June. Spore discharge continued with no apparent diurnal pattern for three days, during which time no further rain was recorded, and the soil gradually dried out again.

a) 1973/74 Experiment

The ergot germination experiment was repeated, with some modifications to its design as described on p 67 at C.M.F. (Fingest). The initial stages of germination were first observed on the surface of ergots sampled on April 11th. Median sections through ergots that had

Figure 12.

Rainfall measurements and results of trapping air-borne ascospores during anthesis of cms Capitole, June 1973.

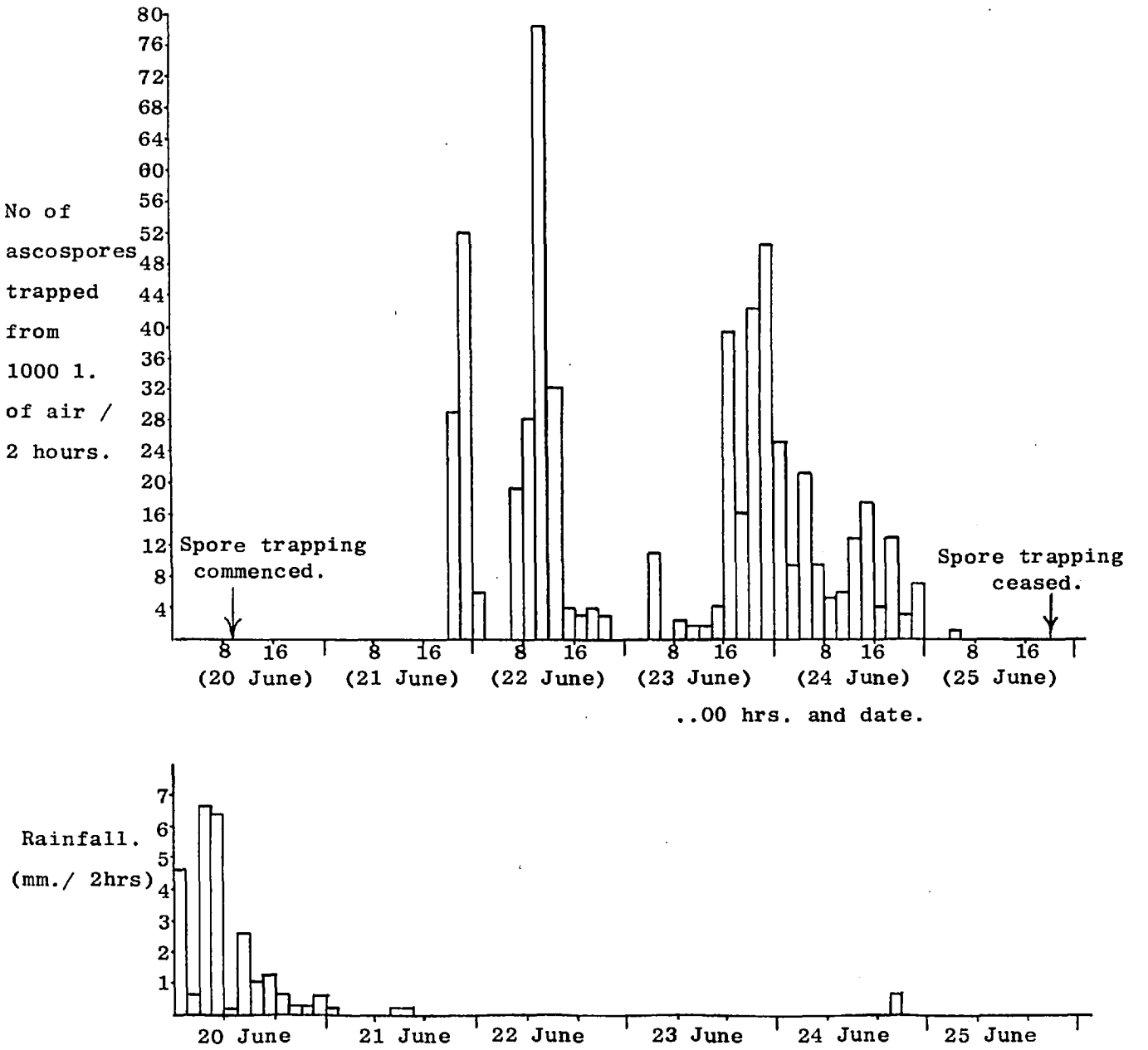
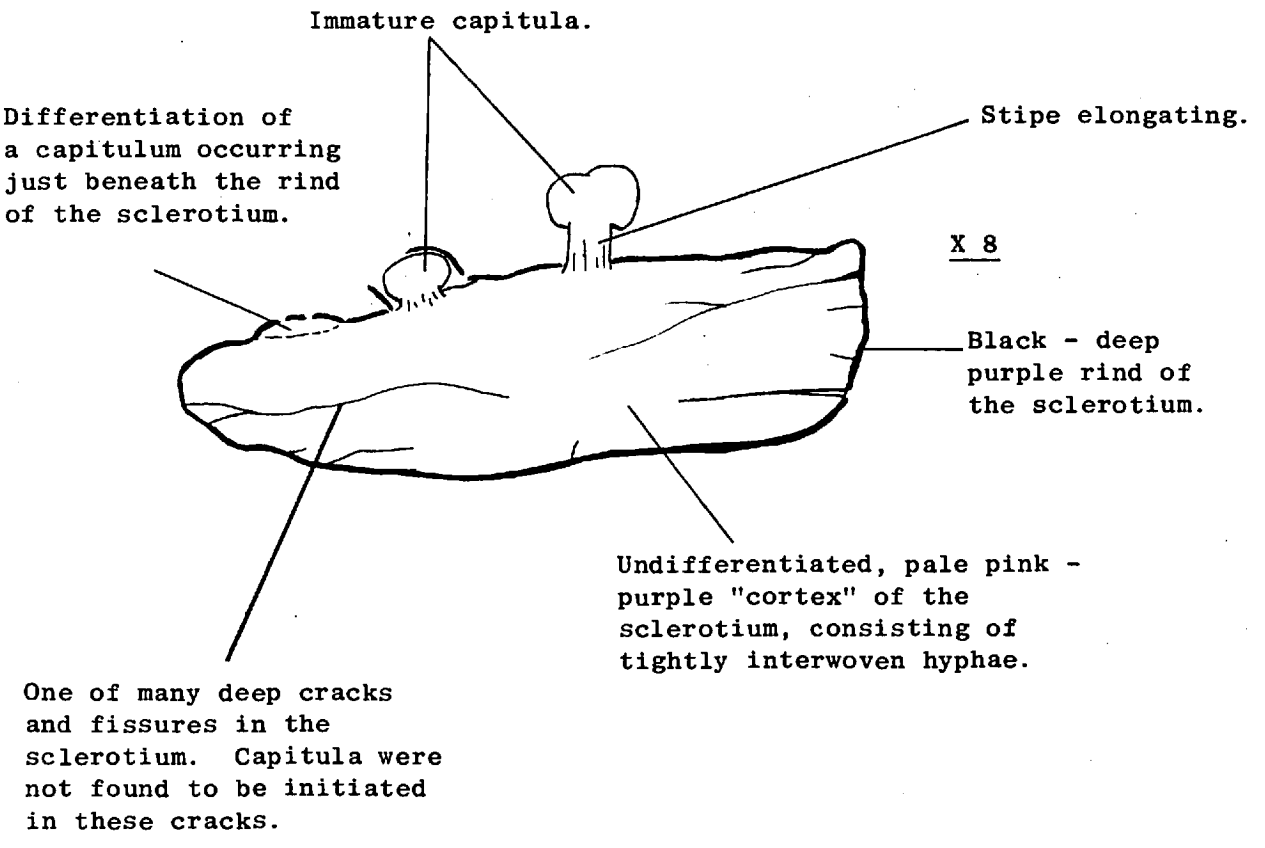


Figure 13.

Composite diagram showing a longitudinal section through an ergot sclerotium and immature capitula.



just started to germinate showed that ascocarp initials originated in the cortex of sclerotia immediately below the pigmented rind. The capitulum developed first, rupturing the rind as it increased in size. There was no macroscopic evidence of reorganisation of the cortical, plectenchymatous mycelium into pathways for translocation of nutrient reserves between the remainder of the sclerotium and the developing ascocarp.

Immature capitula, yellow/light brown whilst protected from sunlight beneath the soil surface, were pushed away from the sclerotia as stipes developed and elongated (Fig.13). As defined previously (p.33) a sclerotium was not regarded as having germinated until it had produced at least one capitulum that was separated from the sclerotial body by a distinct stipe.

April, May and June of 1974 were notable for the lack of rain (figure 14c) and germinating ergots were even more affected by drought than they had been during the previous year. In figure 14a it can be seen that whilst germination of ergots was first recorded at the end of April (as in the previous year) further progress of the germination process was, by comparison with germination of ergots on the same farm in 1973, considerably retarded.

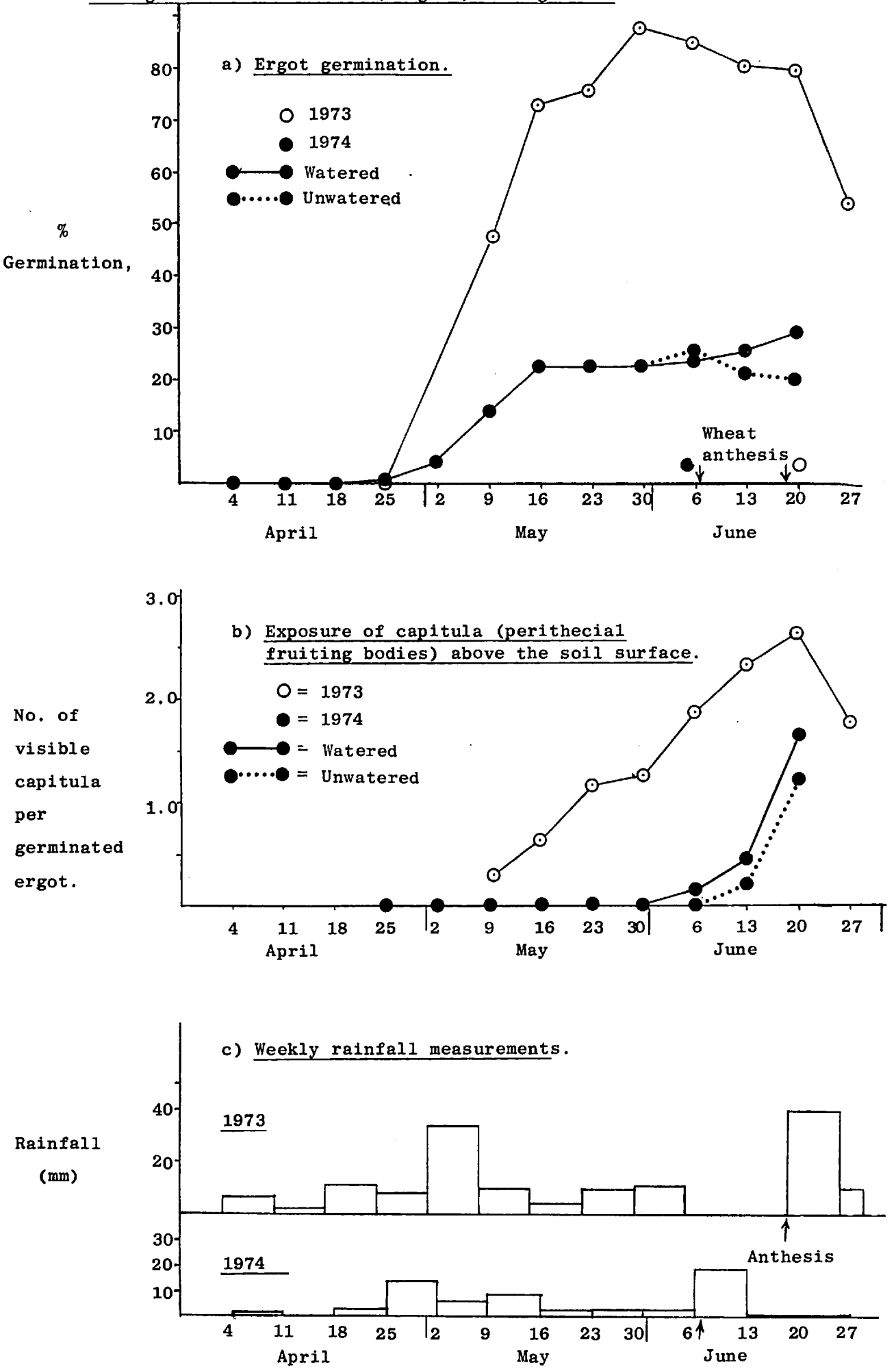
Low soil temperatures (3-15°C) and decreasing soil moisture content during late April and the first two weeks of May probably accounted for this initial retardation. Furthermore, continued drought, and hence extremely dry surface soil, throughout the latter half of May and early June undoubtedly prevented any further development until the onset of wet weather conditions in the second week of June.

Similarly, whereas in the previous year many mature capitula were visible at the soil surface by the third week of May, capitula were not observed in 1974 until the first week of June; and even then few capitula (less than 0.5 per germinated ergot) had reached the soil surface before the end of the followingweek. (figure 14b).

On the 4th June, in the absence of a firm weather report predicting rain, daily watering of half of the experiment commenced. The spore trap was left in position alongside the unwatered half of the experiment. Although drought conditions were interrupted by rain four days later, intermittent periods of dry weather did ensure that a differential between "wet" and "dry" halves of the experiment was maintained for 2-3 weeks. Ergots in both

Figure 14 a-c.

Results of ergot germination experiments at C.M.F.(Stokenchurch) during 1973 and at C.M.F. (Fingest) during 1974.



halves of the experiment showed a rapid response to the termination of drought (figure 14a-c). Nevertheless, some additional response to watering was measurable, both in terms of % germination and the number of capitula reaching the soil surface per germinated ergot. On close examination of ergots collected on the final sampling date, no sign of further initiation of the germination process was found in the majority of the ungerminated ergots.

Anthesis of the wheat cultivar, cms Capitole, was abnormally early in 1974; the first record of flowering of the primary tillers being noted on the 7th June. Continuous sampling of the air by means of a small suction trap (figure 2), at approximately 7 cms above one of the dishes of germinating ergots (within the crop canopy), did not reveal the presence of ascospores until 10th June. Thus, the first opportunity for ascospore-infection of cms Capitole would have been 3-4 days after primary tillers had anthesed. Despite the presence of airborne ascospores during part of the susceptible period of cms Capitole, only a low level of infection (less than 100 ergots per standard drilled plot) was recorded.

21. Results of studies on host restriction of strains of ergot from cereals and grasses

21.1 Cross-inoculation experiments

A total of 47 strains of ergot, isolated from various grass and cereal hosts, were used to inoculate winter wheat (cms Capitole) in two similar field experiments (section 16.2) in consecutive years.

Spore suspensions of eight of the isolates used in the 1972 experiment were taken directly from culture. None of these were highly infective to wheat (table 8) but since it was not possible to check that these isolates had fully retained their pathogenicity during the period of growth in culture, results were not included in any subsequent statistical analyses or summaries.

Table 8

Results of inoculation of cms Capitole with spore suspensions of sclerotial isolates grown only in culture

Accession No.	Original Host species	Av.% Infection (3 samples)
72	Deschampsia caespitosa	1.1
9	Agrostis sp.	0.2
70	Molinia caerulea	0.2
74	Nardus stricta	0.2
76	Brachypodium sylvaticum	0.2
56	Phragmites communis	0.0
87	Ammophila arenaria	0.0

Spore suspensions of all the remaining isolates used in 1972 (table 9) and all isolates used in 1973 (table 10) were prepared from parasitically produced "honeydew" and so they were, at least to their respective original host plants, known to be pathogenic.

In tables 9 and 10, isolates are ranked in order of their average % infection, which has been calculated from the number of ergot sclerotia found in inoculated wheat florets (see definition of an ergot sclerotium, p.33). Secondary infections in the distal florets of each inoculated spikelet, or in adjacent spikelets, were not included in the counts. The data, analysed separately for the two experiments, was first transformed from percentages to degrees (p.85). Since, in both experiments, the cross-inoculation results segregated into two distinct groups of treatments, each experiment was subjected to two analyses of variance; one for each group. Owing to experimental error, results were not obtained for one sample of isolate 102 in the 1972 experiment, and for one sample of isolate 227 in the 1973 experiment. A missing value technique (Snedecor and Cochran, 1972) was applied to estimate these values and the degrees of freedom of the residual S.O.S. was adjusted accordingly.

Table 9.

Results of cross-inoculation of strains of ergot from grasses and cereals to cms Capitole: 1972 experiment.

	Accession No. of isolate.	Original host of isolate.	Mean % infection.	Mean T score.*	L.S.D. of angles (p=0.05)
Group A	47	Triticale	86.3	64.8	5.85
	44	Alopecurus myosuroides.	85.0	67.2	
	25	Wheat	81.7	64.8	
	5	Poa annua.	80.4	63.8	
	17	ms Barley.	79.8	63.3	
	102	A. myosuroides.	78.1	62.5	
	82	Rye	70.2	57.0	
Group B	33	Lolium perenne	31.3	33.9	5.51
	79	Festuca arundinacea.	22.8	28.5	
	2	L. multiflorum.	13.3	21.4	
	13	L. temulentum.	7.9	16.1	
	83	L.perenne.	7.6	15.7	
	3	"	5.9	13.8	
	6	Dactylis glomerata.	3.3	9.0	
	104	Poa annua.	3.3	10.4	
	34	Arrhenatherum elatius.	3.0	9.2	
	7	Phleum pratense.	0.7	2.8	
	52	Holcus mollis.	0.7	4.0	
	77	H. lanatus.	0.2	1.5	
	68	Spartina townsendii.	0.0	0.0	
	73	Anthoxanthum odoratum.	0.0	0.0	

* T score = Arcsine transformation of % infection.

Table 10.

Results of cross inoculation of strains of ergot from grasses and cereals to cms Capitole: 1973 experiment.

	Accession No. of isolate.	Original host of isolate.	Mean % infection.	Mean T score.*	L.S.D. of angles (p=0.05)
Group A	122	Alopecurus myosuroides.	83.0	67.7	No significant difference between treatments.
	132	" "	79.1	63.5	
	143	" "	74.6	61.6	
	330	" "	74.0	60.1	
	149	" "	69.5	57.4	
	25	Wheat	68.7	56.7	
	44	A. myosuroides.	68.4	55,9	
	328	A. pratensis.	63.5	53.0	
Group B	186	Dactylis glomerata.	20.3	26.3	5.62
	210	Arrhenatherum elatius.	10.8	19.1	
	281	Lolium perenne.	4.7	12.0	
	207	Holcus lanatus.	2.8	9.6	
	212	L. perenne.	2.7	9.1	
	227	"	2.0	7.4	
	160	Dactylis glomerata.	1.7	5.7	
	187	" "	0.8	3.0	
	211	" "	0.7	1.9	
	232	H. mollis.	0.6	2.5	
	202	Anthoxanthum odoratum.	0.2	1.4	
	329	A. pratensis.	0.2	1.4	
	301	D. glomerata.	0.0	0.0	

*T score = Arcsine transformation of % infection.

In the 1972 experiment, there was no significant difference between blocks of treatments, whilst between the treatments variation was significant ($p = 0.05$) for the highly infective group of isolates (group A) and was highly significant ($p = 0.001$) for the weakly infective group of isolates (group B). The least significant difference (L.S.D.) between transformed values of the individual treatments has been calculated at the 95% level by the method of Snedecor and Cochran (1972). In table 9, treatments linked by brackets are not significantly different from each other. It is noteworthy that between the least infective isolate of group A and the most infective isolate of group B, there is an interval more than four times greater than the L.S.D. for either of the two groups.

Two-way analyses of variance of the transformed data from the 1973 experiment (table 10) showed that there was a significant different between blocks of treatments, probably as a result of the delay between inoculation of the first and second blocks. Whilst the variation between treatments in the weakly infective group (B) was highly significant ($p = 0.001$) there was no significant difference between treatments in the highly infective group (A). L.S.D., not applicable to the latter, was calculated (as above) for group B, and

again it was found that the interval between the two main groups was more than 4 times greater than the L.S.D.

Combined results of the two experiments are summarised in table 11. All seven isolates from blackgrass (Alopecurus myosuroides) are included in the highly infective group, together with isolates from cereals. All isolates from ryegrasses (Lolium spp) and cocksfoot (Dactylis glomerata) on the other hand are included in the weakly infective group, as are isolates from other common pasture and hedgerow grasses. One isolate from meadow foxtail (Alopecurus pratensis) and one from annual meadow grass (Poa annua) was found to be highly infective to wheat, but a second isolate from each of these two hosts was only weakly infective.

As defined (p33), % infection takes no account of aborted infections of male-sterile florets or development only of the sphacelial stage of the fungus, unless an ergot was subsequently formed. Where an isolate gave only a low percentage of successful infections, it was common to find that some florets contained sphacelia but no ergot sclerotia. Isolates 7, 210 and 281, in particular, produced many sphacelia but few ergots.

Table 11.

Summary of the combined 1972 and 1973 results of cross-inoculation of strains of ergot from grasses and cereals to wheat.

	% INFECTION OF WHEAT.	HOST ORIGIN.	NUMBER OF ISOLATES.
Group A.	63 - 86%	CEREALS.	5
		BLACKGRASS.	7
		MEADOW FOXTAIL.	1
		ANNUAL MEADOW GRASS.	1
Group B.	0 - 31%	RYEGRASSES.	7
		COCKSFOOT.	6
		SWEET VERNAL GRASS.	2
		OTHER PASTURE AND HEDGEROW GRASSES.	10

Exudation of honeydew from florets infected by the group A isolates was extremely variable, both between different isolates on the same host and, to a lesser extent, between different plants of the same host inoculated with a single isolate. Detailed notes on honeydew exudate collected from three ears chosen at random from each treatment during the 1972 experiment are summarised in table 12. There was no correlation between the volume of exudate collected from infected ears and the pathogenicity of isolates to wheat. One of the most highly infective isolates (strain 44) caused only slight exudation of honeydew which contained few spores.

Table 12

Measurements of the volume of honeydew collected from ears of wheat infected with strains of ergot isolated from grasses and cereals.

Accession No. of isolate	Volume of honeydew collected per ear (m.l.) 1	2	3	MEAN.	Notes
47	0.08	0.06	0.13	0.09	
44	0.04	0.02	Trace	0.02	Spore-free exudate
25	0.13	0.04	0.10	0.09	
5	0.07	0.15	0.16	0.13	
17	0.06	0.10	0.11	0.09	
102	0.10	0.05	0.10	0.08	
82	0.08	0.17	0.07	0.11	

Cross-inoculation of a single strain of ergot from wheat (Accession No.25) to various grasses grown in outdoor plots at the C.P.G., was attempted during 1972. Results of these inoculations (table 13) should be interpreted with some caution, since it was not always possible to inoculate individual grass species at the most ideal time (at anthesis) or under ideal weather conditions. In some instances, therefore, failure to obtain infection may have been due to sub-optimal inoculation technique. However, three of the grasses that failed to show infection, Lolium perenne, L. multiflorum and Anthoxanthum odoratum, were inoculated many times, mostly under ideal conditions. Four grasses, Dactylis glomerata, Alopecurus myosuroides, A. pratensis and Festuca pratensis were particularly susceptible to the wheat strain of ergot; a single spray application of a conidial suspension being sufficient to secure infection.

21.2 Analysis of the alkaloid content of sclerotia collected during cross-inoculation experiments

Quantitative analyses of the total alkaloid content of ergot sclerotia, which resulted from cross-inoculation of wheat with isolates of ergot from grass and cereal hosts (table 14), showed no correlation between the pathogenicity of these isolates to wheat and the total alkaloids. Whilst, in some instances, there was considerable variation between samples of the same isolate on replicated hussocks, there was some indication that the total alkaloid content was a feature of the isolate used.

In the second, qualitative, stage of analysis by thin layer chromatography, the relative proportions of the main constituent alkaloids were determined. Figure 15 shows a typical chromatogram in which the major alkaloids have been separated and identified. The alkaloids ergocornine, ergocristine and ergocryptine were not recorded individually, but are referred to collectively as ergotoxine. The ergotamine groups of alkaloids was resolved into 2 components; ergotamine and ergosine. Since the Rf values of ergotamine and ergosine differ only slightly, confirmation of their identification within each spectrum was achieved by examination of their stereo-isomers, ergotaminine and ergosinine, which were more clearly separated.

The initial object of qualitative analysis was to determine whether or not the spectrum of alkaloids, extracted from sclerotia from the original collections, was altered when the fungus parasitised a new host. Cross-infection of the wheat isolate (25) to various grasses resulted in grass ergots which had an almost identical spectrum of alkaloids to that found in the original collection of wheat ergots (figure 16) although there were slight differences in the total alkaloid content (table 13). Similarly, where comparisons could be made between the qualitative alkaloid analyses of natural collections of ergots from Gramineous hosts and of sclerotia collected from wheat after cross-infection, it was found that, with the exception of some variation in the amount of ergosine present, the relative proportions of the principal components of the alkaloid spectra were unchanged (table 15).

In table 15, two main groups of alkaloid spectra have been denoted by A and B:

A, where Ergotoxine group $\gg$ Ergotamine

B, where Ergotamine $>$ Ergotoxine group

A(Ergosine), where ergosine was a major component of the spectrum.

Figure 15.

Photograph of a thin layer chromatogram of mixtures of the three main alkaloids: Ergotamine, Ergosine and Ergotoxine.

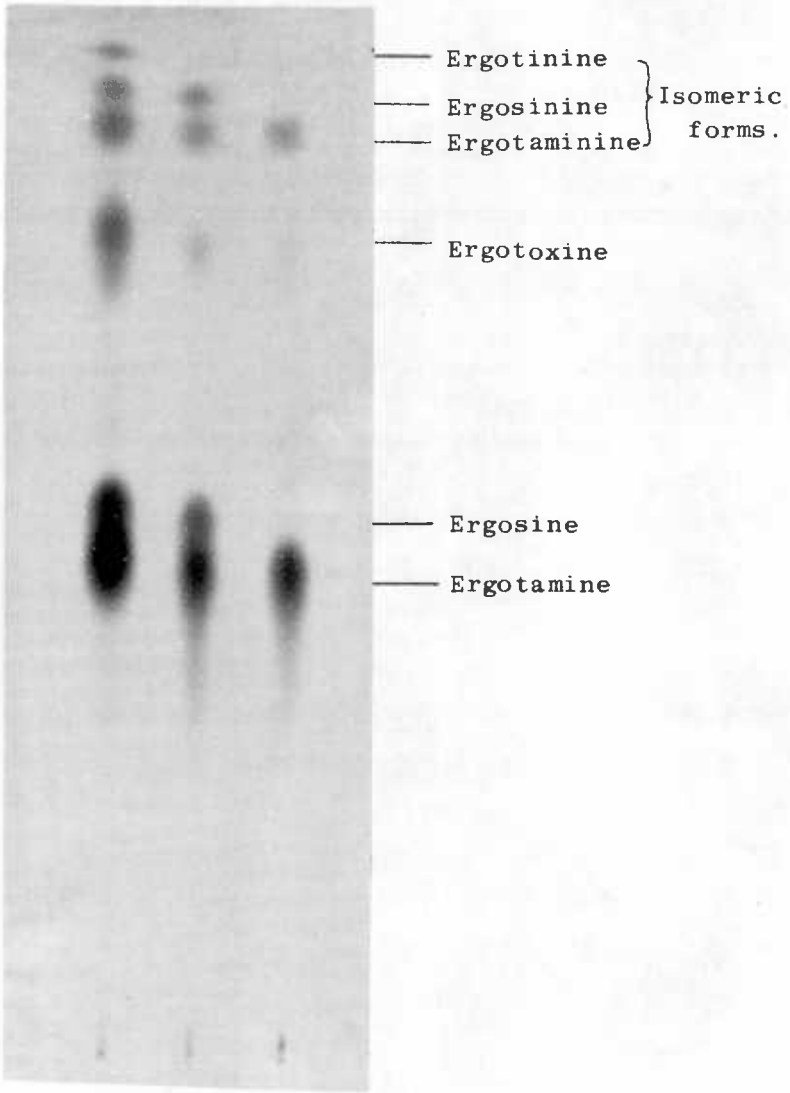
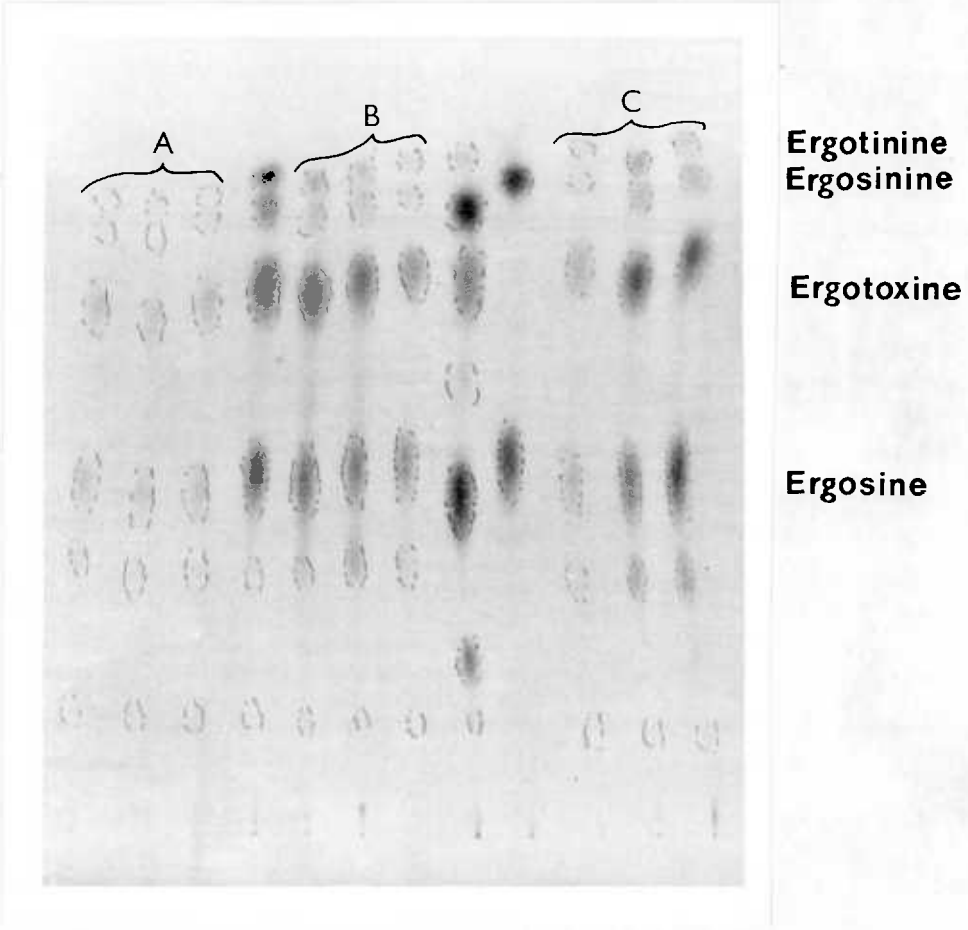


Figure 16.

Photograph of a thin layer chromatogram of the alkaloid extracts of ergots formed by infection of various grasses with strain 25B.



A. Strain 25B on Alopecurus myosuroides.

B. Strain 25B on Dactylis glomerata.

C. Strain 25B on A. pratensis.

Table 13

Results of cross-inoculation of one strain of ergot (25B-ex wheat) to various grasses.

Grass Host.	Results of cross-inoculation.	Total alkaloids (%,w/w) in resultant sclerotia.
Alopecurus myosuroides.	Readily infected by a single spray-application of a spore suspension. Copious honeydew exuded and fully developed sclerotia formed.	0.10%
A. pratensis.		0.08% (c.f.Wheat ergots: 0.17%)
Dactylis glomerata.		0.17%
Lolium perenne (S23)	No cross-infection observed after repeated spraying, injection and immersion of the inflorescences in a spore suspension.	
" " (S24)		
L. multiflorum		
Anthoxanthum odoratum		
Holcus mollis.		
Holcus lanatus.	Less than 1% of inoculated florets bore honeydew, but no ergots were formed.	
Arrhenatherum elatius.	No infection, but inoculation conditions may have been sub-optimal.	
Festuca arundinacea.		
Agrostis canina.		

Table 14.

Results of quantitative analyses of the total alkaloid content of ergot sclerotia resulting from cross-inoculation of strains of ergot from grasses to wheat. (Combined table for 1972 and 1973 experiments)

Accession No. of isolate.	Mean % inf. of wheat. (See tables 9 and 10)	Total alkaloid content of sclerotia (%, w/w): Block 1	Block 2	Block 3	Mean.
47	86.3	0.09	0.09	0.09	0.09
44(1972)	85.0	0.46	0.44	0.41	0.44
122	83.0	0.38	0.31	0.27	0.32
25B(1972)	81.7	0.18	0.15	0.18	0.17
5	80.4	0.21	0.21	0.17	0.20
17	79.8	<0.01	0.05	<0.01	<0.01
132	79.1	0.18	0.17	0.21	0.19
102	78.1	0.39	-	0.39	0.39
143	74.6	0.13	0.07	0.15	0.12
330	74.0	0.21	0.31	0.25	0.27
82	70.2	0.28	0.13	0.31	0.26
149	69.5	0.28	0.25	0.33	0.29
25B(1973)	68.7	0.13	0.15	0.17	0.15
44(1973)	68.4	0.41	0.24	0.30	0.32
328	63.5	0.25	0.26	0.30	0.27
33	31.3	0.18	0.18	0.22	0.19
79	22.8	0.22	0.21	0.24	0.22
186	20.3	0.23	0.19	0.25	0.22
2	13.3	0.36	0.31	-	0.34
210	10.8	0.03	0.001	-	0.02
13	7.9	-	0.28	0.20	0.24
83	7.6	-	0.20	0.31	0.26
3	5.9	-	0.20	-	0.20
281	4.7	0.31	0.19	-	0.25
6	3.3	-	0.09	-	0.09
34	3.0	0.42	-	-	0.42
207	2.8	0.16	-	0.25	0.21
212	2.7	0.42	-	-	0.42
227	2.0	0.23	-	-	0.23
160	1.7	0.31	-	-	0.31

Table 15

Qualitative analysis of alkaloid extracts of ergot sclerotia from wheat inoculated with strains of ergot from various grasses.

Accession No. of isolate.	Type of alkaloid spectrum. Original.	After cross-infection.	Gross changes in the alkaloid spectrum following cross-infection to wheat.
47	A	A	NIL
44(1972)	A	A	NIL
122	A	A	Loss of ergosine.
25(1972)	A	A	NIL
5	-	A	-
17	A	-	-
132	-	A	-
102	-	A	-
143	A	A	NIL
330	-	A	-
82	-	A	-
149	A	A	NIL
25(1973)	A	A	NIL
44(1973)	A	A	Sl. increase of ergosine
328	A	A	NIL
33	B	B	NIL
79	-	B	-
186	B	B	NIL
2	B	B	NIL
210	B	B	NIL
13	B	-	-
83	B	B	NIL
3	B	-	-
281	B	B	NIL
6	C	-	-
34	B	B	NIL
207	B	-	-
212	-	B	-
187	A(ergosine)	-	-

A= Predominantly ergotoxine.
B= " ergotamine.
C= Ergotoxine and ergotamine approx. equal.
A(ergosine)= Ergotoxine $>$ ergotamine, but predominantly ergosine.

Clearly, for the isolates used in the cross-inoculation experiments, there was a close relationship between the predominant alkaloid group and the degree of cross-infection to wheat. Highly infective isolates generally produced ergots that had a high ergotoxine content in relation to ergotamine (figure 17). Where sufficient material was available for analysis, weakly infective isolates were found to give ergots with a high ergotamine content in relation to ergotoxine (figure 18). Whilst it may not be justifiable to extrapolate these results (which are based on a relatively small number of samples) to give a meaningful assessment of the likely amount of cross-infection in a wide range of field situations, two general statements can reasonably be made:

1) Each isolate gave sclerotia having a characteristic spectrum of alkaloids, and parasitism of a different host species did not alter the proportions of the main alkaloid groups.
2) There was a correlation between high ergotoxine content of ergot sclerotia and high infectivity of sclerotial isolates to wheat.

These statements provide a basis for interpretation of the data collected from a nation-wide collection of ergots described in the following section.

Figure 17.

Photograph of a thin layer chromatogram of alkaloids extracted from ergots collected during cross-inoculation experiments.

I. Ergots formed as a result of infection of wheat by the highly infective isolates.

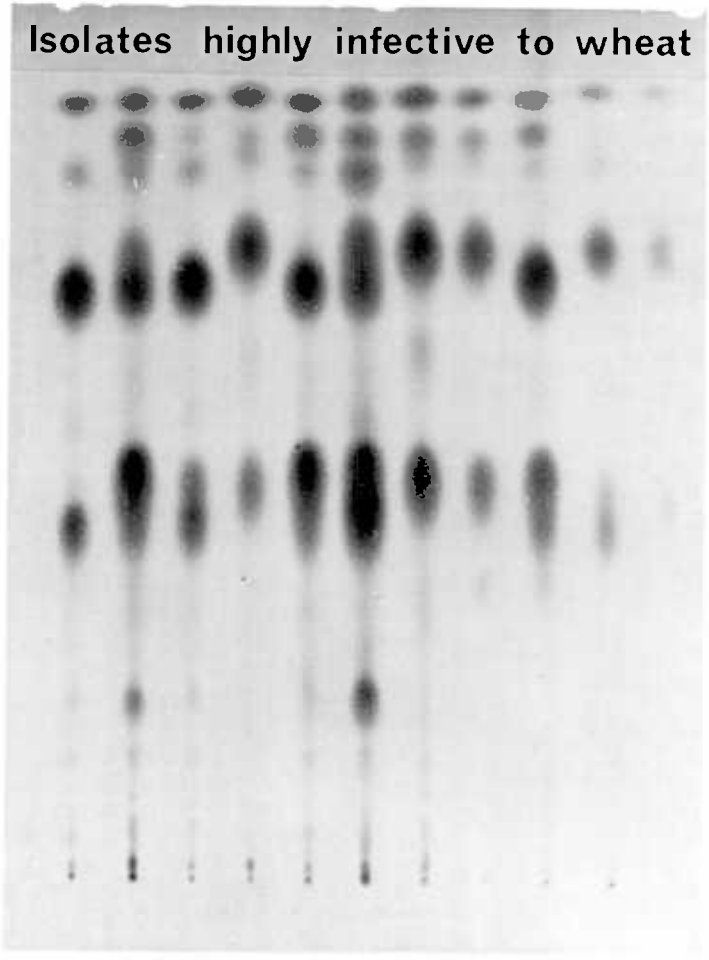
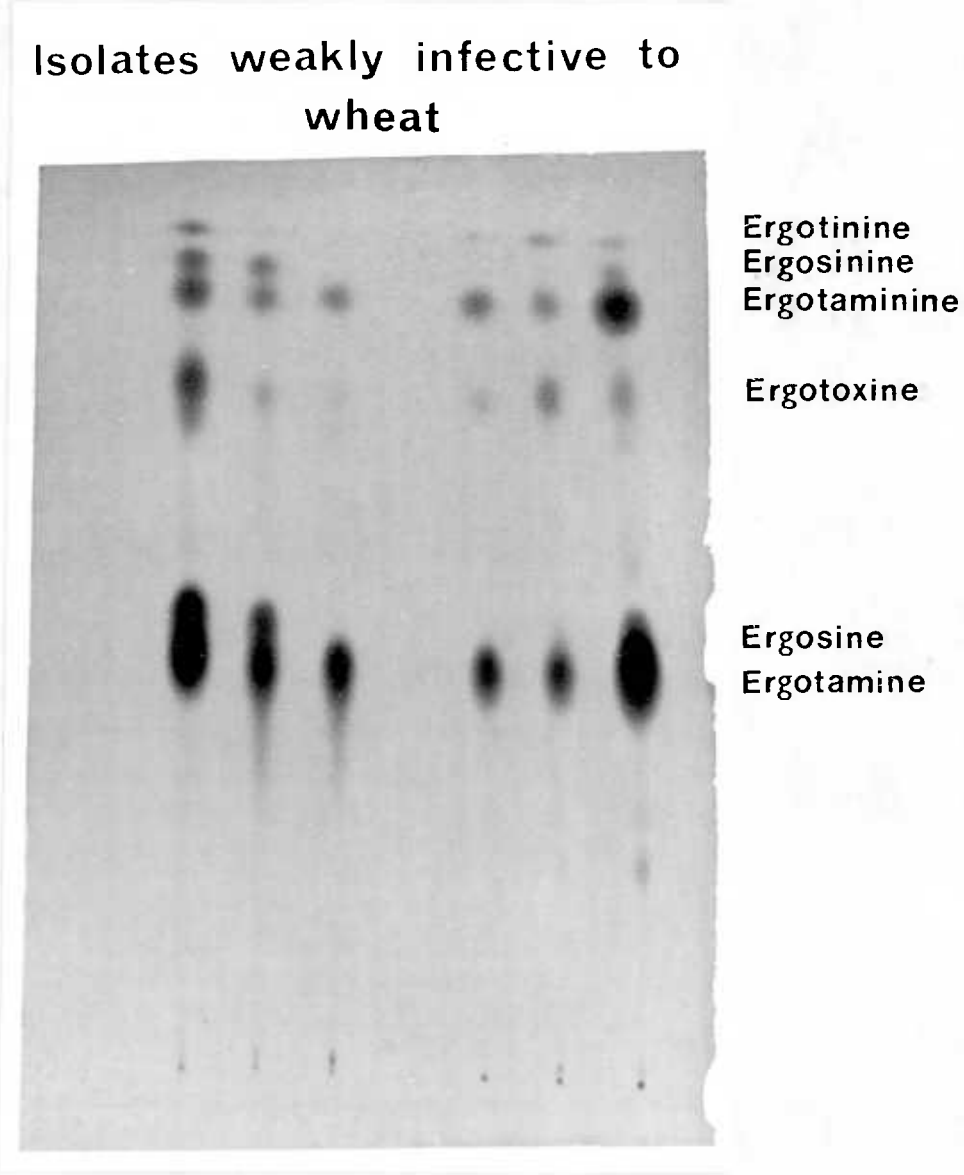


Figure 18.

Photograph of a thin layer chromatogram of alkaloids extracted from ergots collected during cross-inoculation experiments.

II. Ergots formed as a result of infection of wheat by the weakly infective isolates.



21.3 Analysis of the alkaloid spectra of naturally occuring ergot sclerotia

From a collection of over 450 samples of ergots throughout the British Isles, 241 samples were selected and subjected to qualitative analysis of their alkaloid content by thin layer chromatography. Selection was based mainly on sample size: all samples weighing approximately 0.2g or more were analysed, except where more than one sample was obtained from the same host in the same locality; in which case the first selected sample was generally used. Both from the intensity of fluorescent alkaloid spots on the chromatograms viewed under U/V light, and also from the intensity of blue colouration which developed as a result of spraying the plates with P.D.A.B., assessments were made of the relative proportions of individual alkaloids in each chromatogram. Considering the large number of samples examined, it was not within the scope of this investigation to undertake quantitative assessments of the individual alkaloid components of each sample extract. However, the whole spectrum of alkaloids in each sample was examined by a) visually assessing the relative proportions of the main alkaloid components; and b) recording the presence or absence of minor alkaloids.

A system of coding was devised which simplified the comparison of alkaloid spectra of ergots collected from groups of hosts. Individual chromatograms were scored as shown on the following page to give an Alkaloid Spectrum Code (A.S.C.).

Alkaloid Spectrum Code

Ratio of ergotoxine to ergotamine	Ergotoxine > Ergotamine	A
	Ergotoxine < Ergotamine	B
	Ergotoxine ~ Ergotamine	C
	No Ergotoxine	D
	No Ergotamine	E
	No Ergotoxine or Ergotamine	F

Amount of Ergosine	No Ergosine	0
	Ergosine << Ergotoxine and < Ergotamine	1
	Ergosine < Ergotoxine but > Ergotamine	2
	Ergosine > Ergotoxine but << Ergotamine	3
	Ergosine > Ergotoxine and Ergotamine	4

Presence of Ergometrine	1
" " Lysergic amide	2
" " Ergometrine and Lysergic amide	3
" " Unidentified alkaloid "X"	X
" " " " "Y"	Y

Example:-

Accession No.258

Ergotoxine < Ergotamine	B
Ergosine ~ Ergotamine but > Ergotoxine	3
Ergometrine absence	0
Lysergic Amide - trace	2
Unidentified alkaloid "X" present	X
	B3. 2X

In this system, the gross proportions of the alkaloid spectrum are described by a letter and a digit before a decimal point, whilst the minor alkaloid components of the spectrum are described by a digit and a letter following the decimal point. It is important to note that the score for minor components of the spectrum was, to some extent, dependant on the sample size. Ergometrine, as a small percent of the total alkaloids, for instance, may not have been detected in the chromatography of a small sample.

The alkaloid spectrum codes for all analysed samples of ergots are listed in table 16, and are represented by histograms in figure 19. Later figures (20 and 37) show the same information in a summarised form, and are, therefore, more easily interpreted. However, figure 19 does serve to emphasise the variability of alkaloid spectrum types both within groups of samples from the same host genus, and also between samples from different host genera.

Since, as explained above, the minor alkaloid components could not always be resolved in small samples, their usefulness in characterising individual spectra is limited. Nevertheless, close examination of figure 19 in conjunction with the key to spectrum codes (p129) does reveal a number of

Table 16.

Table of Alkaloid Spectrum Codes representing the results of analyses of samples of grass and cereal ergots collected throughout the British Isles.

See p.129 for a key to the Alkaloid Spectrum Codes (A.S.C.), and Table 2 for a list of the ergot accessions.

Host Plant.	Accession number.	A.S.C.	Accession number.	A.S.C.	Accession number.	A.S.C.
Wheat.	16	A2.0	124	A2.3	391	A2.1
	19	A2.3	145	A2.3	393	A2.0
	22	A2.3	146	A4.1	404	A2.0
	25	A2.2	147	A1.0	435	A2.1
	28	A2.3	148	A2.3	436	A2.0
	39	A2.3	223	A2.3	437	A2.0
	40	A2.0	235	A2.1	440	A2.3
	53	A2.3	323	A2.3	441	A2.0
	88	A2.3	327	A2.0	442	C1.0
	89	A2.1	344	A2.0	443	A2.0
	90	B0.0	348	A4.0	444	A2.0
	93	A2.0	360	A2.3	445	A2.0
	94	A2.0	370	A2.0	450	A2.0
	95	A2.0	375	A2.0	451	A2.0
	100	A2.0	376	A2.0	453	A2.1
	101	A2.0	381	C1.0	454	C1.0
	108	A2.1	383	A1.0		
	123	A2.1	384	A2.1		
Barley.	17	A2.1	126	A2.3	244	A2.3X
	55	A2,2	240	A4.0		
Rye.	27	C0.1	134	A0.0	452	C1.0
	107	A0.0	140	C0.1		
Agropyron pungens.	166	C0.0Y				

/continued.

Table 16 (continued)

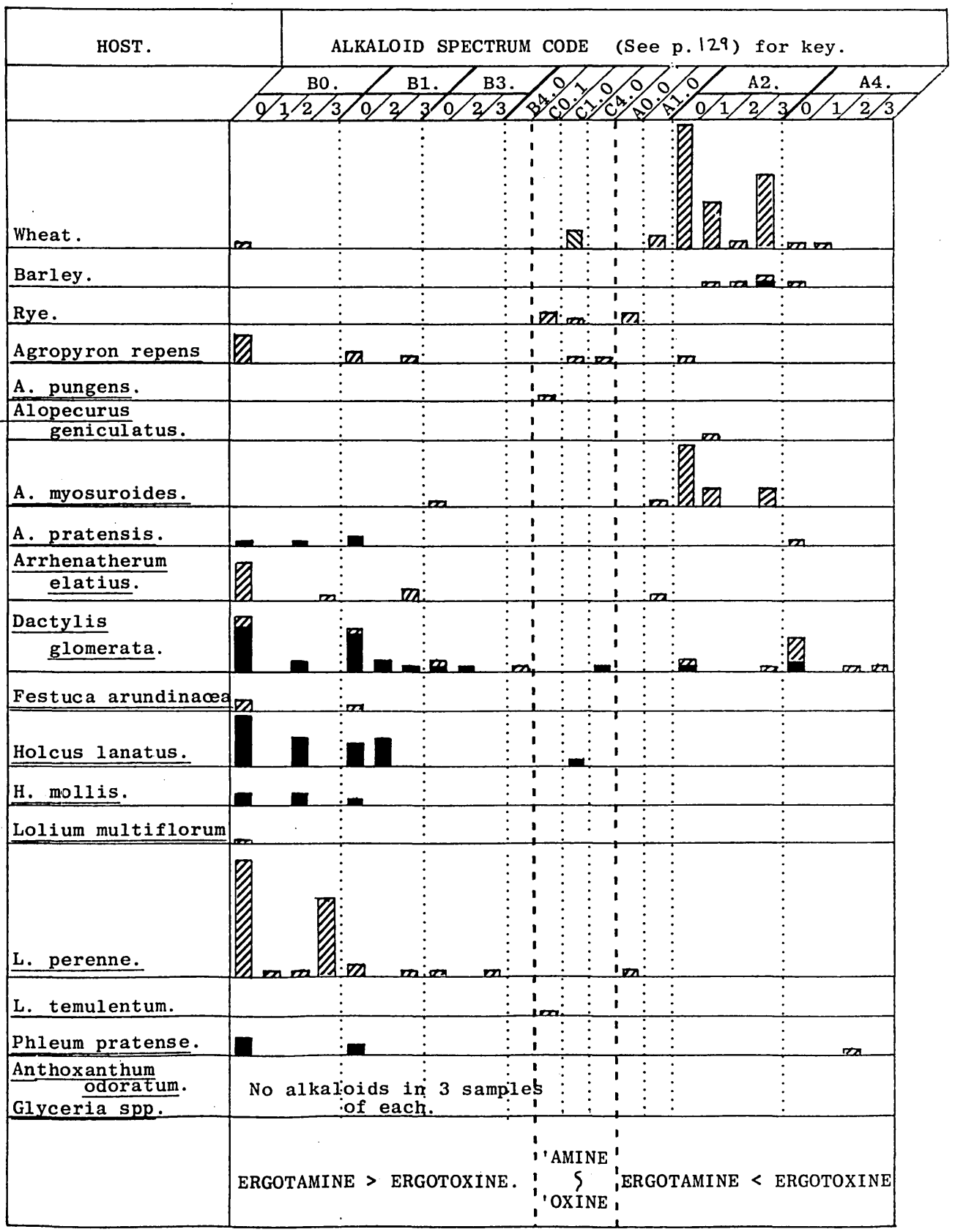
Host Plant.	Accession number.	A.S.C.	Accession number.	A.S.C.	Accession number.	A.S.C.
Agropyron repens.	172	C1.0	250	B0.0	364	B0.0
	174	B0.0	265	B1.0	397	C4.0
	233	B1.3	354	A2.0	401	B0.0
	243	B1.3	363	B0.0		
Alopecurus geniculatus.		A2.1				
A.myosuroides.	122	A2.1	352	A2.0	394	A1.0
	131	A2.3	371	A2.1	428	A2.0
	143	A2.0	374	A2.0	429	B3.0
	149	A2.3	377	A2.0	440	A2.3
	150	A2.0	378	A2.0	449	A2.1
	345	A2.0	379	A2.0		
	349	A2.0	388	A2.0		
A.pratensis.	251	B0.2X	308	B1.0X	411	B1.0X
	273	B0.0X	385	A4.0		
Anthoxanthum odoratum.	202	NIL	399	NIL	422	NIL
Arrhenatherum elatius.	34	B0.0	210	B0.0	366	B0.0
	36	B0.3	225	B0.0	392	B0.0
	80	A1.0	335	B0.0	415	B0.0
	130	B1.3	361	B1.3		
Dactylis glomerata.	6	C4.0X	187	A4.0X	263	B1.0X
	14	B1.3X	193	A4.0	268	B4.0
	37	B1.0X	199	B0.0	274	B3.0X
	121	A4.2	206	B0.2X	277	B0.0X
	127	A4.3	219	B0.0X	279	A4.0
	159	B0.0X	226	B0.0X	284	A4.0X
	160	B0.0	229	B0.0X	301	A2.0
	171	B1.0X	246	B1.0X	337	A4.0
	177	A2.0X	257	B1.0X	353	A2.3
	181	B1.0	258	B3.2X	362	B0.0X
	184	B0.2X	260	B1.2X	369	B0.0X
	186B	B1.0X	261	B1.2X	396	B0.2X

Table 16 (continued).

Host Plant.	Accession number.	A.S.C.	Accession number.	A.S.C.	Accession number.	A.S.C.
D.glomerata. (cont.)	407	A4.0	418	B3.0	423	B0.0X
	414	B1.0X				
Festuca arundinacea.	97	B0.0	249	B1.0	276	B0.0
Glyceria spp.	185	NIL	247	NIL	294	NIL
Holcus lanatus.	62	B0.2X	201	B1.2X	280	B1.0X
	129	B0.2X	207	B1.2X	286	B0.2X
	173	B1.2X	213	B1.2X	288	B0.2X
	178	B1.0X	253	B0.0X	292	B1.0X
	183	B1.2X	266	B0.0X	293	B0.0X
	188	B0.2X	270	B1.0X	367	B0.0X
	189	B0.0X	275	C1.0X	395	B0.0X
	192	B0.0X	278	B0.0X	405	B0.0X
Holcus mollis.	161	B0.0X	208	B1.0X	365	B0.0X
	196	B0.2X	232	B0.2X		
Lolium perenne.	1	B0.3	125	B0.3	254	B0.3
	3	B0.0	153	B0.3	255	B0.3
	8	B0.0	158	B0.0	256	B0.0
	18	B0.0	170	B0.0	259	B0.2
	26	B1.0	175	B0.0	264	B0.0
	38	B1.3	191	B0.3	267	B0.3
	41	B0.3	195	B0.3	281	B0.3
	46	B0.0	203	B0.3	282	B0.0
	61	B0.3	204	B0.0	291	B0.0
	67	B0.0	216	B0.0	347	A0.0
	78	B3.0	220	B1.0	355	B0.0
	81	B0.0	227	B0.0	380	B0.0
	83	B0.1	231	B0.3	419	B0.0
	98	B0.0	239	B0.3		
	106	B0.0	252	B3.3		
Lolium multiflorum.	2	B0.0				
Phleum pratense	7	A4.2	180	B0.0X	262	B1.0X
	179	B0.0X	224	B1.0X	368	B0.0X

Figure 19.

Histograms showing the frequency of occurrence of alkaloid spectrum types of ergots collected from grasses and cereals.



■ = Alkaloid spectra containing alkaloid "X".
▨ = " " not " " " .
Scale of histograms: 1mm = 1 sample.

interesting associations between these minor alkaloid components of ergot and their hosts. Shaded areas of the histograms represent those spectra in which the unidentified alkaloid "X" was found. This alkaloid had an Rf value slightly less than ergotaminine (the isomer of ergotamine), fluoresced under U/V light (254 nm) and gave a blue colour when chromatograms were sprayed with P.D.A.B. It was detected in every sample of ergots from Holcus spp. and was also common in the spectrum of alkaloids extracted from ergots from Dactylis glomerata, Alopecurus myosuroides and Phleum pratense. At the Welsh Plant Breeding Station (p149) "X" was also detected in the spectrum of alkaloids extracted from ergots that had been collected from male-sterile barley. Where present in the extracts from Dactylis ergots, "X" was often found to be a major component of the total alkaloids.

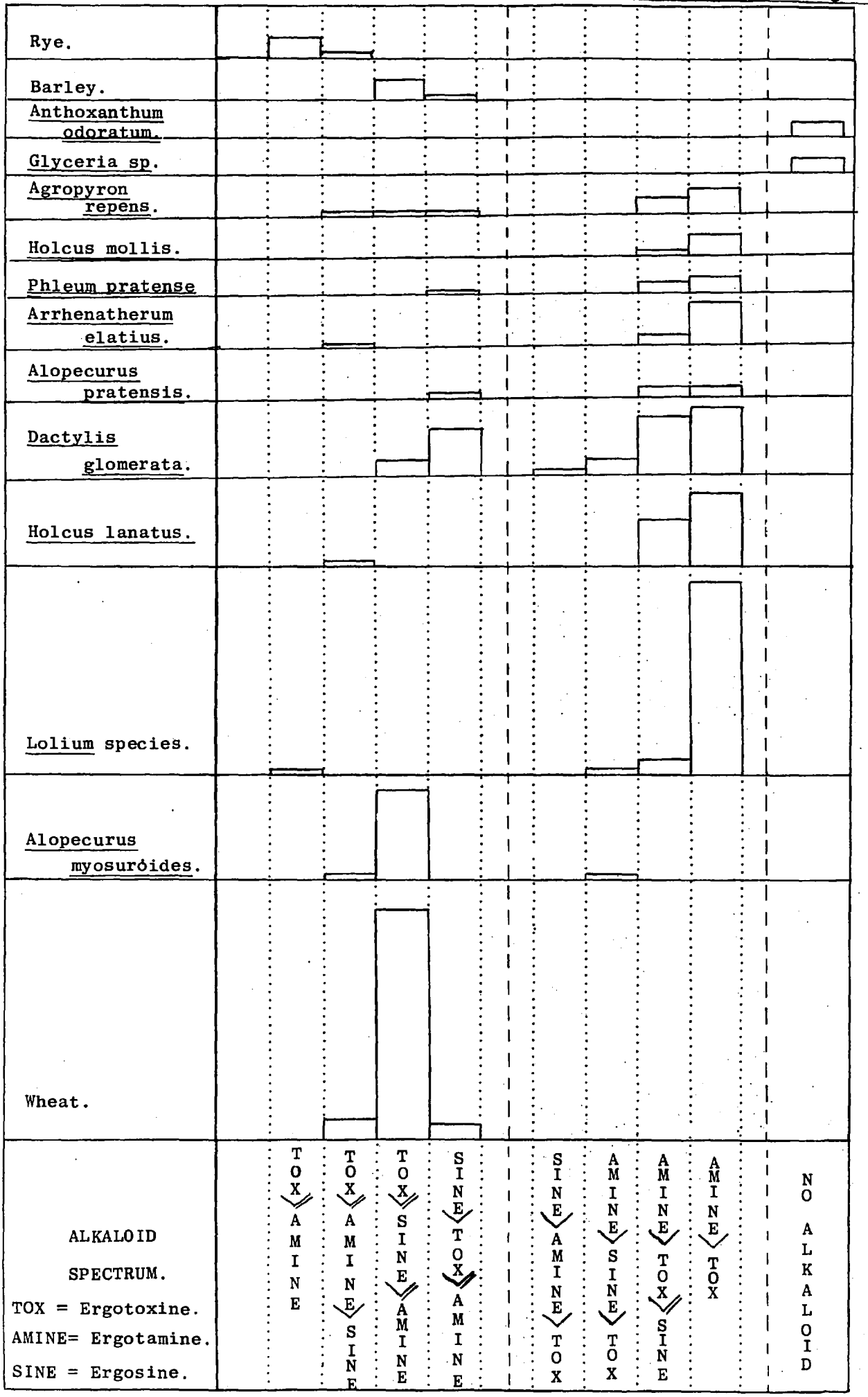
A collection of ergots from cocksfoot (Accession No.186), in which "X" was detected, provided one of the strains used in the cross-inoculation experiments (Section 21.1). Although only weakly infective to wheat, some ergots were formed, and subsequent analysis showed that these too contained a detectable amount of "X", which must, therefore, be a characteristic product of the fungal strain rather than of growth on a particular host plant. Considering this

cross-infection result, it is remarkable, but not altogether surprising, that only one out of 62 collections of ergot from cereal hosts was found to have an alkaloid spectrum in which "X" was present. Thus, it would appear, that strains of ergot which characteristically produce this unidentified alkaloid rarely infect wheat under natural conditions.

Many collections of ergots from Lolium and Holcus species were taken from the same locality; often from mixed stands of the two grasses. Although the alkaloid spectra of these samples were similar in their gross proportions (ergotamine was the predominant alkaloid), evidence of two distinct, host restricted strains was found by examination of their minor alkaloid spectra (figure 19). Ergots from ryegrass were commonly found to contain both ergometrine and lysergic amide (A.S.C. --.3) but none of the samples analysed contained the unidentified alkaloid "X". In contrast, the presence of "X" was demonstrated in all samples of ergots from Holcus species, and, although many samples were large enough to contain an appreciable amount of lysergic amide (A.S,C. --.2), none were found to contain ergometrine.

Figure 20is also compiled from the results listed in table 16. The alkaloid spectra have been grouped into nine main categories, based on the relative proportions of the major alkaloids. The similarity of the general type of alkaloid spectra

Figure 20. Summary of qualitative alkaloid analyses of grass and cereal ergots



Scale of histograms :- 1mm.= 1 sample.

found in natural wheat ergots to those extracted from blackgrass ergots is clearly demonstrated. Only one sample of ergot from ryegrass in Britain was found to have an ergotoxine/ergotamine ratio greater than one. In this instance a single, infected spikelet was found after a thorough search amongst a ryegrass headland of a wheat crop that was heavily infected with ergot; the common source of infection having been from ergot-infected blackgrass in the same area of the field (page 147.).

In respect of the ergotoxine/ergotamine ratio, some collections of ergot from cocksfoot were similar to those collected from wheat, but high ergosine content and/or the presence of the alkaloid "X" served to distinguish all but two of them from the most common wheat types.

One sample of ergot from each of the two early flowering grasses, meadow foxtail and annual meadow grass, had an alkaloid content similar to that found in some of the collections of ergots from wheat. However, other collections of ergots from these two grasses were found to have alkaloid spectra in which the ergotoxine content was low by comparison with ergotamine. Similarly, most samples of ergots from timothy grass and false oat grass were found to be more like those from Holcus spp. and Lolium spp., respectively,

than wheat ergots. In three small samples of ergot from each of two grasses, Anthoxanthum odoratum and Glyceria sp., the total alkaloid content was so small (determined quantitatively for two samples as less than 0.01% w/w) that thin layer chromatography was not possible.

22. Further investigations of the role of weed grasses as alternative hosts for wheat-infective strains of ergot.

22.1 Cycle of infection experiments

The design of these experiments is described in section 16. At the Grassland Research Institute (G.R.I.), where the role of ryegrass and cocksfoot in the wheat ergot/weedgrass/wheat infection cycle was investigated, severe bird damage before harvest prevented quantitative assessments of ergot infection of cms Capitole. However, prior to the loss of the experiment at this site, it had been noted that, in spite of the close proximity of ryegrass to germinating ergots bearing mature capitula, no ryegrass inflourescences became infected. Cocksfoot, on the other hand, although free from outward signs of honeydew exudation at the time when hussocks of cms Capitole were flowering, did eventually bear normal ergots. By analysis of their alkaloid content, these sclerotia were found to be similar to one of the collections of wheat ergots that had earlier germinated alongside the cocksfoot.

Ergot infection was also noted in hussocks of cms Capitole for all treatments except the control. In the wheat ergot/ryegrass/wheat treatment the levels of ergot infection of wheat were markedly higher than in other treatments. A possible explanation may be that ryegrass plants, which tended to overhang some of the dishes of germinating ergots, maintained a micro-climate more favourable to germination and may, therefore, have increased both the amount

and duration of ascospore discharge, especially during dry weather.

At C.M.F. (Stokenchurch) the wheat ergot/blackgrass/wheat combination was investigated. The ergots gave rise to capitula that were well formed and visible above the soil surface by 2nd - 3rd week of May, and honeydew was observed on the blackgrass on 6th June. A sample of this honeydew was found, by inoculation of wheat test plants in the glasshouse, to be infective to wheat. A further sample, taken after a short period of dry weather, showed that spores remained viable even in dried honeydew. During the flowering period of cms Capitole (in hussocks alongside the germinating ergots and infected blackgrass), wet, humid weather conditions were favourable for infection and further exudation of honeydew. The total number of ergots found on cms Capitole in each treatment is shown in table 17.

Table 17. Results of an investigation of the infection cycle of wheat ergot, C.M.F. (Stokenchurch) 1973

Treatment	Number of ergots collected from cms Capitole			
	Rep.1	Rep.2	Rep.3	Mean
Wheat control	13	3	6	7.1
Wheat ergot/wheat	9	1	14	8.0
Wheat ergot/wheat/ blackgrass	249	39	126	138.0

Although, as a result of considerable variation between replicates of treatments, the difference between treatments, was only statistically significant at the 10% level (Appendix I), the results do appear to have much greater biological significance.

A similar experiment was established at Throws Farm, Essex in the autumn of 1973. However, owing to prolonged drought during April, May and early June, 1974, ergots failed to germinate and no infection was found in either wheat or blackgrass.

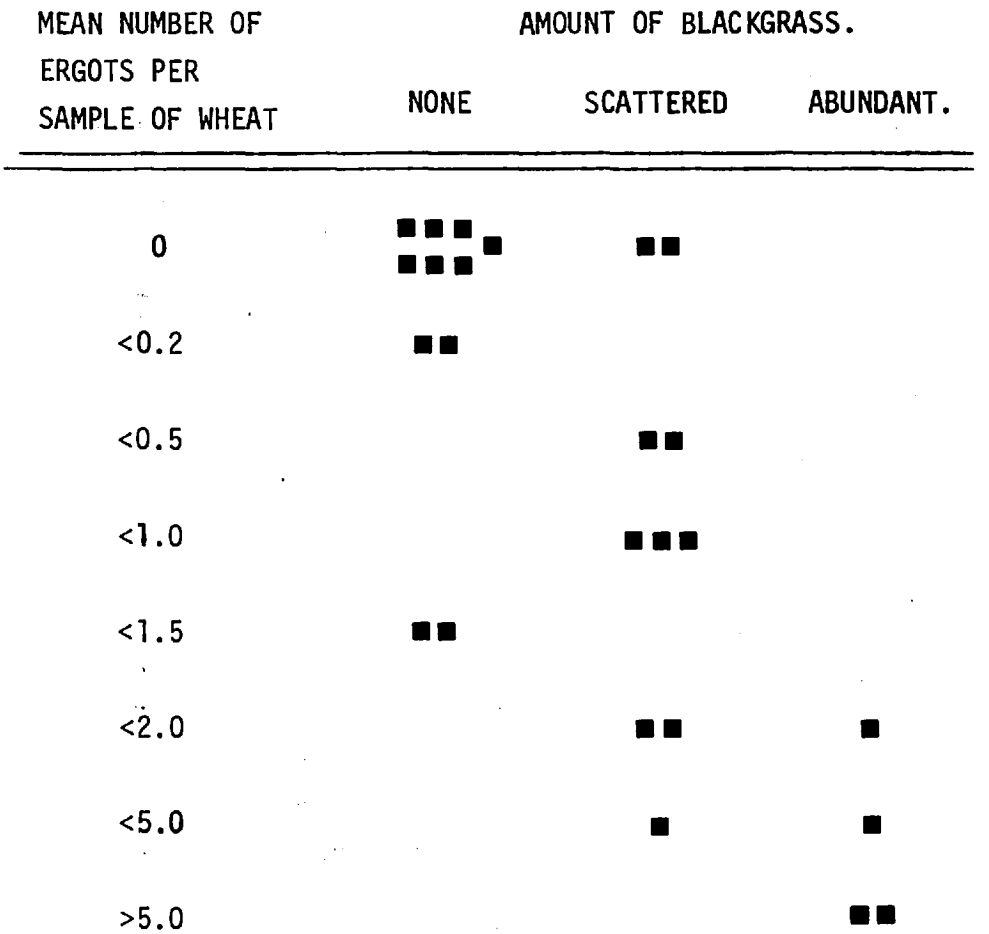
22.2 Survey of blackgrass and ergot in Maris Huntsman seed wheat crops

In September, 1973, reports from seed merchants indicated that a number of winter wheat seed crops, particularly of the cultivar Maris Huntsman, had been infected with ergot. A survey of all the seed lots of this cultivar cleaned by two seed companies was undertaken.

In figure 21 the abundance of blackgrass in fields from which seed lots were harvested is compared with the amount of ergot contaimination found in seed samples before these seed lots were cleaned by the seed merchants. Clearly, there was a tendency for more wheat ergot to be found where crops had been abundantly infested with blackgrass.

Figure 21.

Results of a survey of the incidence of blackgrass and wheat ergots in seed crops of the wheat cultivar Maris Huntsman, 1973.



Each black square represents one farm site.

22.3 Notes on field observations of ergot in blackgrass

During the summers of 1972 and 1973 ergot on blackgrass was observed in many farm situations, especially on heavy land in an area from the South West Midlands to the East Coast (see distribution map, figure 1).

In early July, 1972, at Lane End Farm (L.E.F., p.152) where there had been a history of ergot infection of both pasture and cereal crops, exudation of honeydew was observed on ears of winter wheat in the headland of a crop that was also heavily infested with blackgrass. At the same time, partially developed ergot sclerotia, estimated to be at least three weeks post infection, were found on the blackgrass, suggesting that these grass inflorescences had been infected before wheat. At harvest, wheat from the headland was found to contain approximately 0.06% (w/w) ergot.

Having discovered an area within the farm where ergot was to be expected under favourable weather conditions in the following year, weekly observations commenced in May, 1973. However, dry weather conditions hindered the spread of infection during most of May and early June (see section 22.5), and, in spite of the discovery of some germinating ergots within the crops, no infection of blackgrass was recorded until 28th June, by which time the wheat crop had passed through its susceptible phase.

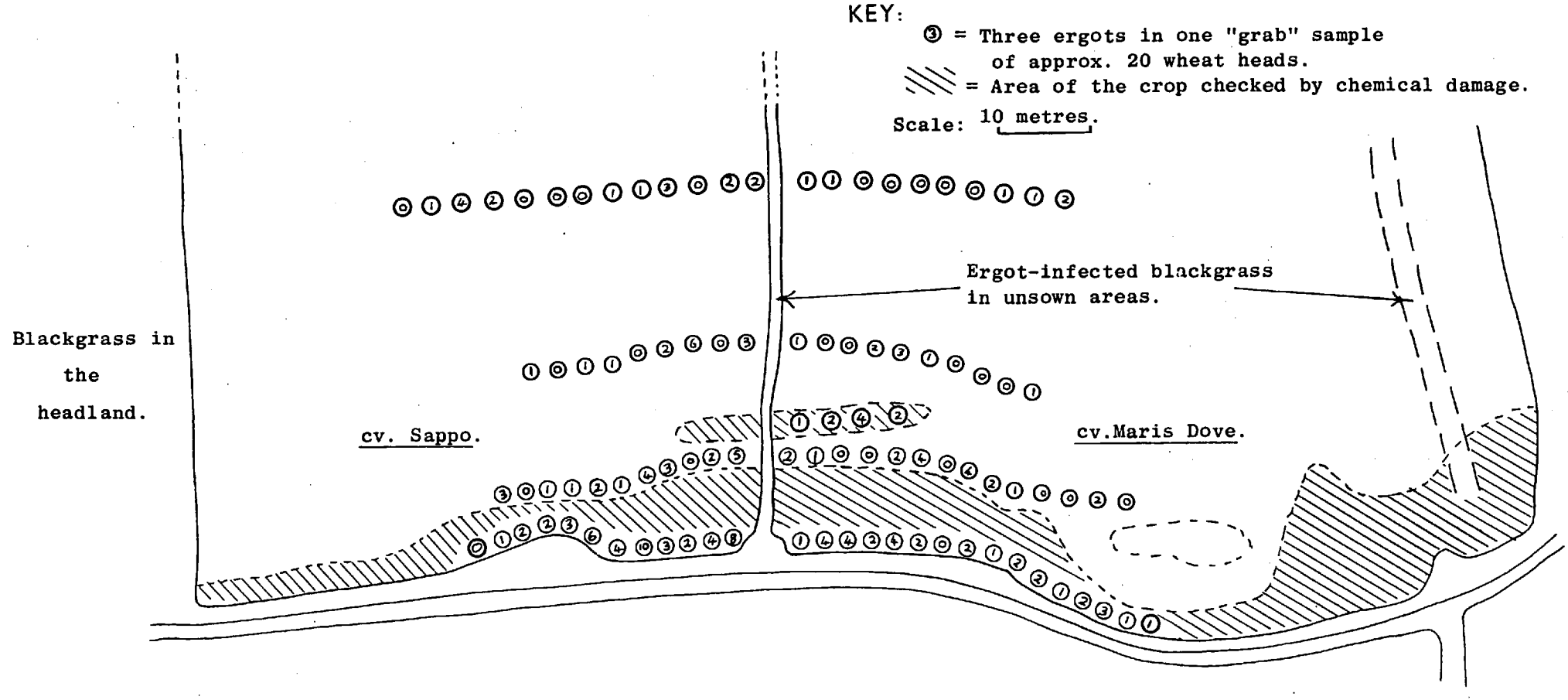
During a visit to Rothamsted on the 14th June, 1973, blackgrass ergots and honeydew were collected from the edge of Stackyard field. Alongside a boundary fence, where the ground had remained undisturbed, blackgrass was heavily infected with ergot, and adjacent wheat plants were, at a later date, also found to be infected. Samples of honeydew were also taken from blackgrass within a crop of field beans and were found to be highly infective to a spring wheat cultivar, cms Rask, which was inoculated in the glasshouse on the following day.

At Throws Farm, Essex, ergot-infected blackgrass was commonly found in cereals. Before harvest in 1973 one field of spring wheat, which had suffered some chemical damage, was found to be heavily infected with ergot (figure 22).

Examination both of hedgerow grasses and of samples of the seed used to sow the crop did not reveal a source of inoculum. Ergots were found on small areas of blackgrass within the crop; but this grass did not appear to have carried enough inoculum to account for the widespread infection of the whole of the wheat crop. Nevertheless, the abundance of blackgrass in small patches of the field that had been missed as the wheat was drilled indicated that blackgrass had been present in the previous year's crop (Maize). Since ergot of blackgrass had been very common on this farm during the previous year, it is inferred that the wheat may have been sown in soil contaminated with blackgrass ergots.

Figure 22.

Map of the distribution of ergot-infected wheat heads within two adjacent spring wheat crops; Throws Farm, August 1973.



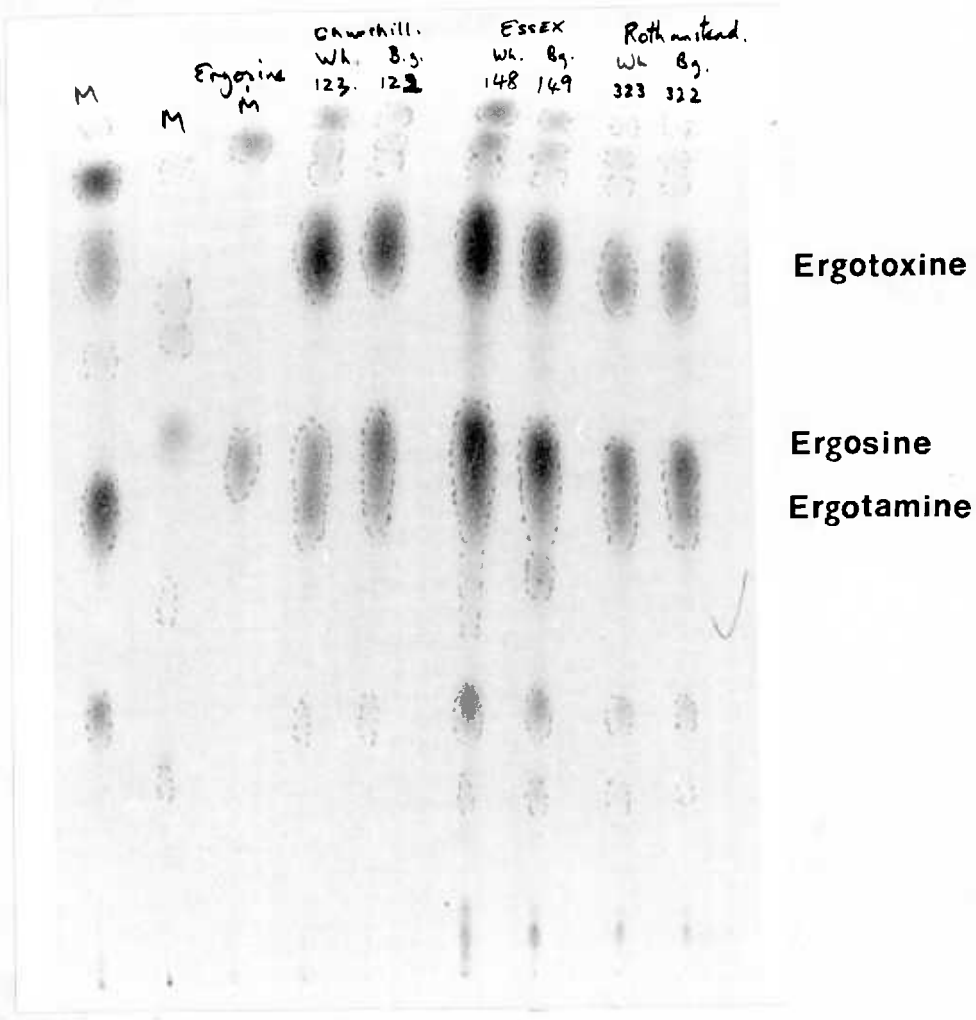
A number of farms were visited because, on the advice of R.H.M. crop inspectors, they were known to be in areas where blackgrass was abundant. On four out of five Northamptonshire farms visited on July 22, 1973, ergot was found in both blackgrass and wheat. At one of these sites, where only one small area of blackgrass was found within a wheat crop, ergot was found on wheat alongside the blackgrass but, after an intensive search, could not be found in other areas of the field. Similarly, on four out of six Bedfordshire farms, which had been used as trial sites for blackgrass herbicide chemicals, ergot was found on both blackgrass and wheat.

Whenever sufficient sclerotial material was collected, the alkaloid spectra of adjacent samples of wheat and blackgrass ergots were compared. Three pairs of chromatograms, shown in figure 23, indicate that, in each locality, the two hosts had been parasitised by the same strain of the ergot fungus.

At many sites it was noted that wheat ergot was most common in the "wheelings" where tractors had damaged the crop during spring application of agro-chemicals. Here, late tillers from damaged plants were commonly infected. Similarly, chemical damage to wheat crops was often associated with high levels of infection by ergot. Thinly populated blackgrass within a wheat crop was often more heavily infected than dense stands of the grass which, even in localities where ergot disease was prevalent, often escaped severe infection.

Figure 23.

Photograph of a thin layer chromatogram of alkaloid extracts from collections of ergot from blackgrass and wheat in three localities.



Alkaloid spectra of wheat (Wh) and blackgrass (Bg) ergots from Churchill, Oxfordshire; White Colne, Essex; and Rothamsted; Herts.

22.4 Investigations of the progression of ergot disease of cereals and grasses in two localities

22.4.1 The Welsh Plant Breeding Station, Aberystwyth (W.P.B.S.)

At the end of the summer of 1972, samples of ergots from field plots of male-sterile barleys and from grasses around these plots were received from the W.P.B.S. Most ergots in the barley had been found at the edges of plots, near to grassy headlands and banks, indicating that a source of inoculum had possibly been the ergot-infected grasses (Foster, Personal Communication). Results of alkaloid analyses of these ergot samples are summarised in table 18. On the basis of the alkaloid spectra, it would appear that a number of different strains of ergot were present in and around the male-sterile barley plots.

In Cae Banadl field, alkaloid spectra of barley ergots most resembled the spectrum of alkaloids from Marsh foxtail (Alopecurus geniculatus), except that a trace of the unidentified alkaloid "X" (not present in ergots from A.geniculatus) was extracted from the barley ergots. This may indicate that the collection of ergots from barley were of mixed strain origin, some inoculum possibly having spread both from infected marsh foxtail and some from one or both of the Holcus species and/or Dactylis glomerata. In Cae Ruel field, the only heavily infected grass species, Lolium perenne, carried ergots having an alkaloid content dissimilar to the ergots found within the plot of barley, and was therefore unlikely to have been the source of infection. Analysis of ergots from cocksfoot (D.glomerata) in Cae Newydd field

Table 18

Results of analyses of the alkaloid spectra of ergots collected at the Welsh Plant Breeding Station in 1972; expressed as Alkaloid Spectrum Codes (see p 129)

Host Plant.	Field name:- Cae Banadle.	Cae Ruel.	Cae Newydd.
Barley.	A2.3X	C4.1	A4.0
Alopecurus geniculatus.	A2.1	-	-
Arrhenatherum elatius.	B0.0	-	B0.0
Dactylis glomerata.	A4.0X	-	A4.0
Holcus lanatus.	B1.2X	-	B0.0X
H. mollis.	B1.0X	-	-
Lolium perenne.	B0.3	B0.0	B0.0

showed that the spectrum of alkaloids they contained was similar to the alkaloid spectrum of barley ergots in this field; whilst ergots from other grasses had a completely different alkaloid content.

The above observations indicate that certain grasses did play a role in the aetiology of ergot in male-sterile barley at the W.P.B.S. during 1972. However, it should be remembered that the sequence of cross-infection could, conceivably, have been from infected barley to the grasses rather than - as is suggested above - from grasses to barley.

The aim of further observations at the W.P.B.S. during 1973 was to establish more clearly the time sequence of infection of male sterile barley and adjacent grasses. On 11th June, grasses alongside four plots of male-sterile barley were examined. Although the grass species Anthoxanthum odoratum, Poa pratensis, Alopecurus geniculatus, Bromus spp and a few plants of D.glomerata all had some anthesing inflorescences, ergot honeydew was only found in a few, mature heads of A.pratensis, which appeared to have anthesed a few weeks earlier.

The first observed flowering dates of male-sterile barleys were between 21st and 26th June. On 22nd June grasses near to the barley plots were re-examined for signs of ergot infection. L.perenne, Holcus lanatus, H.mollis, Arrhenatherum elatius, Agropyron sp., Poa sp., Agrostis sp. and Anthoxanthum odoratum were all apparently free from ergot disease, whereas clumps of Dactylis glomerata and Alopecurus geniculatus, near to two

of the barley plots, were both occasionally found to be infected. After a thorough search of the male-sterile barleys during harvest, no barley ergot was found. This was not an altogether unexpected result since the June/early July weather had been extremely dry and unsuitable for spread of the disease.

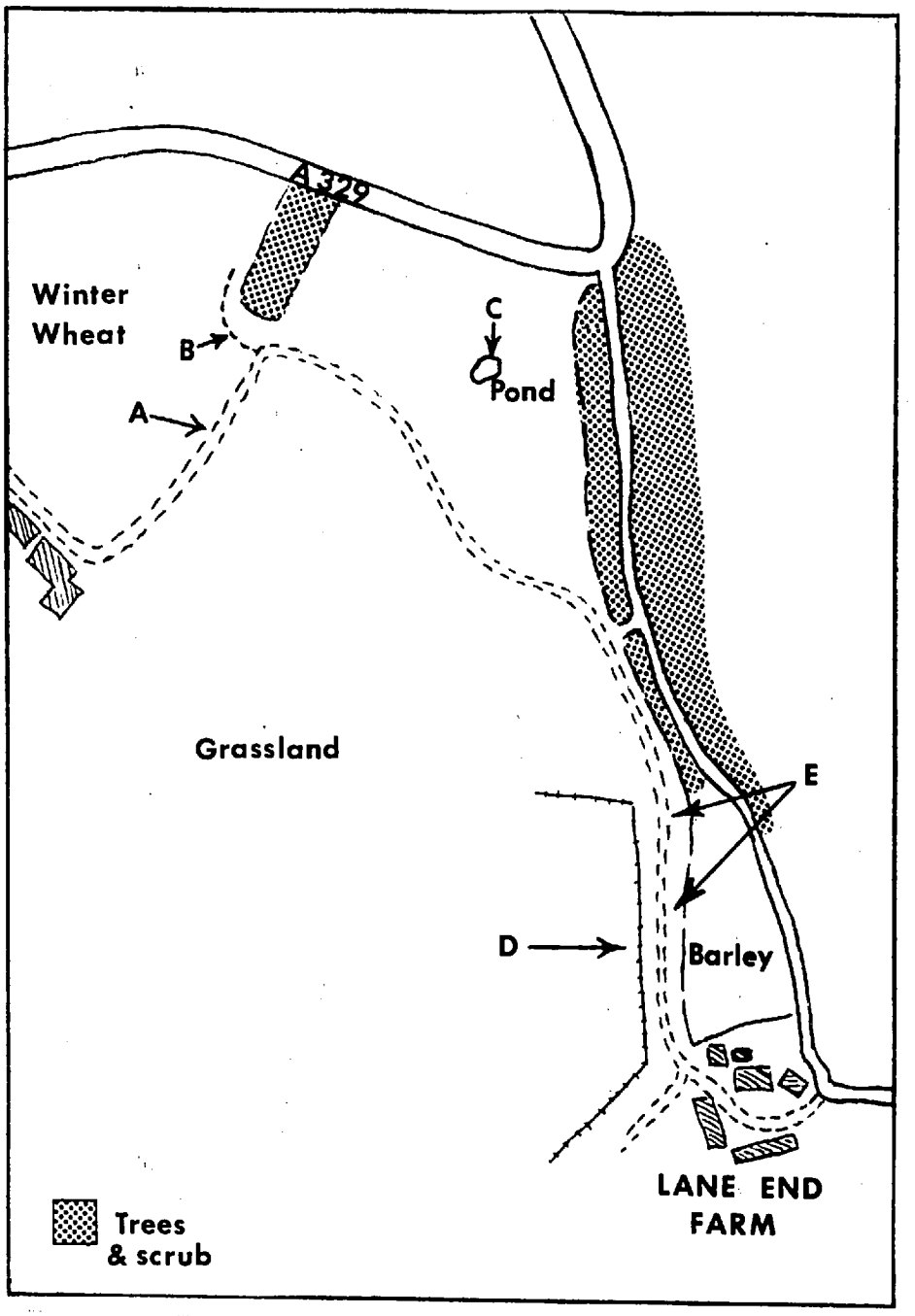
22.4.2 Lane End Farm

A summary of the aetiology of ergot disease during two consecutive years at this farm site is given in figures 24,25 and 26. When this locality was first visited on 13th July, 1972, ergot sclerotia were observed both on blackgrass (Alopecurus myosuroides) and on cocksfoot (Dactylis glomerata) at site "E" near to the farm buildings (figs. 25 and 26). Although most tillers of the adjacent barley crop had reached anthesis, no sign of ergot-infection of barley was noted at this time.

On subsequent visits to the farm, a larger area was investigated. At site "A" on the 20th July, both blackgrass and wheat ears were found to be ergot-infected; though parasitism of the former had, on the evidence of their stage of sclerotial development been initiated earlier. Nearby, a small clump of cocksfoot also bore the early (sphacelial) stage of ergot, whilst at site "E", traces of honeydew were found on a few inflorescences of false oat grass (Arrhenatherum elatius). On August 3rd, well formed sclerotia were evident on most plants on which honeydew had been noted previously, but no sign of infection of other grasses (most

Figure 24

Distribution of grasses and location of sites for collection of ergot at Lane End Farm, Warborough, Oxon.



Grasses.	Ergot-infected plants (See figures 25 and 26)	Grasses present at sites: A	B	C	D	E
Cereals	⊕	+	+			+
Alopecurus myosuroides	●	+	+	+		+
A. pratensis.	■			+		+
Dactylis glomerata.	▲	+		+		+
Lolium spp.	○				+	+
Arrhenatherum elatius.	△					+
Holcus spp.	□			+		+

Fig. 25

Distribution of ergot-infected grasses and cereals at L.E.F. during July - August 1972.

See Fig. 24 for key to symbols.

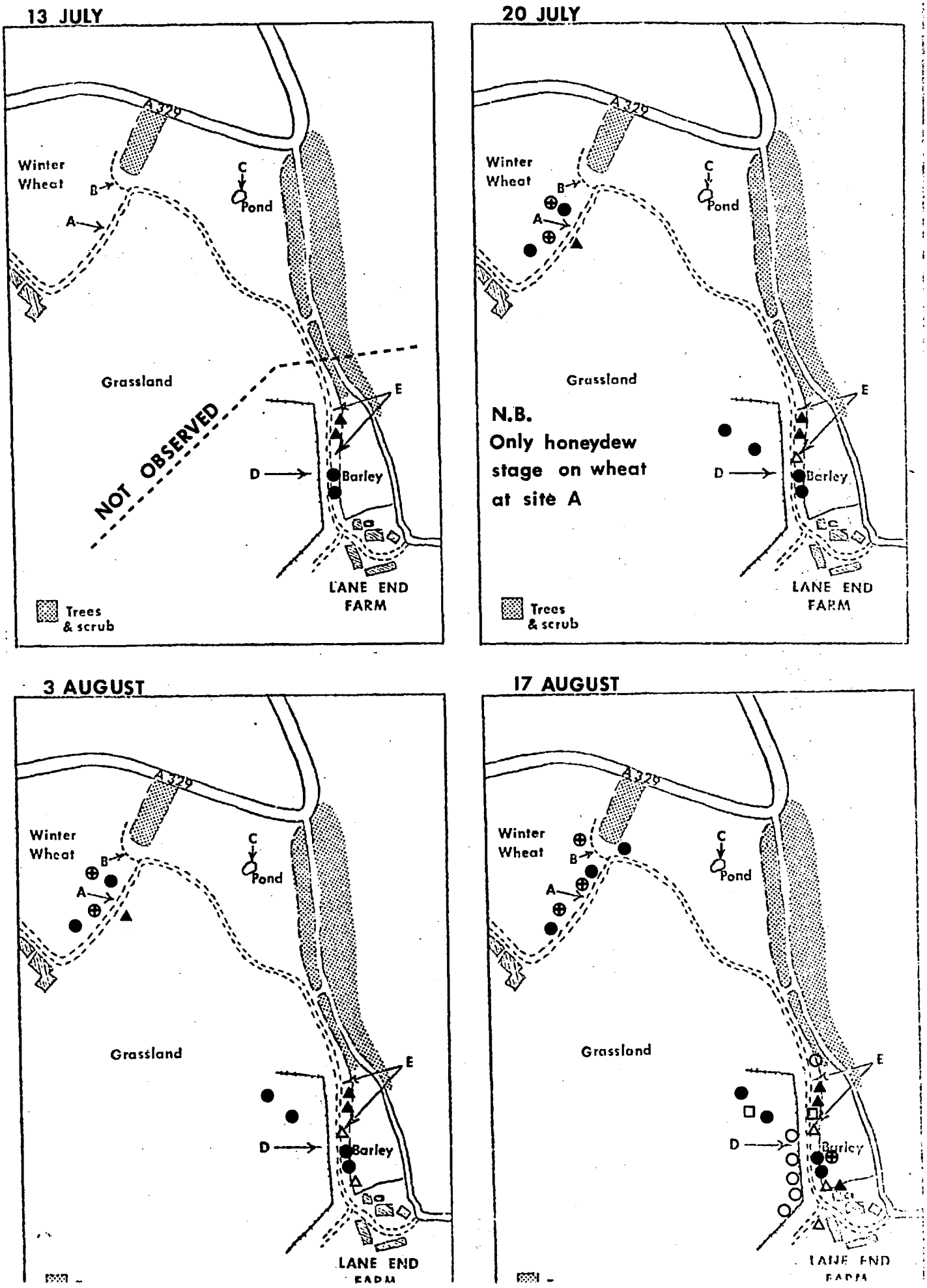


Fig. 26
Distribution of ergot-infected grasses at L.E.F. during June - July 1973.

See Fig. 24 for key to symbols.

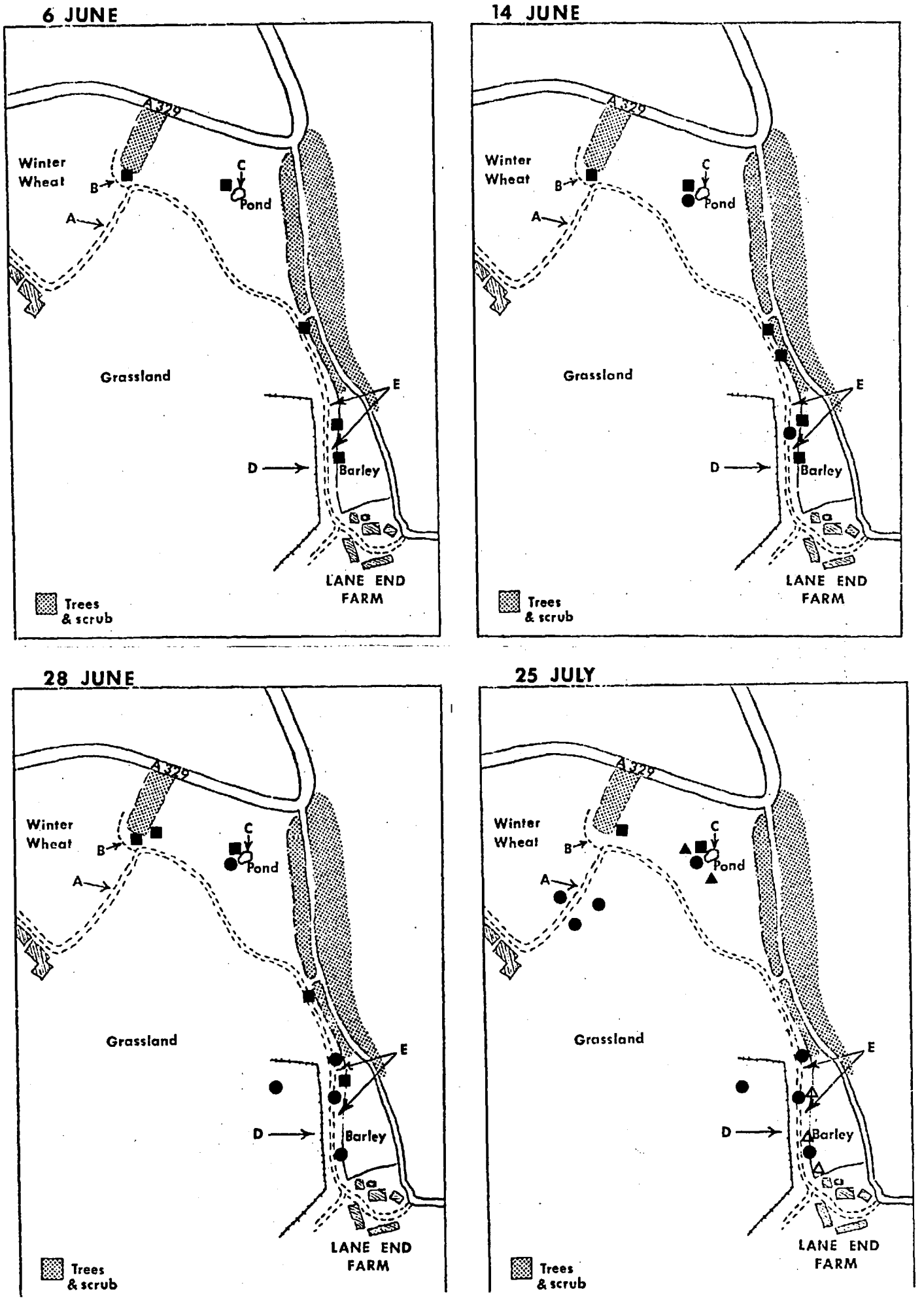
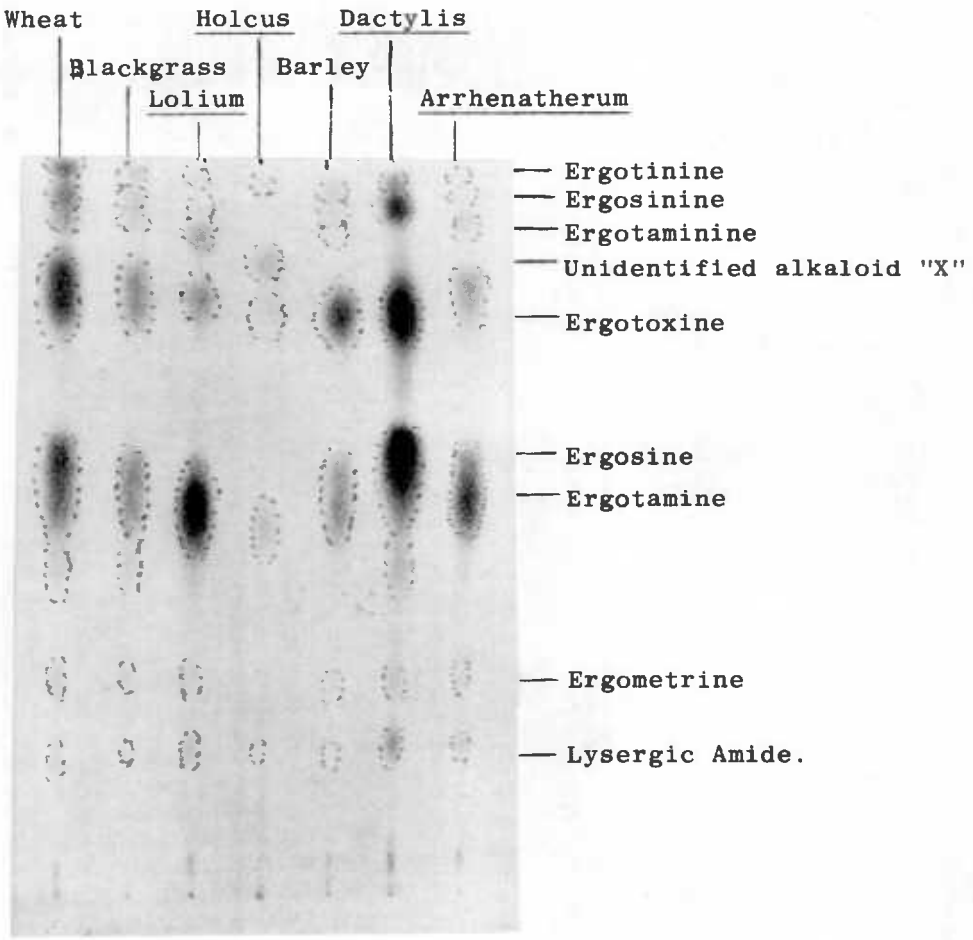


Figure 27.

Photograph of a thin layer chromatogram of alkaloid extracts of ergots collected from grasses and cereals at Lane End Farm during 1973



of which had reached anthesis) was observed until the final visit on August 17th. By this time, ryegrasses near to a permanent fence at site "D" had become heavily ergotised, as had a few heads of Holcus lanatus in the same area.

Alkaloid analyses (figure 27) showed that possibly four distinct strains of the ergot fungus at L.E.F. could be distinguished on the basis of differing sclerotial alkaloid spectra. One strain appeared to have been common to blackgrass and wheat at site "A" (see also, section 22.3). At sites "D" - "E", ergots collected from L.perenne, and A.elatius had almost identical alkaloid spectra, whilst ergots from H.lanatus were distinguished from all other strains by the presence of the unidentified alkaloid (p.135). Although both barley and cocksfoot ergots had an alkaloid spectrum in which the ratio of ergotoxine to ergotamine was high, they differed markedly in the amount of ergosine present. Too few blackgrass ergots were collected from site "E" for alkaloid analysis, but following experimental cross-infection to wheat of an isolate taken from one of these ergots (Section 21.1 - Accession number 132 in table 10), sclerotia were formed which had an alkaloid spectrum (A.S.C. = A2.0) similar to the barley ergots from site "E".

A period of dry weather during late May and early June 1973, together with early anthesis of wheat at site "A" probably accounted for the absence of cereal ergots at L.E.F. during the second summer of observation at the farm. Nevertheless, ergot honeydew

was first observed on meadow foxtail (Alopecurus pratensis) on May 30th at site "C" (figure 24). By June 6th this grass was found to bear honeydew at many other parts of the farm (figure 26a) at a time when all other grasses, including early flowering blackgrass and Glyceria sp, were apparently still uninfected. It may be of epidemiological significance that when a sample of honeydew from meadow foxtail (taken on 6th June) was used in cross-inoculation experiments (section 21.1, Accession No.328 in table 10) it was found to be highly infective to wheat. Further progress of the disease is summarised in figures 26 b - d.

23. The susceptibility of wheat to ergot during the flowering period.

23.1 Duration of the period of susceptibility

Puranik and Mathre (1971) demonstrated that florets of male-sterile wheats, though initially very susceptible to ergot, begin to lose their susceptibility from 5-10 days after "anthesis". The aim of glasshouse and field experiments (Section 16.4) has been to establish more clearly the nature and timing of this phase of declining susceptibility, especially in relation to the epidemiology of ergot disease in male-sterile wheat cultivars. The combined results of four experiments are shown in figure 28 and in table 19. Susceptibility is expressed as the % of inoculated florets yielding sclerotia (as previously defined, p.33). The following conclusions (a-c) are drawn from figure 28:

a) In the glasshouse, susceptibility to ergot infection of self-pollinated cultivars started to decline within 24 hours after anthesis.

Figure 28.

Graphs showing the changes in susceptibility to ergot of male-sterile wheat florets with time after anthesis.

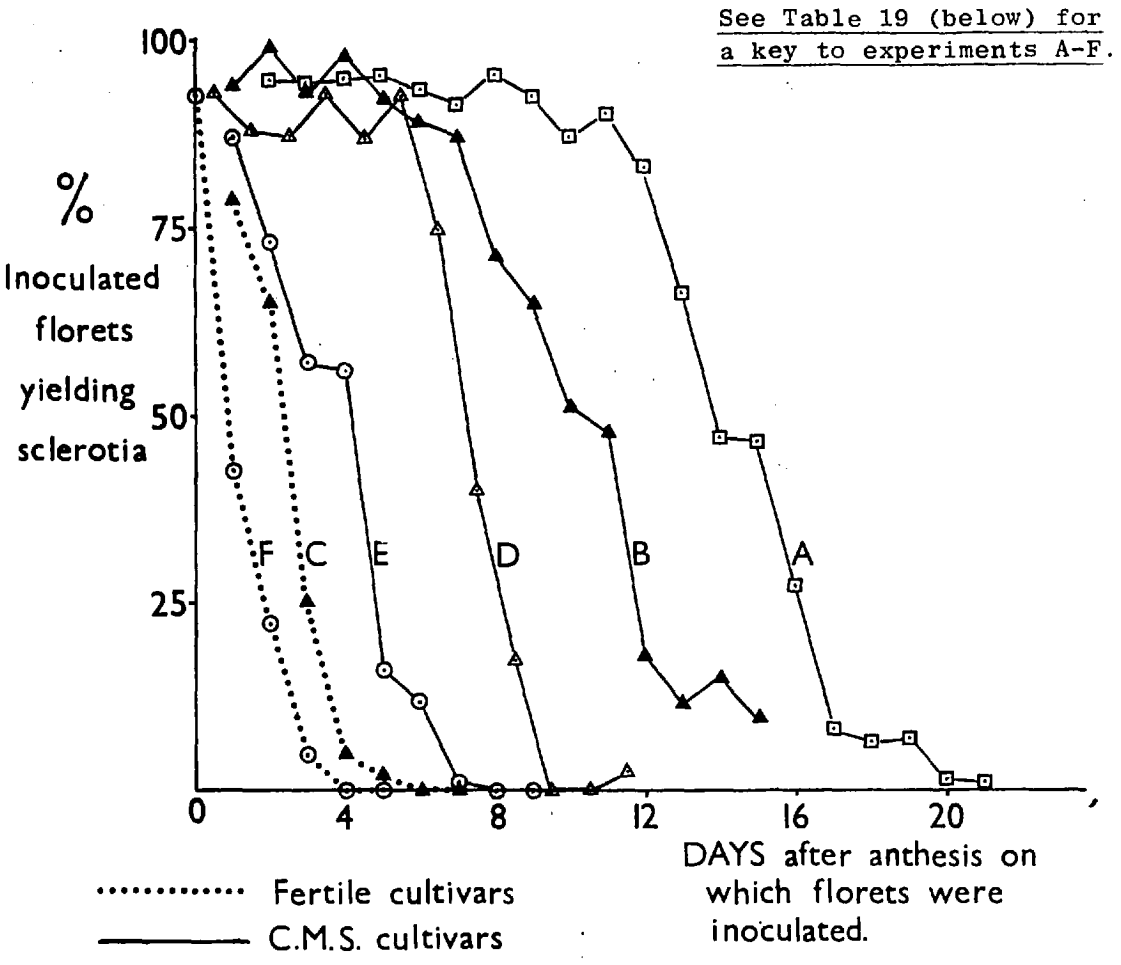


Table 19.

Glasshouse and Field conditions during experiments to investigate the duration of the period of susceptibility of wheat to ergot.

Graph	Year	Location	Cultivar	Temperature °C				Humidity R.H.%			
				Max.		Min.		Max.		Min.	
A	1972	Field	c.m.s. Capitole	15·2	±1·3	8·8	±2·8	98·7	±1·4	71·6	±18·3
B C	1972	Glasshouse	" Capitole [fertile]	21·6	±1·6	11·0	±0·9	98·9	±1·6	51·8	±11·3
D	1972	"	c.m.s. Capitole	23·6	±1·2	11·1	±1·0	94·2	±3·1	53·0	±11·0
E F	1973	"	" Capitole x Beacon	22·5	±1·7	14·4	±0·5	83·9	±4·2	67·3	±5·8

Examination of the contents of the floral cavities of inoculated spikelets revealed that whilst susceptibility, in terms of the number of sclerotia formed, declined rapidly after anthesis, many fertilized ovaries developed into partially infected seeds. Structures that appeared to be part seed and part sphacelial tissue were common, as were dark brown seeds which showed signs of superficial fungal infection and, frequently, had a water-soaked appearance (table 20).

b) In experiments, A, B and D (figure 28) cms Capitole was readily infected by ergot for 6-10 days after "anthesis", depending on environmental conditions. Thereafter, susceptibility rapidly declined until by 10-20 days, respectively, male-sterile ovaries did not support infection.

Examination of the contents of floral cavities of the inoculated spikelets of cms Capitole from the field experiment (figure 29) showed that as the numbers of sclerotia decreased, so the frequency of occurence of sphacelia (aborted infections) increased. Thus, the loss of susceptibility appears to have resulted from the inability of the fungus to proceed beyond a well-established sphacelial phase to form sclerotial tissue.

c) The duration of the period of maximum susceptibility of cms Capitole was influenced by the ambient temperature.

Table 20.

Results of inoculation of fertile wheat (cv.Capitole) with ergot from 1 to 7 days after anthsesis.

Days after anthesis on which florets were inoculated.	% of inoculated florets yielding:-		
	Ergots	Sphacelia	Damaged seed.
1	79	2	9
2	65	17	10
3	25	25	21
4	5	4	34
5	2	10	44
6	0	3	41
7	0	0	47

Regression equations were calculated for the approximately linear portions of graphs A, B, D and E (figure 28). From the regression lines obtained (figure 31) the values of x (days after anthesis on which florets were inoculated), where y = 50 (50% of inoculated florets yielding sclerotia), were calculated. For each of these experiments the mean temperature and humidity was also calculated (as $\frac{1}{2}$ x (Average daily maximum + Average daily minimum)) from data shown in table 19. The values of x (at y = 50%) were then plotted against a) mean temperature and b) mean humidity (figure 30). Whilst the number of samples was insufficient to provide a meaningful test of statistical significance, the results suggest that there is a negative correlation between the ambient temperature and the duration of the susceptible phase.

The average time intervals between inoculation and the first sign of exudation of honeydew are shown in figure 31. This time interval was greatest in experiments conducted at lower temperatures when the rate of development of the fungal infection was retarded. It was notable that in each experiment honeydew started to exude from first infected florets during the phase of declining susceptibility of the remaining, uninoculated florets. In the field experiment, exudation of honeydew was first observed during the early part of this decline phase.

Figure 29

The effect of inoculation of male-sterile wheat florets with ergot from 2 - 21 days after anthesis. (Field experiment, 1972).

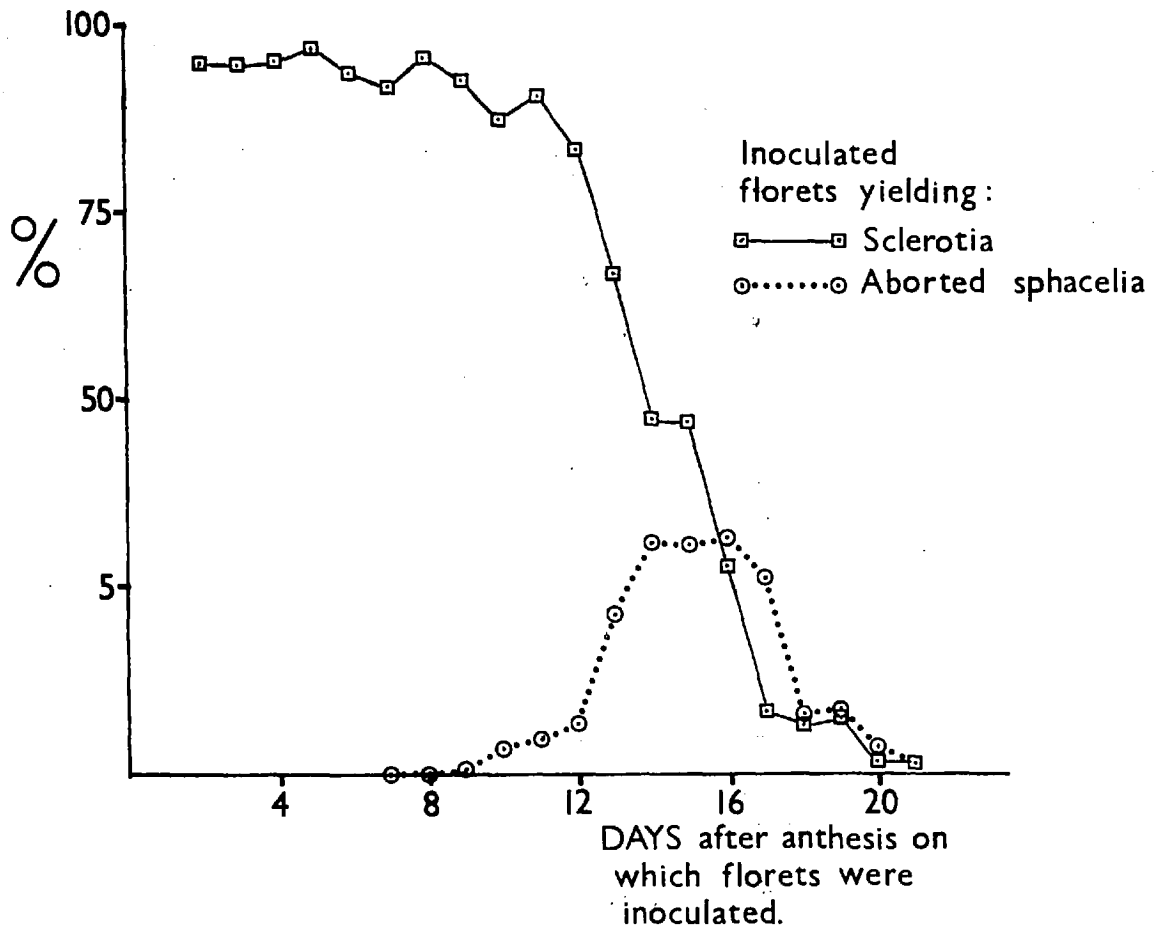


Figure 30

The effects of a) temperature and b) relative humidity on the duration of the period of high susceptibility of male-sterile wheat to ergot.

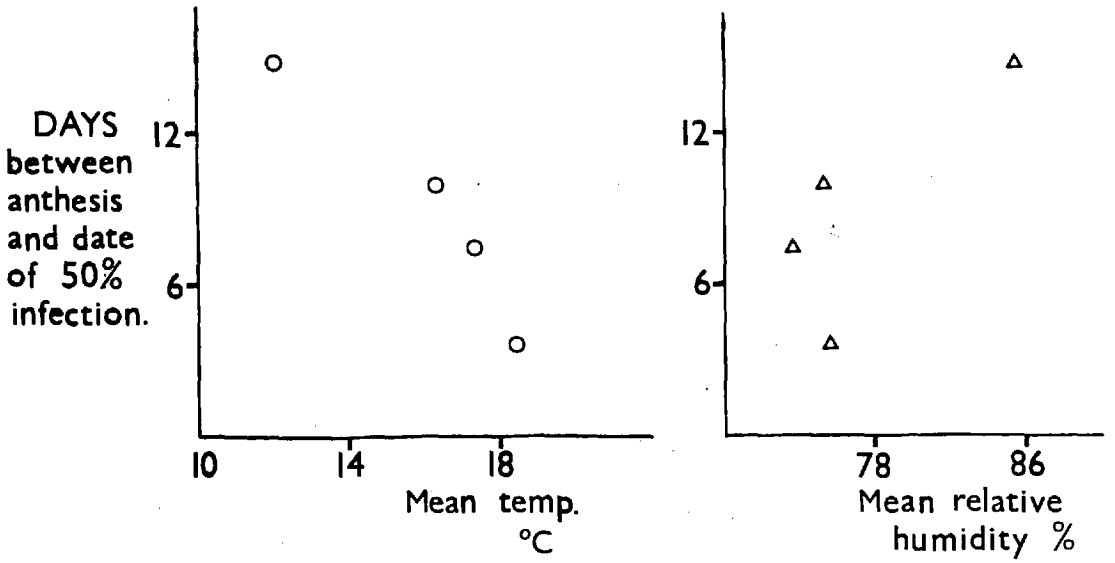
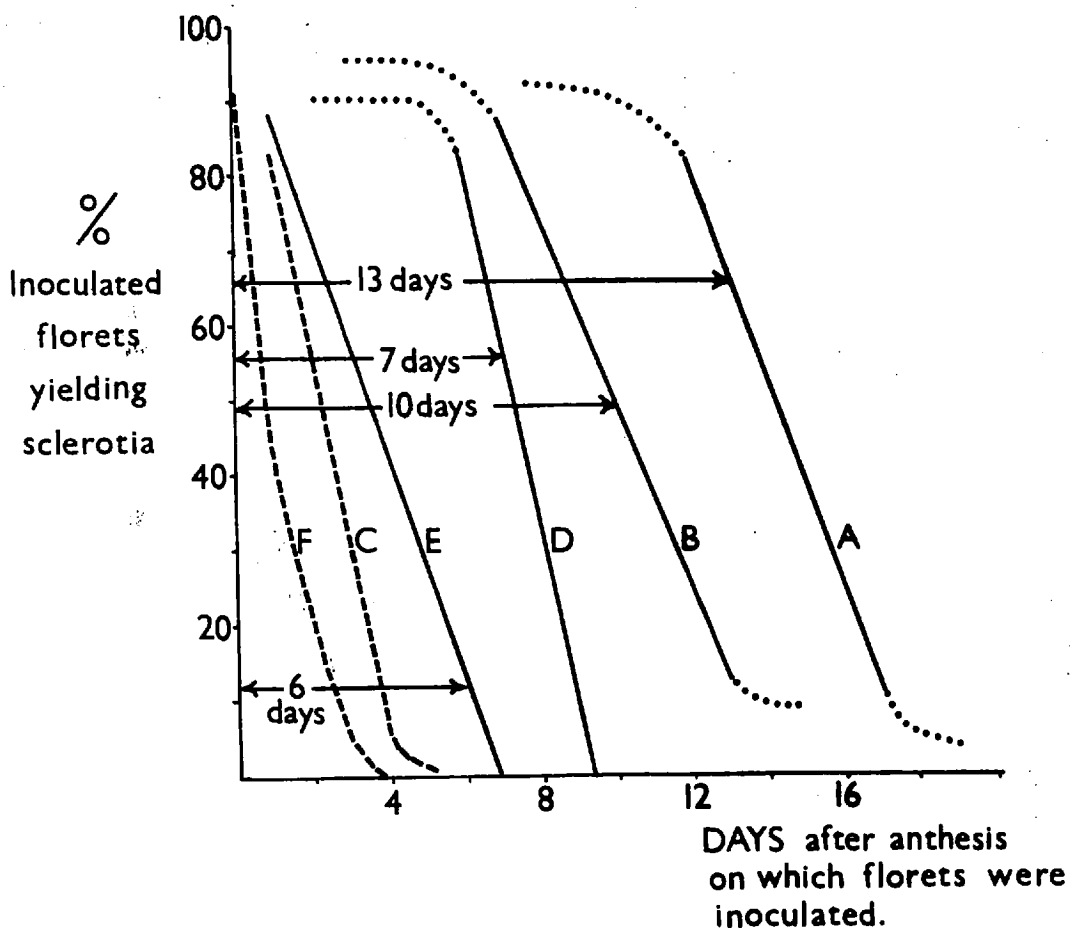


Figure 31

The relation of the time interval between ergot-infection and first production of honeydew to the duration of susceptibility of male sterile wheats.



←——— x ———→ = x days between inoculation of the first anthesing heads and production of honeydew.

Regression equations :

Graph A. $y = 50.05 - 2.81x$

Graph B. $y = 34.86 - 2.48x$

Graph D. $y = 45.51 - 4.85x$

Graph E. $y = 20.62 - 3.00x$

23.2 The period of susceptibility of male-sterile wheat to ergot disease, in relation to seed set

Designs of both the 1973 and 1974 experiments are described in Section 16.4.2 In the 1973 experiment a gradient of seed set within the plots of cms Capitole was established. The amount of seed set decreased with increasing distance from the source of pollen, as shown in figure 32. The distribution and amount of pollen-trapped within the plots (figure 32) followed a trend similar to seed set. Wheat pollen was first trapped on 18th June, two days before the primary heads of cms Capitole commenced flowering. Following this, with the exception of the period 19-21 June (when weather conditions were cool and wet), air-borne pollen was trapped on each successive day until 29th June when sampling was discontinued. Furthermore, wind direction recordings (table 21) showed that the prevailing winds had generally been in a favourable direction for the dispersal of pollen from the pollinator, across the male-sterile plots.

In the preceding section it was shown that fertilized wheat ovaries lose their susceptibility to ergot more rapidly than unfertilized ovaries. It might have been expected, therefore, that samples of wheat heads having the highest seed set would have been the least susceptible to secondary infection by ergot. Results shown in figure 32 neither reject nor uphold this prediction. Although the mean number of ergots in hussocks near to the pollinators showed

Figure 32.

Graph showing a) the amount of pollen trapped, and b) seed set and infection by ergot in hussocks of cms Capitole, at varying distances from a pollinator. (1973, field experiment.)

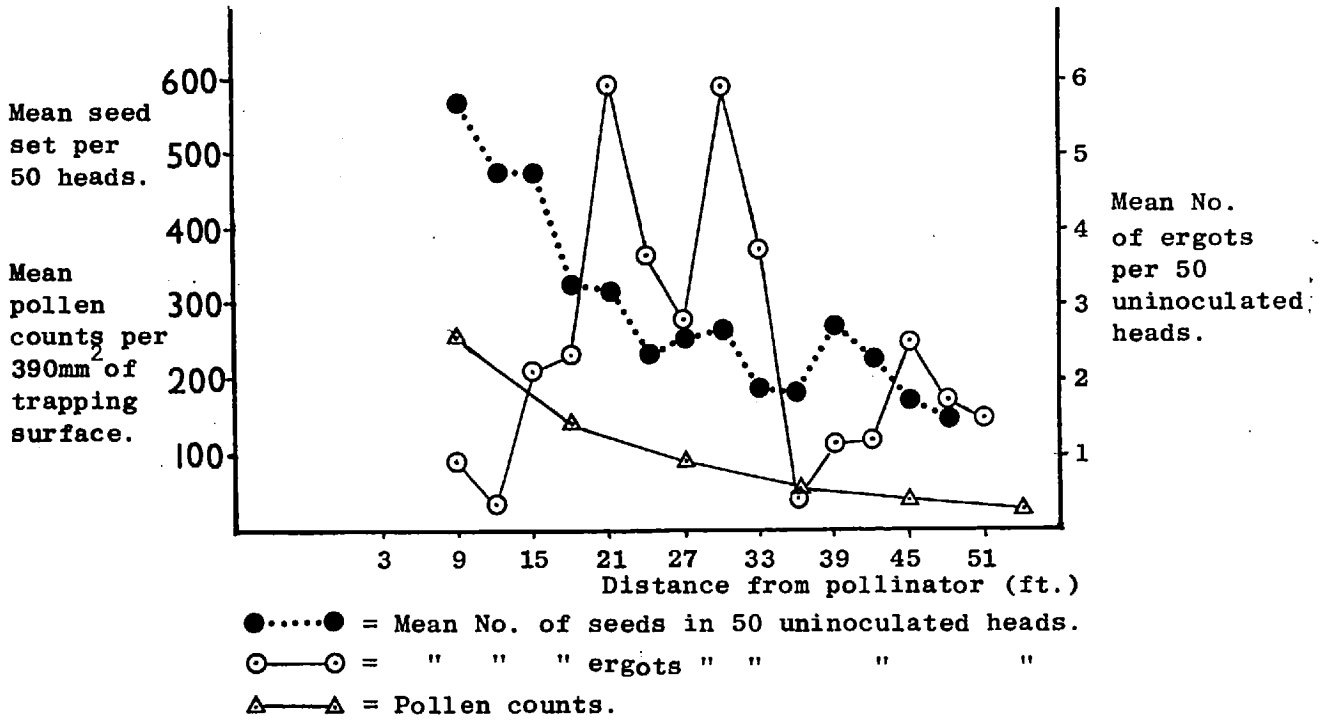


Table 21.

Daily pollen counts and wind direction during the experiment to investigate the effect of seed set on infection of male-sterile wheat by ergot, 1973.

Date: (June, 1973).	18	19-21	22	23	24	25	26	27	28	29
Total daily pollen count.*	135	7	142	172	292	564	52	32	99	347
Prevailing wind direction.**	230°	170-190°	150-200°	170-190°	80-160°	180-250°	90-210°	90°	-	220-240°

* No. of pollen grains trapped on a total of 700mm² of trapping surface.

** Where 180° = direction of wind blowing from the pollinator to the male-sterile plants (see figure 8).

some correlation with the amount of seed set in these hussocks, levels of ergot infection of hussocks at greater distances from the pollinator were extremely variable. Surprisingly few ergots were found in hussocks that had the least seed set. Considering that the highest rate of secondary infection in any hussock was less than 13 ergots per 50 ears, the most reasonable explanation of these results was that, for the most part, they reflect no more than the distribution of a few, heavily infected ears which may not have been representative of the general level of infection in each hussock.

As a result of staggered inoculation dates in rows D, E, F and G (figure 8) honeydew was first observed in these rows 10,12, 15 and 18 days after anthesis, respectively. Despite the low levels of infection observed, comparison of treatments D - G (figure 33) did serve to demonstrate that the amount of secondary infection was influenced by the timing of exudation of honeydew from the primarily infected heads.

Treatments within hussocks in rows A, B and C (figure 8) were designed to investigate the effect of seed set on the relationship between the susceptibility of wheat ovaries to infection and the time after anthesis at which they were inoculated. Results are shown graphically in figure 34. By comparison of the amount of infection of hussocks nearest to the pollinator with ergot infection of the control hussocks (in which individual ears were bagged during the period of pollen release to prevent

fertilization before they were inoculated), it was clear that the effect of pollination and seed set had been to shorten the susceptible period. However, it is emphasised that since the maximum level of seed set recorded in the male-sterile hussocks was equivalent to less than thirteen seeds per ear, these results cannot be regarded as being indicative of the effects of a commercially viable level of seed set on ergot infection in a normal crossing block situation.

In the light of problems encountered in the above experiment, the design of the 1974 experiment (figure 9) had been modified to encourage both increased seed set and higher levels of secondary infection. Results (figure 35) show that neither objective was achieved. As a consequence of poor "nick" between the flowering period of cms Capitole and the dates of anthesis of the pollinator varieties, seed set was even lower than it had been in 1973. Furthermore, since weather conditions were unfavourable for natural spread of the disease within male-sterile plots, the amount of secondary infection was too small to provide meaningful results.

A secondary aim of this experiment had been to investigate the effect of ergot infections on seed quality. In experiments described in section 23.1, it was noted that where infection occured after ovaries had been fertilized, partially infected seeds were commonly formed. Samples of seed from inoculated

and uninoculated plots of the seed set experiments were compared. Although a few seeds from the ergot-infected plants were found to have been colonized by the white, spore-bearing sphacelial stage of the fungus, the remaining seeds, from both infected and control plots, were too shrivelled and heavily contaminated with saprophytic fungi to permit further comparisons to be made.

Figure 33.

Histograms showing the amount of secondary infection by ergot in plots of male-sterile wheat, both in relation to distance from a source of pollen and to the earliness of primary infection.

Treatments: Secondary infection by natural spread of ergot honeydew from four wheat heads in each hussock, inoculated at:-

D - anthesis.
E - anthesis + 3 days.
F - anthesis + 6 days.
G - anthesis + 9 days.

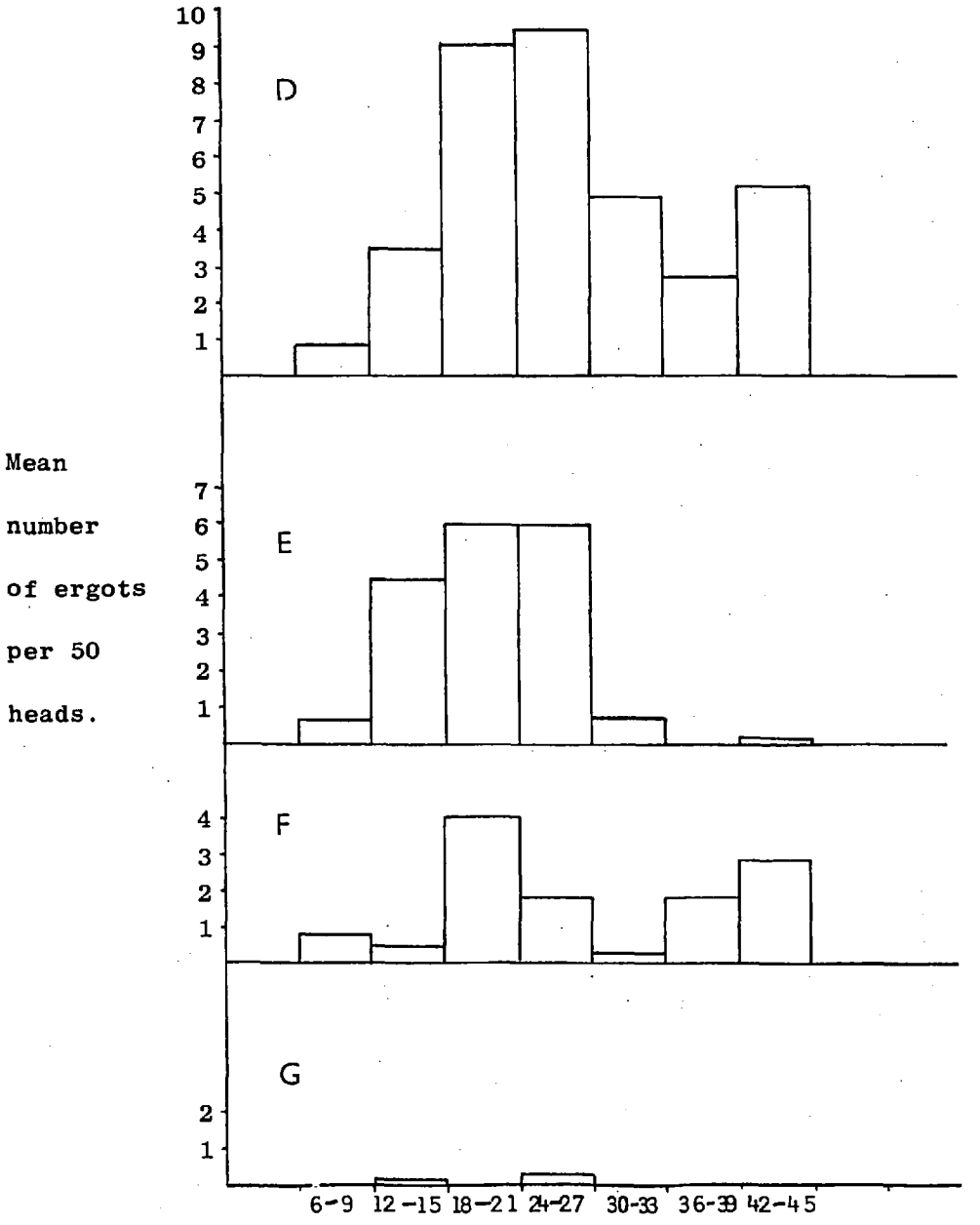


Figure 34.

The effect of seed set (as determined by the distance from a source of pollen) on the relationship between ergot infection and time after anthesis at which male-sterile florets were inoculated (1973, field expt.)

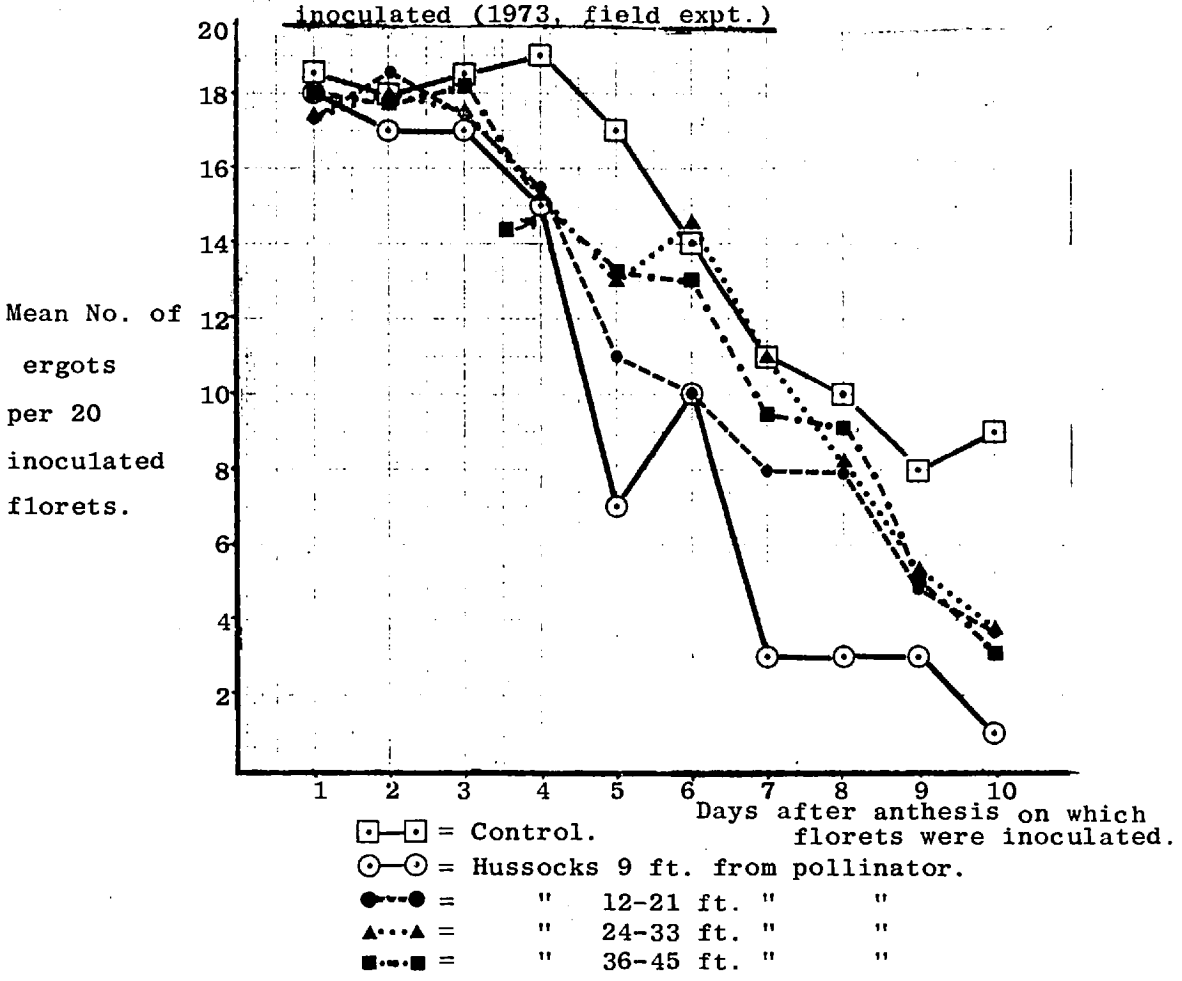
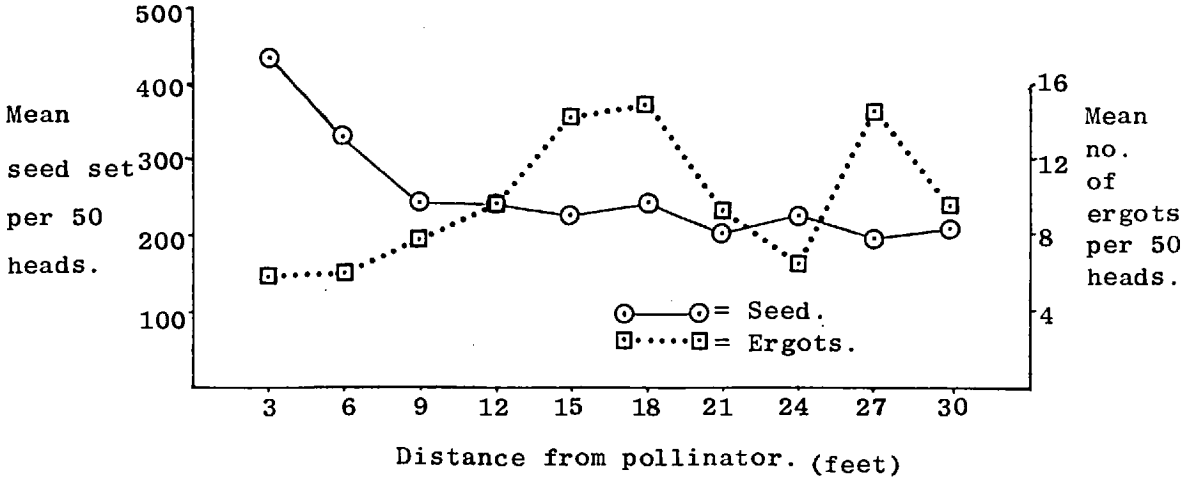


Figure 35.

Graphs showing a) seed set and b) 2^{o} infection by ergot in hussocks of cms Capitole at varying distances from a source of wheat pollen. (1974, field experiment).



24. Results of screening of conventional and male-sterile wheat cultivars for resistance to ergot

24.1 Screening in the glasshouse

Five male-sterile winter wheat varieties, together with two conventional wheats (cvs. Kenya Farmer and Carleton), described by Platford and Bernier (1970) as having resistance to ergot, were tested by inoculation with strain 25. Spore concentration was measured in honeydew collected from one inoculated head from each sample, and the remaining three heads were used to assess the average number of ergots formed in 60 inoculated florets (table 22). Cultivars have been ranked in order of a) spore production and b) numerical yield of sclerotia. The cultivars cms 4 6 and Carleton occupy the upper and lower extremities of the rankings for each parameter, but only in ergot production was Carleton significantly different from all other cultivars. Surprisingly, the other reputedly resistant cultivar, Kenya Farmer, was only found to be significantly less susceptible than one of the cms cultivars, cms 46, and then only marginally different at the 5% level.

24.2 Varietal susceptibility to ergot in a winter wheat variety observation trial

At Garford, near Oxford, a winter wheat observation trial was found to be infected with ergot. The presence of mature ergots on blackgrass, which was thinly distributed throughout the trial area, suggested that honeydew on the blackgrass may have been the primary source of inoculum for infection of the wheat varieties.

Table 22

Results of a glasshouse experiment to screen seven wheat cultivars for resistance to ergot.

Cultivars are ranked in order of a)spore production and b) number of ergots formed in 60 inoculated florets.

a)

Cultivar	Mean spore count*
cms 46	274
cms Shawnee	252
cms 42	198
Kenya Farmer	187
cms Capitole	171
cms Cappelle D.	156
Carleton	142

L.S.D. = 82.8 (p= 0.05)

b)

Cultivar	Mean No. of ergots.**	Mean T score.
cms 46	58.1	80.9
cms Cappelle D.	57.1	79.7
cms Capitole	56.4	79.0
cms 42	55.4	75.4
Kenya Farmer	54.6	72.7
cms Shawnee	54.1	72.4
Carleton	48.6	64.5

L.S.D. of T scores = 7.29 (p = 0.05)

N.B. No significant difference between treatments linked by brackets.

* Total conidiospores collected per ear may be calculated as: Mean spore count x 10^7

**Mean number of ergots formed in seven replicate samples of 60 inoculated florets.

Unreplicated counts of ergots, expressed as ergots per 10Kg of uncleaned seed of each cultivar, are shown in table 23. Cultivars are listed in order of their spatial arrangement in the field.

Table 23. Counts of ergots in seed samples of winter wheat cultivars grown at Garford, Oxford

Cultivars	No. of ergots per 10Kg sample
Maris Nimrod	1.5
Maris Ranger	3.4
West Desprez	6.0
Maris Widgeon	6.4
Atou	9.7
Chalk	31.6 (late flowering)
Flinor	0.9 (early flowering)
Maris Huntsman	9.3
Ceppelle Desprez	7.9
Champlein	0 (" ")
Joss Cambier	0.7 (" ")

24.3 Screening for field resistance

The design of this experiment (p82) proved to be unsuitable for the dry weather conditions that prevailed during the early summer at Throws Farm. In particular, inoculation of the blackgrass by an external spray of inoculum was not completely successful, with the result that sources of honeydew from blackgrass were not evenly spread throughout the experiment. Furthermore, many of the hussocks of male-sterile cultivars were severely affected by drought. In consequence, a considerable degree of variation

between ergot infection of samples of the same variety in different blocks was encountered. Nevertheless, in terms of ergot number per head in each test hussock, some fairly significant variation between varieties ($p = 0.01$) was encountered (table 24). An analysis of variance of these results is given in Appendix I.

Table 24

Results of an experiment to screen twelve male-sterile, spring wheat cultivars for field resistance to ergot.

Cultivars are ranked in order of a) ergot number per head, and b) ergot weight per head. Values within brackets are not significantly different.

a) Ergot number per head.

Cultivar	Mean
RHM 578	0.767
Maris Ranger	0.805
Sirius	1.203
Rask	1.423
Trident	1.503
Clarion	1.650
RHM 576	1.933
R. Sprite	1.973
Maris Dove	2.150
Kleiber	2.417
Cardinal	2.947
RHM 573	3.083

L.S.D.= 1.214 (p = 0.01)

Block 1	1.657
Block 2	1.944
Block 3	1.959

N/S

b) Ergot weight per head.

Cultivar	Mean weight(g)
Maris Ranger	0.0205
Clarion	0.0367
Trident	0.0413
RHM 578	0.0527
R. Sprite	0.0567
RHM 576	0.0717
Sirius	0.0743
Maris Dove	0.0747
Rask	0.0803
Kleiber	0.0857
Cardinal	0.1043
RHM 573	0.1103

F-ratio not significant for varieties ∴ no L.S.D.

Block 1	0.0463
Block 2	0.0747
Block 3	0.0858

L.S.D. = 0.0266 (p= 0.025)

DISCUSSION

The present investigations of the aetiology of ergot disease of wheat have followed a number of distinct lines, many of which have provided a better understanding of some of the factors involved. Whilst discussing these studies it will be useful to refer to four hypothetical ways by which ergot disease may spread within a cereal crop (figure 36).

Whilst preparing figure 36, two factors were taken into account. First, it was assumed that the ergot fungus can rarely, if ever, overwinter in Britain by continuous parasitism of gramineous hosts. Only one common British grass, Poa annua, flowers throughout the winter months, and there is no evidence of winter-infection of this species (section 18). It would appear, therefore, that the annual initiation of the disease cycle is dependant on the release of ascospores from germinating sclerotia in the spring or early summer. Secondly, through observations at many experimental sites it was generally apparent that whilst ergot disease was often widely dispersed within the trial areas (presumably by insects), most secondary infection occurred within a metre radius around primarily infected plants.

In the field situation A (figure 36) an arable site that is more or less free from grass weeds is considered. Here, in the absence of alternative grass hosts, the earliest date of establishment of the fungus in its parasitic state would be at anthesis of the cereal crop. In this instance it is assumed that the amount of primary inoculum (either as ascospores from

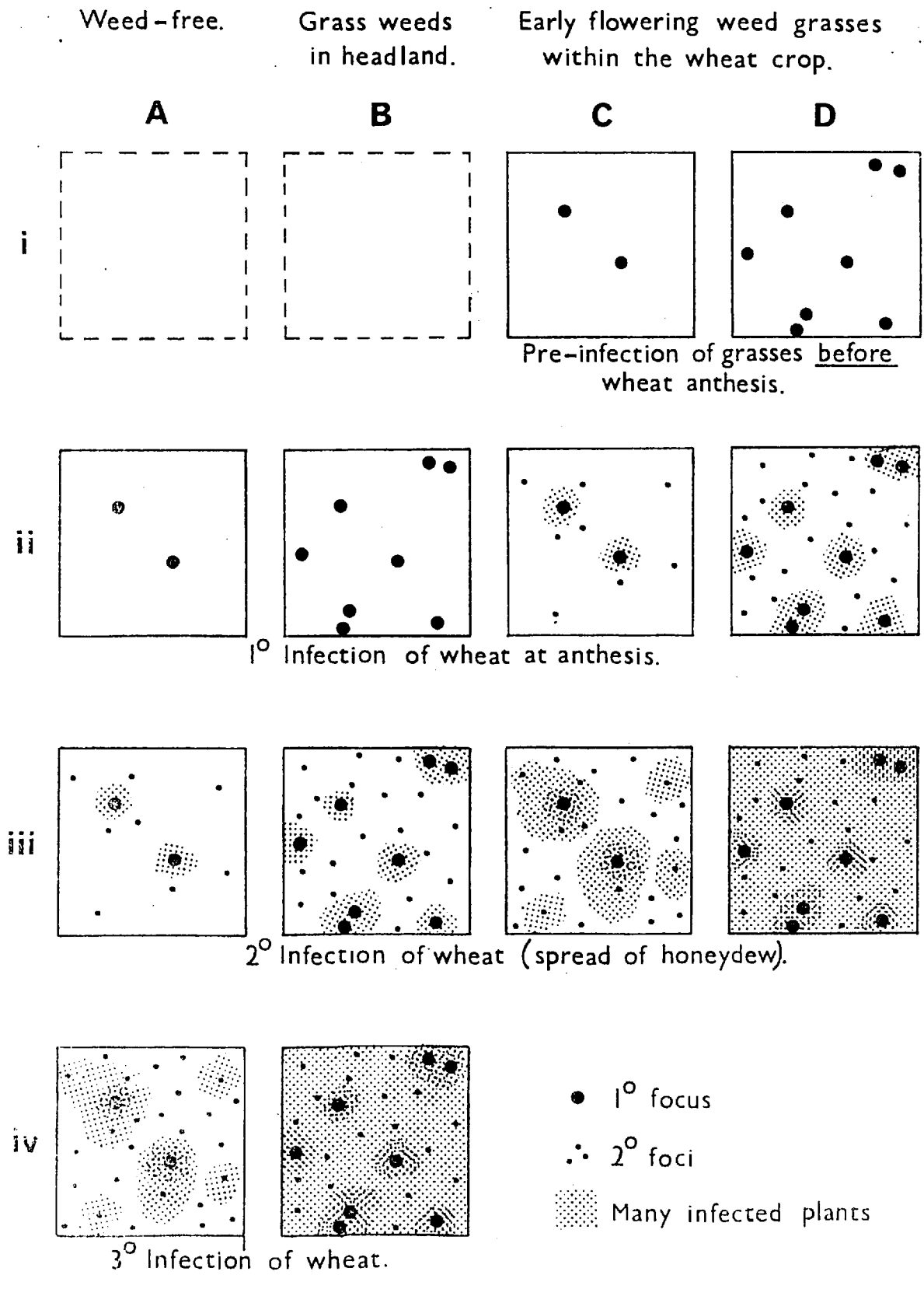
residual cereal ergots remaining from a previous cereal crop, or as honeydew carried by insects from infected grass hosts in other localities) would be relatively small, and would therefore give rise to few primary foci of infection. At these foci, honeydew (containing conidiospores of C.purpurea) may be exuded from infected florets some 7-14 days after primary infection (section 23.1). Thus if some ears of wheat are still susceptible at this time, secondary infections may occur; mainly on plants adjacent to honeydew-bearing tillers but also, possibly as a result of insect transmission, on a few plants scattered throughout the crop. Severe infection, as illustrated in part A.iv is not anticipated, except in the unlikely event of widespread tertiary infection.

Situation B (i - iv), is a weed-free cereal crop surrounded by grass headlands. Whenever early flowering grasses are present in the headland there is a possibility that such grasses may become infected and exude honeydew before wheat reaches anthesis. This honeydew exudate could thus provide a source of inoculum for the establishment of many foci of infection of the earliest anthesing wheat plants, mostly around the perimeter of the crop. The epidemiological significance of this is particularly apparent when secondary infection within the crop is considered (B.iii).

In the two hypothetical situations described so far it has been suggested that wide-spread and serious ergot infection of a cereal

Figure 36.

Hypothetical types of progression of ergot disease in a wheat crop.



crop is unlikely unless the fungus successfully completes the secondary phase of infection. In situations C and D, the presence of early flowering grasses within the crop provides an opportunity for the establishment of foci of infection of the disease even before the cereal reaches anthesis, thereby placing the crop at greater risk of a high rate of secondary infection.

It has been assumed for situation C that primary foci of infection of weed grasses would be few, but there are two possible ways in which even the early flowering grasses could be widely infected (D). First, there may be a large number of grass ergots carried overwinter in the soil from a previous weedy crop. These may have the potential to discharge many ascospores, especially when lying at the surface of undisturbed soil in headland areas. Secondly, there are a few very early flowering grasses, notably Alopecurus pratensis, Anthoxanthum odoratum and Poa annua, which could conceivably be infected early enough to provide a source of honeydew for more widespread infection of other early flowering grasses amongst a cereal crop. As illustrated in C and D (i - iv) the consequence of primary foci of infection within a crop before anthesis could, under favourable weather conditions, be a severe epidemic of ergot disease.

In constructing the above hypothetical situations many assumptions concerning both the life cycle and the epidemiology of ergot have, of course, been made. Not the least of these is the assumption that the seasonal timing of ascospore discharge from germinating ergots would permit an early infection of gramineous

hosts. The previous observations (Jenkinson, 1958) that ryegrass ergots germinate during the June-July period does not, at first sight, support the aetiological hypothesis of a disease progression from ergot sclerotia to early flowering grass to wheat. However, cross-infection studies (section 21.1) indicate that strains of ergot collected from ryegrasses are only rarely associated with ergot of wheat and indeed were only found to be weak parasites of wheat under experimental conditions.

Despite having been undertaken in France, studies on the germination and ascospore discharge of wheat ergots (Rapilly, 1969) may have more significance. Rapilly found that the majority of wheat ergots had germinated and had discharged ascospores before the end of May, and thus appears to support the view that wild grass species are important as an alternative host of wheat-infective strains of ergot.

In the present studies (section 20), the first series of observations of sclerotial germination indicated that the peak of ascospore discharge from wheat ergots occurred at about the end of May/early June 1972. Under experimental conditions one early flowering grass, blackgrass, became infected with ascospores discharged from wheat ergots and bore wheat-infective honeydew before and during anthesis of the early flowering winter wheat, cms Capitole.

Observations, during the second week of April 1973, of the presence of ergot sclerotia showing early stages of germination showed that wheat ergots had the potential to germinate and discharge

ascospores at an even earlier date than had been observed during the previous year. However, as a result of prdonged dry weather conditions, the germination process was so severely checked that it was not until early June that ascospore discharge was first recorded. The influence of weather conditions on the timing of ascospore discharge was thus amply demonstrated.

Germinating ergots were observed at three separate sites during 1972. Again, the varied timing of germination was apparent. Ergots in the sheltered environment of the Chelsea Physic Garden reached their peak of production of perithecial fruiting bodies 2-3 weeks before ergots at the most exposed site at C.M.F. (Stokenchurch).

Since the earliness of ascospore-infection of grasses and cereals is a fundamental factor in the hypothetical types of disease progression described earlier, it is apparent that the progress of an ergot epidemic may be largely influenced by the local weather conditions that prevail throughout April, May and June. Nevertheless, results have demonstrated that (abnormally dry seasons excepted) ascospores from wheat ergots can constitute a primary source of inoculum for those early flowering grasses which anthese in late May or early June. The question of whether or not even earlier flowering grasses can be infected during April remains unanswered.

To be a potential source of inoculum for the infection of wheat crops, honeydew, exuding from the infloresences of infected grass florets,

must be present at the time of anthesis of wheat. Since honeydew is not produced until 1-2 weeks after infection (p 162), only those grasses flowering 1-2 weeks before wheat will initially be important as alternative hosts of wheat-infective inoculum. Results of past and present investigations of flowering of various grass species have been partly discussed in section 18. Alopecurus geniculatus, A. myosuroides, A. pratensis, Anthoxanthum odoratum, some Bromus species, Glyceria fluitans, Lolium perenne (early flowering varieties only), Poa annua and P.pratensis are common British grasses that have all been described as "early flowering" grasses (Table 3) and as such are capable of bearing honeydew at a time when it can act as inoculum for primary infection of winter wheat. Three of these grass species, Anthoxanthum odoratum, Alopecurus pratensis and Poa annua have been found to flower early enough to allow time for the ergot fungus to complete more than one multiplication phase of its life cycle on the grass before infecting wheat in mid June.

Many other grass species reach the flowering stage at about the same time as most winter wheats. In most field situations it is not envisaged that these grasses could be important carriers of ergot inoculum for infection of winter wheats, except where abundant late tillering and/or secondary growth has occurred. However, if spring wheats are also to be considered, the generally later anthesis dates of spring wheat cultivars - extending to the

end of June in some cases - could elevate some of the June-flowering grasses into a more aetiologically important role.

Even allowing for late tillering of wheat crops, it would be exceptional to find more than a scattering of susceptible (anthesing) ears of wheat in a crop after the first week of July. Grasses flowering after the end of June are, therefore, most unlikely to play a major role in the aetiology of ergot disease of wheat except, possibly, by bearing ergot sclerotia which fall to the ground and discharge ascospores in the following spring. It is noteworthy that couch grass (Agropyron repens), a common arable weed that is frequently parasitised by ergot, is included in this category. Referring once again to figure 36, it is clear that although ergot-infected June-flowering grasses may contribute some inoculum for infection of late tillers of wheat, increased primary infection of wheat by the spread of ergot honeydew from the earlier flowering grasses is potentially of much greater epidemiological importance.

Three of these early flowering grass species, Alopecurus myosuroides, A. pratensis and Anthoxanthum odoratum have in common a markedly protogynous flowering mechanism. This is particularly suited to their potential role as hosts of ergot since cross-pollination is encouraged by the exsertion of stigmas from florets a few days in advance of dehiscence of the anthers.

This means that florets are, in effect, male-sterile for a while and are accordingly more susceptible to ergot. It is, therefore, curious that one of these grasses, A. odoratum, was seldom found to be ergot-infected. The infrequency of occurrence of ergot on Poa annua is, on the other hand, more understandable since this grass is generally self pollinated.

A high frequency of occurrence of ergot on early flowering grasses near to arable land does not necessarily prove that such grasses play a role in the aetiology of wheat ergot. Some grass hosts may carry strains that are relatively inocuous to wheat. The cross-infectability of grass-borne strains of ergot to wheat has been investigated by many workers (section 5). Two of these Stäger (1903, 1905, 1907, 1908, 1923) and Campbell (1957) came to somewhat different conclusions. Whereas Stäger had concluded that there existed some host restriction of strains of ergot, Campbell claimed to have successfully cross-infected barley, wheat and rye with strains of ergot from 47 different gramineous host species. By way of an explanation of these conflicting results, Campbell suggested that, in some instances, Stäger had failed to secure infection only because of poor inoculation technique and/or impersistence. However, Campbell's results may also be open to criticism, from the opposite viewpoint: successful cross-infection appears to have been claimed, in most instances, regardless of the degree of difficulty with which it was achieved.

In the present studies, cross-inoculation of strains of ergot between gramineous hosts have been investigated on a quantitative basis (section 21.1). As shown in table 11, when the percentage of successful infections of wheat by different strains of ergot were compared, isolates could be divided into two main groups, i.e. into "highly" and "weakly" infective isolates. It was, however, notable that only 3 out of 40 different isolates failed to give any infection at all - a result which, if interpreted on a non-quantitative basis, would not have appeared to have differed greatly from Campbell's observations. Significantly, all seven isolates of ergot from blackgrass readily cross-infected wheat, as did isolates from other cereals. Seven isolates from ryegrass, six from cocksfoot grass and twleve isolates from other common hedgerow and pasture grasses were found to be only weakly infective to wheat.

Except, perhaps, for the three grass species named above, most host species were represented by too few isolates to contribute much to the evidence supporting host restriction of ergot strains. However, subsequent analysis of the alkaloid content of ergots formed during cross-infection studies revealed two features which greatly enhanced their significance. First, there was a close correlation between high ergotoxine content (compared with ergotamine) and a high frequency of successful infections of wheat by an isolate. Secondly, it was clear that parasitism of a new host did

not markedly affect the relative proportions of the main alkaloids produced by particular ergot strains. In other words, there was a characteristic spectrum of alkaloids for each isolate, which normally remained unchanged during cross-infection. It is, therefore, reasonable to assume that if, under natural conditions, strains of ergot carried by a particular grass host constitute a common source of inoculum for infection of wheat, then a proportion of a large collection of naturally occurring wheat ergots ought to be found to have similar alkaloid spectra to those ergots most commonly collected from the grass. The ability to "fingerprint" strains of ergot by sclerotial alkaloid analysis was thus utilized in relating the results of analysis of over 250 samples of naturally occurring cereal and grass ergots to incidence of natural cross-infection.

Results of these analyses have been partly discussed in section 21.3. It remains to re-examine them in relation to the degree of risk of cross-infection of ergot between particular grass species and cereals. In figure 37, the frequency of occurrence of samples of naturally collected ergots in each of the three main alkaloid categories (Ergotoxine $\gg$ ergotamine, Ergotamine $>$ ergotoxine and no alkaloids) is shown by histograms. Only commonly infected grasses from habitats in and around arable land have been included, and these are grouped according to their flowering dates: these being generally earlier, similar to or later than anthesis of wheat.

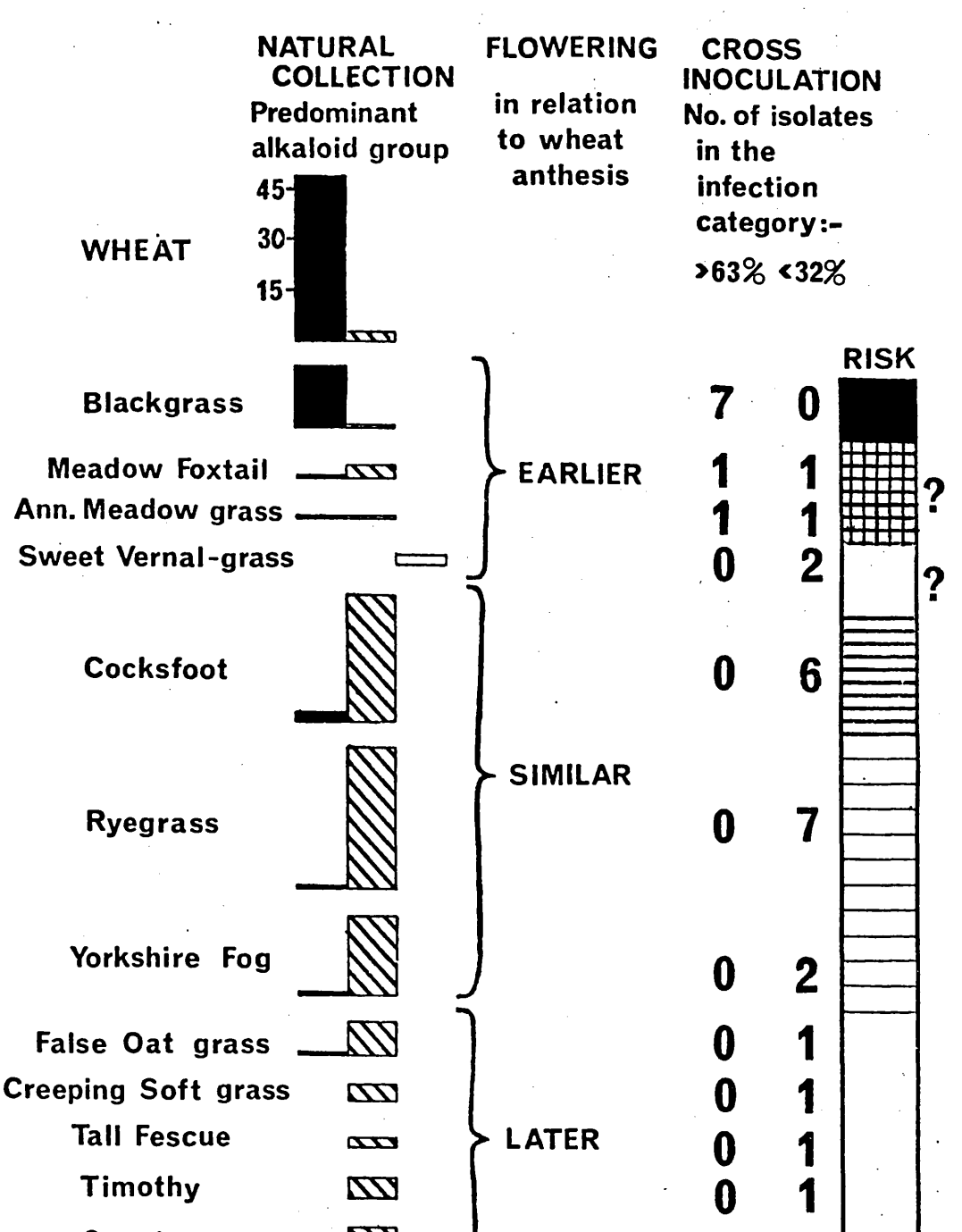
In terms of risk of natural cross-infection of ergot to wheat, the presence of blackgrass in a crop appears to be unassailably at the top of the list. This common arable weed has been found to carry alkaloid strains of ergot that are similar to those found in wheat, and it has an ideal flowering date both for infection and a build-up of inoculum to occur before wheat reaches anthesis. Furthermore all seven isolates investigated readily cross-infected wheat under experimental conditions.

Although the two early flowering grasses, annual meadow grass and meadow foxtail have each been found to carry one strain of ergot that was highly infective to wheat, other strains tested were only weakly infective. These grasses may be of some importance in the aetiology of wheat ergot but more samples, especially from arable land, are required before any firm conclusions can be made. Similarly, the limited evidence of analysis of ergots from sweet vernal grass, which were found to be almost totally devoid of any alkaloids, suggests that infections of this grass are of little consequence.

None of the common grasses that commence flowering at about the same time as anthesis of winter wheat have provided a highly wheat-infective strain of ergot. However, the apparent habit of cocksfoot, in particular, to carry a number of different alkaloid strains (as shown by analysis of a large, natural collection of cocksfoot ergots) does not preclude the possibility that a cereal-infective

Figure 37.

Summary of some of the factors contributing to an assessment of the degree of risk of natural cross-infection of strains of ergot from grasses to cereals.



Scale of histograms; 1mm = 2 samples.

strain may occasionally be carried by this grass. Indeed, this may have occurred when male-sterile barley was naturally infected at the W.P.B,S. (p.149). During the course of the present studies, the common belief that ergot on ryegrass is a source of infection of wheat has been reiterated, particularly in conversations with arable farmers, but all experimental evidence supports the view that, in Britain, there is little, if any, aetiological association between cereal and ryegrass hosts of ergot. Alkaloid spectra consisting of a very low level of ergotoxine and ergosine and a high percent of ergotamine were common to almost all samples of rye grass ergots. In addition it was found that not only did ryegrass isolates exhibit low pathogenicity to wheat, but also an isolate of ergot from wheat did not, after repeated inoculations, infect ryegrasses.

Direct comparisons of the results of analysis of the natural collection of ergots with similar surveys that have previously been reported elsehwere, are not possible. In general, however, other workers have encountered much greater variability of the alkaloid content of different collections of ergot from the same, or related, host species. This may, in part, be a reflection of the geography of sampling. For example, samples of perennial ryegrass ergots collected throughout Europe have been analysed (Rochelmeyer, 1949; Silver and Bischoff, 1955 and Meinicke, 1956). Results of these analyses showed that a whole range of alkaloid spectrum types, ranging from predominantly ergotoxine to predominantly ergotamine, have been collected from this one host

species. It is perhaps significant that, during the present studies, the only large sample of ryegrass ergots found to have an alkaloid spectrum in which ergotoxine predominated was also the only sample of ryegrass ergots received from outside the British Isles, from Sweden.

It may well be that the English Channel has been sufficient to maintain some degree of geographic isolation of British strains of ergot from those on the mainland of Europe. If this is so, then the possible risk of introduction of new, more virulent strains of ergot into this country in imported grass and cereal seed ought to be considered.

The only previously reported comprehensive study of host restriction of British strains of ergot (Loveless, 1971) was based solely on comparisons between the dimensions of conidia produced by isolates of C.purpurea from different gramineous hosts. By bringing together one group of hosts of an apparently host-restricted conidial form of ergot (see page 25, group 1), Loveless indicated that Lolium perenne, Holcus lanatus, Dactylis glomerata, rye and wheat are all naturally infected by a similar strain of ergot. Clearly, neither the cross-infection studies, nor the alkaloid spectrum analyses reported here lend support to the view that ryegrass and wheat are normally common hosts to one strain of ergot. Furthermore, alkaloid analyses of all samples of ergot from Holcus species were found to differ from Lolium ergots by the presence of an unidentified alkaloid - a feature that was shared by most alkaloid spectra of ergots from Dactylis glomerata.

As a parameter for determining host restriction of strains of ergot, Loveless's usage of variation of the size of conidiospores seems to have had two major limitations. First, there was considerable variation even between the dimensions of conidia within a sample of one isolate. During the present studies, similar, if not greater, variation has been observed whilst counting both culture and honeydew - derived spores on a haemocytometer. Secondly, in the absence of a) cross-infection studies on host-restricted conidial forms and b) studies on the effect of host species on the size of conidia, the implications of observed differences in conidial size in the aetiology of ergot disease in the field can only be speculative.

The role of blackgrass (Alopecurus myosuroides) in the aetiology of ergot of wheat, has already been the subject of some discussion. In addition, results of a survey of blackgrass infestation in relation to ergot infection of seed wheat crops (figure 21) have provided some evidence that the experimental observations are compatible with the limited knowledge of the aetiology of the disease in a variety of arable situations.

Whereas the currently reported survey was limited, both in terms of the number of crops sampled and in its scope (having been instigated only after the crop was harvested), a larger scale survey of wheat crops inspected before and after harvest could be broadened to include such factors as previous cropping, chemical treatments, earliness and type of sowing, varietal differences and eveness of ripening.

During field investigations of ergot on blackgrass it has been observed that a few, scattered inflorescences of blackgrass in, or around, a crop are often more heavily infected (i.e. more ergots per head) than are inflorescences within a dense stand of the grass. An explanation of this phenomenon may lie in the fact that blackgrass has a protogynous flowering mechanism (see p. 88). Whereas, in a dense stand of the grass, it might be expected that all but the most precocious flowering heads would be efficiently fertilized by cross-pollination, a plant that is spatially isolated from other blackgrass plants may bear unfertilized, ergot - susceptible florets for a longer period of time. The same reasoning may also provide an explanation of why, according to J.M. Thurston (Personal Communication), spring germinating blackgrass, which tends to be sparse, is generally more extensively infected with ergot than autumn germinating blackgrass.

Collectively, experimental and observational evidence of the involvement of blackgrass in the aetiology of ergot disease of wheat in some localities is almost complete. However, successive progression of the disease from the release of ascospores from overwintering ergot sclerotia in the soil to infection of wheat ears, with an intermediary stage on blackgrass, has yet to be demonstrated conclusively. Observations of ergot on blackgrass, wheat and other grasses at Lane End Farm in 1972 clearly demonstrated the latter part of the succession, but had commenced too late for the first stage of infection of blackgrass to be seen. In the following year, despite suitable earliness of flowering of blackgrass

and the presence of germinating ergots at this site before the end of May, prolonged dry weather conditions appeared to have prevented an early infection of the grass. Hence, further progression of the disease onto wheat did not occur.

The disease transmission from ergot sclerotia to blackgrass to wheat was also investigated by field experiments. In one treatment (p 141), the presence of blackgrass alongside germinating wheat ergots and male-sterile wheat plants was found to have been associated with increased incidence of the disease on the wheat. Since blackgrass had been found to be infected with ergot before wheat anthesis and subsequently gave rise to ergots with an identical alkaloid spectrum to ergots later collected from the wheat, it is concluded that this grass probably acted as an intermediate host of the disease. However, owing to an error in experimental design, the possibility that blackgrass had also had a shading effect on the germinating ergots (thus creating conditions more favourable to ascospore - infection of the wheat) could not be discounted. Thus, although the first stage of transmission of the ergot fungus from sclerotia to blackgrass was observed under experimental conditions, the second stage from blackgrass to wheat was not shown conclusively. Nevertheless, on the basis of other evidence, including alkaloid studies and observations of the disease in natural field situations, it is reasonable to predict that whole sequence of infection from ergot sclerotia, via blackgrass, to wheat could be followed under suitable experimental field conditions.

Another major factor influencing the hypothetical types of disease progression discussed earlier (figure 36), is the duration of the ergot-susceptible phase of development of a wheat ear. Obviously, secondary and tertiary infection of a wheat crop by honeydew exuding from the first infected ears will only continue for as long as some ears within the crop remain susceptible. Soon after a series of experiments to investigate the duration of the period of susceptibility of male-sterile wheats had been instigated, it emerged that very similar work had been undertaken in the United States (Puranik and Mathre, 1971). In general, results of the present studies (section 23.1) have confirmed those of Puranik and Mathre - the period of maximum susceptibility of male-sterile wheat florets (cms Capitole) to ergot was considerably longer than that of their fertile counter parts.

The actual duration of the period of maximum susceptibility, after anthesis, was found to be dependant on environmental conditions; varying from three days to thirteen days for the male-sterile cultivars, and from half a day to two days for the fertile cultivar. On the basis of limited evidence collated from four separate experiments it appears that the duration of the period of susceptibility of cms Capitole to ergot was negatively correlated with temperature, but not with relative humidity. Regrettably, experimental conditions were not fully described by Puranik and Mathre, so detailed comparison with their results is not possible.

Ambient temperature was also found to influence the speed of development of an ergot infection. Time intervals between inoculation of wheat heads and the earliest observations of exudation of

honeydew from infected florets varied from six days (in a warm glasshouse) to thirteen days (in the field, under abnormally cool weather conditions for early summer). In each case, it was found that honeydew did not begin to exude from the inoculated wheat heads until the majority of wheat ears had entered the phase of declining susceptibility to ergot.

Considering the relationship of the above results to epidemiological aspects of ergot disease in a field-grown male-sterile cultivar, it appears to be predictable that, assuming a primary source of inoculum at anthesis, secondary infection within crop is likely to be most severe when:

a) weather conditions are favourable both for the timely dissemination of 1° inoculum and for subsequent 1° infection of the earliest anthesing ears. These might then be expected to exude honeydew (2° inoculum) before the bulk of the unpollinated florets in the crop lose their susceptibility.

b) weather conditions are again favourable for dissemination of 2° inoculum as soon as it is produced by the primarily infected ears.

c) fertilization of the ovaries of male-sterile florets is delayed and/or seed set is low.

However, if dry weather should persist even for one or two days at either of the two stages mentioned in a) and b), thereby increasing the time interval between first anthesis and the earliest production of secondary inoculum, a marked reduction in the amount of secondary infection may be expected (see figure 31).

Detailed examination of the contents of inoculated male-sterile florets of wheat used in the above experiments showed that a reduction of the number of ergots formed was, during the phase of declining susceptibility, accompanied by a sharp increase in the numbers of infections aborted at the sphacelial stage (figure 29). This indicated that, at least during the early stage of decline of susceptibility of a male-sterile wheat ear, the ergot fungus is still able to infect ovary tissue but florets are no longer capable of sustaining the protracted development of a sclerotium. An explanation of this phenomenon is that at some date after anthesis (possibly coincident with the stage at which an ovary loses its ability to be fertilized and form a seed, Abramova (1966)) an unfertilized ovary, may, as a result of necrosis and blockage of the vascular tissue of the receptacle, become isolated from the rest of the plant. This, in turn, may render the ovary unsuitable for ergot parasitism by depriving the invading ergot fungus of an adequate source of nutrients.

Whilst investigating the susceptible period of male-sterile wheat many partially infected seeds were observed (table 20), either as

half seed - half ergot (or sphacelium) or as a seed with a water soaked appearance. The latter was similar to the description (Campbell, 1958) of ergot-infected barley seeds which were found to have a sub-epidermal layer of mycelium.

It is commonly assumed that losses caused by ergot in cereals are directly proportional to the number of sclerotia produced. However, seed spoilage (as described above) must also be taken into account. The significance of partial infection of grain was first demonstrated by Seymour and McFarland (1921) who found that in ergotised spikes of rye there was, in addition to a percentage of florets yielding sclerotia, a much larger percentage of florets that contained "blasted kernels" or were empty. Furthermore, there was always a greater percentage of the latter in ergotised spikes than in healthy spikes. Potential damage to seed set in male-sterile wheat cultivars, particularly in crossing blocks used to produce F1 hybrid seed, is deserving of further investigation. It was, indeed, unfortunate that experiments to investigate the effects of seed set on the amount of ergot infection and seed spoilage (section 23.2) yielded so few results. In both the 1973 and 1974 seed set experiments, seed collected from unergotised ears was too shrivelled and damaged by other (mainly saprophytic) fungi to be effectively compared with seed collected from ergotised wheat ears.

Finally, the factor of host resistance to ergot disease must be considered. Screening for resistance to ergot in male-sterile wheat cultivars, both in the field and in the glasshouse (section 24), has

provided evidence that there is at least a significant variation in the degree of susceptibility of these cultivars to the disease, particularly in terms of the numbers of ergots formed and in the amount of spore production. Despite this, the term "ergot resistant", however qualified, could not justifiably be used to describe any of the cultivars investigated - certainly not if this were to be taken to imply that a particular cultivar has been found to have useful field resistance to the disease.

In Canada, Platford and Bernier (1970) were less reluctant to describe the wheat cultivars Kenya Farmer and Carleton as having resistance to ergot disease. However, as explained in section 7.3 subsequent reports have indicated that their claims may have been premature. Carleton is no longer deemed to have satisfactory resistance, whilst Kenya Farmer is described as exhibiting "only moderate" resistance to ergot (Ratnopas and Bernier, 1972). When, in the present studies, these two fertile cultivars were included in a greenhouse experiment to screen five male-sterile wheats for resistance, cv Kenya Farmer was only found to be significantly less susceptible than one of the male-sterile wheats. Carleton, whilst significantly less susceptible than all other cultivars, still produced an ergot in over 80% of inoculated florets.

Nevertheless, it is concluded that the observed variations in both size and number of ergots produced, together with decreased spore production by the fungus on some wheat varieties, should be

considered as possible modes of expression of resistance which, if more highly developed, could amount to useful horizontal resistance to the disease. A plausible physiologic mechanism of resistance could, for example, be a defence reaction by the host plant through which the exudation of host sap from the vascular system in the receptacle may be restricted. This, of course, would reduce the volume of spore-bearing honeydew and may also lead to the production of smaller ergots. In addition there are varying degrees of open floweredness of male-sterile cultivars which, together with differences in the arrangement and structure of floral parts, may determine the ease by which spores are dispersed to, and deposited on, an unfertilized ovary.

The present studies can provide guidelines for the design of a more comprehensive screen of a greater number of male-sterile cultivars for resistance to ergot, when they are available. On balance, it seems to be most practicable to carry out primary screening trials in the field. Whilst field trials have the disadvantage of being at the mercy of climatological and disease factors, they do impose few limitations on the size and design of experiments. Costly glasshouse tests on wheat plants grown to maturity may best be reserved for more stringent second stage resistance screening of selected cultivars. Furthermore, it is considered to be preferable that, at least at the primary screening stage, natural infection of test plants should be encouraged. It is possible that some forms of resistance, attributable to morphological characteristics (as

described above), may be by-passed by the injection of spores into the floral cavities of wheat spikelets. However, the technique of clipping back the glumes of a wheat heads prior to spray-inoculation (Cunfer et al, 1974) may also remove some morphological aspects of resistance, and so alter the micro-environment within the florets that spore germination and growth of the fungus, particularly during the early stages of infection, are affected.

The spreader bed technique (p 82)which has been used as a method of evaluating the field resistance of male-sterile wheats to ergot is recommended as it provides for the natural infection of small hussocks of a large number of varieties, all in a relatively small field plot. At Throws Farm the use of blackgrass to form the "spreader" rows was not very successful, mainly because of difficulties encountered when attempting to inoculate this grass with ergot during exceptionally dry weather. Replacement of blackgrass with a very early flowering winter wheat, or with a winter-sown spring wheat cultivar, would alleviate the problem of inoculation, but may not always ensure the production of honeydew in the spreader rows before the earliest flowering dates of all the cultivars under test.

One major disadvantage of screening cultivars for field resistance to ergot is that in any one year a cultivar may escape infection solely by virtue of passing through its flowering period during a spell of dry weather, and thus appear to be resistant. It may, therefore, be advisable to include a male-sterile wheat variety in two or three consecutive field trials before selecting it for

more intensive investigation. Alternatively, results of primary screening tests could be augmented by collated evidence of natural occurrence of ergot in field-grown male-sterile cultivars during the early stages of breeding and multiplication.

Some conclusions can be made concerning the prospects for ergot in F1 hybrid cereals. Since male-sterile wheat cultivars have been found to have much greater susceptibility to ergot than have their fertile counterparts, the question arises: is there a risk that ergot disease will become prominent in all areas in which these susceptible plants are grown?

Evidence that there is already a widespread distribution of Claviceps purpurea on grasses and conventional cereals throughout Great Britain is embodied in the geographical distribution of the large collection of ergot samples received during the course of this study. The high frequency of natural occurence of ergot is evinced by the ease of location of ergot sclerotia on at least one host species in almost every locality investigated. Although infection of conventional, self-fertile wheats was seldom found to be severe, some wheat ergot was collected from more than 75% of arable farms visited in 1973.

It seems to be inevitable, therefore, that in many areas of Great Britain the use of male-sterile cultivars in F1 hybrid seed production will, from time to time, be accompanied by some increase in the incidence of ergot disease. Whether or not the disease will achieve prominence is, however, open to debate. Cross-infection and alkaloid studies on naturally occuring ergots have demonstrated

the possibility that in some localities the proportion of endemic strains of ergot that are highly infective to wheat may be less than in others. Furthermore, the disease severity has been shown to be influenced by additional factors, including the weed-grass situation, efficiency of pollination and crop husbandry, which can, by careful selection and preparation of F1 hybrid seed production sites, be modified to reduce the risk of an ergot epidemic.

It is clear that weather conditions, particularly at anthesis, but also during the two months preceding anthesis, will be a critical factor in determining the general level of ergot infection. To minimise the risk of a large proportion of all F1 hybrid production sites being affected in an "ergot year" it would be advisable to choose a variety of sites and sowing dates in an attempt to stagger the flowering dates of the male-sterile lines. Whilst control of ergot by sanitation and crop husbandry may be usefully employed to reduce the amount of ergot infection in male-sterile cultivars, more effective control measures may have to be developed, particularly for control of the disease in crossing blocks when wet weather conditions prevail throughout the flowering period. At the present time, possibilities for the effective control of ergot, either by the use of resistant varieties or by the application of fungicidal chemicals, promise little for the immediate future. It may, therefore, be expedient to investigate methods of improvement of techniques for extraction of ergot by seed cleaning machinery, for it is the seed merchants who may feel the greatest impact of increased contamination of wheat seed if and when commercial production of F1 hybrid varieties commences in this country.

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APPENDIX I.

Analysis of Variance Tables.

1. Cross-inoculation experiments, 1972. (Table 9) .

Group A.

Source of variation.	d.f.	Sum of Squares	Mean Square.	F-ratio.	Sig.
Between treatments.	6	246.1	41.0	3.8	5.0%
" blocks	2	13.4	6.7	0.6	N/S
Error	11*	117.5	10.6		
Total	19	377.0			

* One missing value calculated.

Group B

Source of variation.	d.f.	Sum of Squares	Mean Square.	F-ratio.	Sig.
Between treatments.	13	4321.8	332.4	30.7	0.1%
" blocks.	2	26.6	13.3	1.2	N/S
Error	26	282.6	10.8		
Total.	41	4631.0			

2. Cross-inoculation experiment, 1973. (Table 10).

Group A.

Source of variation.	d.f.	Sum of Squares	Mean Square.	F-ratio.	Sig.
Between treatments.	7	418.8	59.8	1.08	N/S
Between blocks.	2	1354.8	677.4	12.3	0.1%
Error.	14	770.0	55.0		
Total.	23	2543.6			

Group B.

Source of variation.	d.f.	Sum of Squares	Mean Square.	F-ratio	Sig.
Between treatments	12	2131.5	177.6	16.0	0.1%
Between blocks.	2	163.0	81.5	7.3	1.0%
Error.	23*	256.7	11.1		
Total.	37	2551.2			

* One missing value calculated.

Analysis of Variance Tables (continued.)

3. Cycle of infection experiment - blackgrass. (Table 17).

Source of variation.	d.f.	Sum of Squares.	Mean Square.	F-ratio.	Sig.
Between treatments,	2	33974.2	16987.1	4.95	10%
Between blocks.	2	8690.9	4345.5	1.27	N/S
Error.	4	13713.8	3428.5		
Total.	8	56378.9			

4. Resistance Screening Experiment. (Glasshouse, 1973) (Table 22).

a) Transformed % infection data.

Source of variation.	d.f.	Sum of Squares.	Mean Square.	F-ratio.	Sig.
Between treatments.	6	1360.31	226.72	5.01	0.1%
Between blocks.	6	250.78	41.80	0.92	N/S
Error.	36	1628.73	45.24		
Total.	48	3239.82			

b) Spore counts.

Source of variation.	d.f.	Sum of Squares.	Mean Square.	F-ratio.	Sig.
Between treatments.	6	101296	16882.7	2.9	5.0%
Between blocks.	6	30886	5147.7	0.88	N/S
Error.	36	209525	5820.1		
Total.	48	341707			

5. Resistance Screening Experiment. (Field, 1974) (Table 24).

a) Ergot Number per head.

Source of Variation.	d.f.	Sum of Squares.	Mean Squares.	F-ratio.	Sig.
Between varieties.	11	18.3887	1.6717	3.27	1.0%
Between blocks.	2	0.5328	0.2664	0.52	N/S
Error.	21	10.7244	0.5107		
Total.	34	29.6459			

b) Ergot Weight per head.

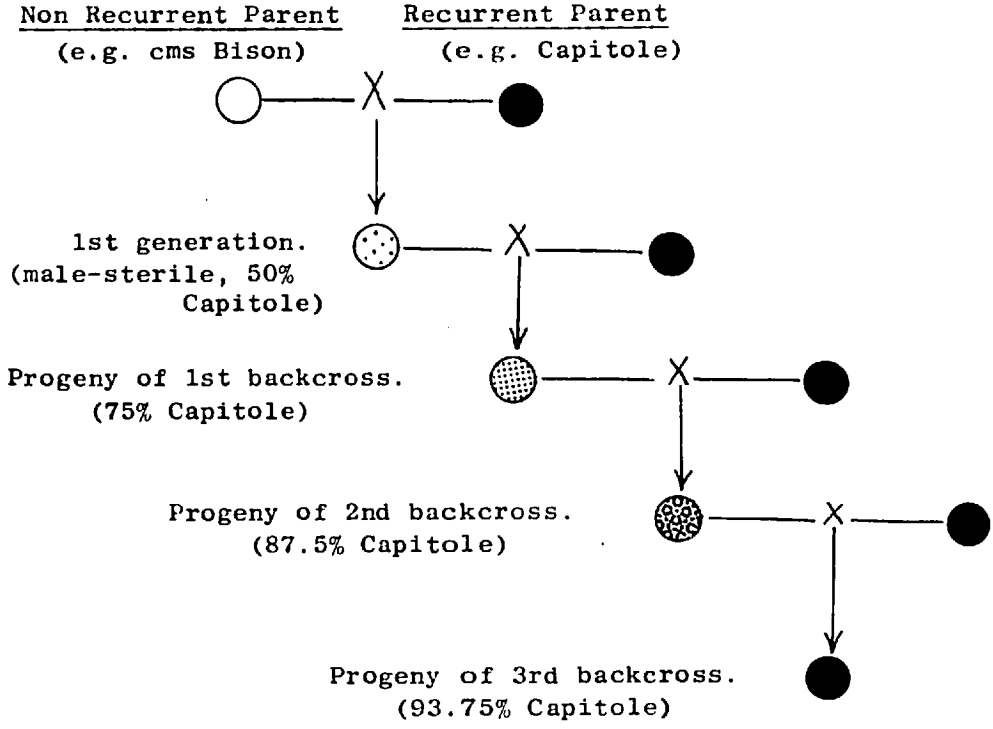
Source of variation.	d.f.	Sum of Squares.	Mean Squares.	F-ratio.	Sig.
Between varieties.	11	0.02353	0.00214	2.17	N/S
Between blocks.	2	0.00949	0.00474	4.82	2.5%
Error.	21	0.02067	0.00098		
Total.	34	0.05369			

Appendix II

Methods of Production of Male-Sterile Cereal Cultivars.

a) Backcross method used to develop and increase cytoplasmic male-sterile lines of wheat cultivars.

As outlined previously (p 8), male-sterility in cms wheat cultivars used in the present studies was initially inherited from the cytoplasm of Triticum timopheevi Zhuk. For the introduction of male-sterility into European wheat cultivars a N.American wheat, cms Bison (originally produced by recurrent backcrossing of Bison with T.timopheevi), has been commonly used as the non-recurrent parent in the following breeding programme :

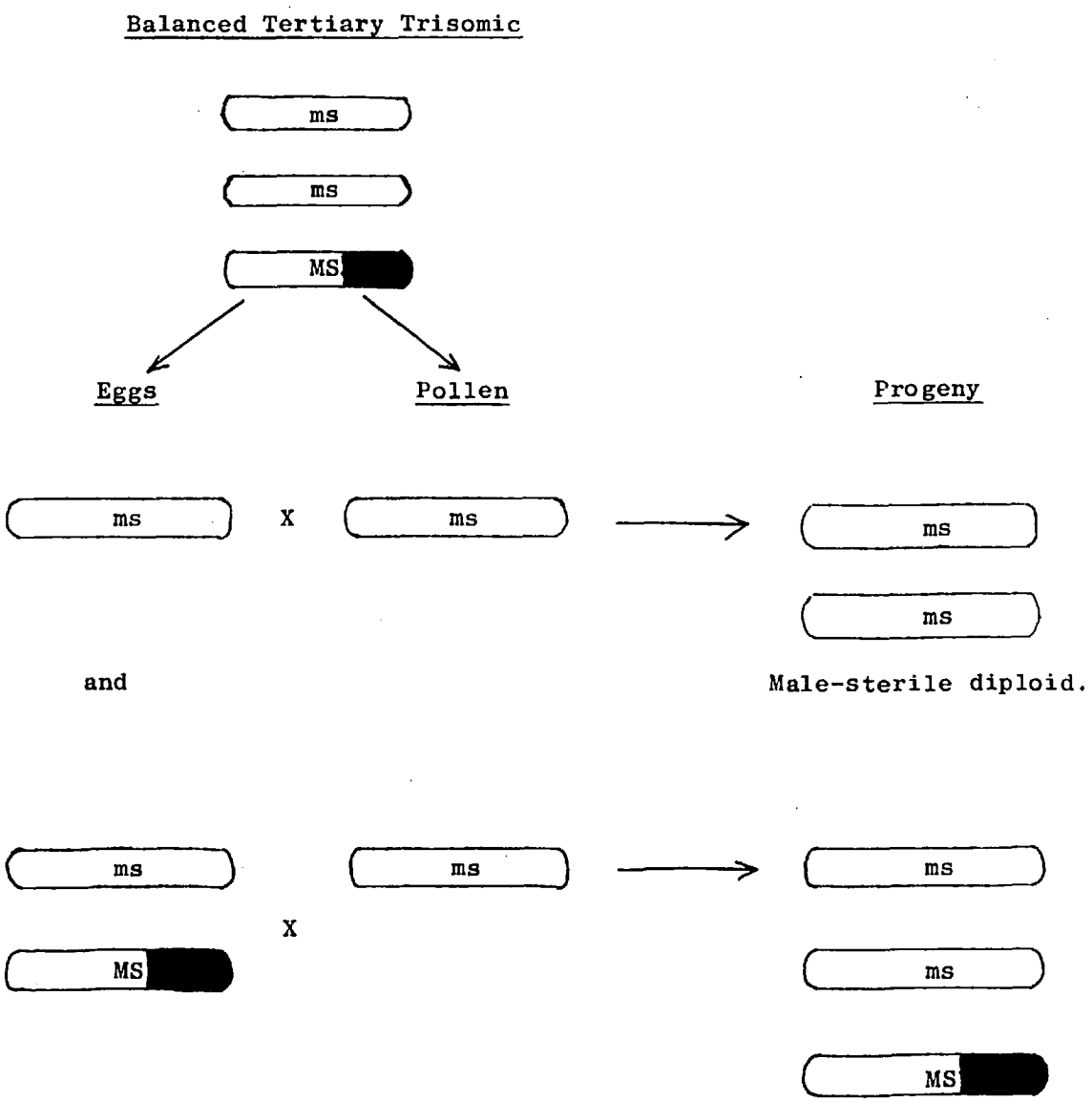


Backcrossing continues until the final progeny is identical to the recurrent parent (Capitole) in most characteristics except that it will be male-sterile instead of fully fertile.

b) Balanced Tertiary Trisomic (BTT) method used to develop and increase genetic male-sterile lines of barley cultivars.

Male-sterility in diploid barley plants mentioned in the present

studies is the phenotypic expression of genetic recessive male-sterile genes. In balanced tertiary trisomic individuals, recessive alleles of male-sterility (ms) are carried on the two normal chromosomes that carry the normal diploid complement. However, BTT plants have an extra chromosome on which is carried the dominant allele for male-sterility (MS) and are thus male fertile. This extra chromosome is only homologous up to the locus for MS; the remainder of the chromosome being an interchanged segment. Male or female gametes having the chromosome with the interchanged segment alone are genetically deficient and, therefore, non-viable. The extra chromosome is rarely transmitted through the pollen because of slower development of microgametophytes containing an extra chromosome. Consequently, BTT plants produce only two types of viable eggs and one type of viable pollen. As shown below, selfing of a balanced tertiary trisomic gives rise to mixed BTT and male-sterile diploid progeny:-



The typical progeny of selfed, balanced tertiary trisomics consists of about 30% BTT and 70% male-sterile diploid individuals. It is therefore necessary to distinguish between adult plants of the two genotypes so that they can be separated at harvest. To do this, the interchanged segment of the extra chromosome may be marked with a dominant plant character such as red colouration, resistance to a phytocide or short plant character. Following harvest, seed from BTT plants is used to produce more trisomics and male-sterile diploids, whilst seed harvested from the male-sterile diploids may be sown in crossing blocks alongside conventional, male-fertile plants inorder to produce F1 hybrid seed.

Reference

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ACKNOWLEDGEMENTS.

I wish to express my thanks to Dr P.G.Mantle and Dr D.A.Doling for their constant advice, encouragement and helpful discussion throughout this study and during the preparation of the thesis.

This research was supported by the Science Research Council for two years during the tenure of an S.R.C. Cooperative Award in Pure Science. Financial support during the final year was generously provided by R.H.M. (Research) Ltd., High Wycombe.

Laboratory and field facilities have been provided by the following, to whom I am indebted: Professor A. Spicer and Dr J. Edelman, The Lord Rank Research Centre; Mr W. Mackenzie and Mr A. Patterson, curators of the Chelsea Physic Garden; the Director of the Grassland Research Institute and Mr Derryk, Lane End Farm, Warborough.

Helpful discussion and collaboration with the following has been much appreciated: All members of staff of the Crop Science Department, L.R.R.C.; Dr J.Heard, The Grasslands Research Institute; Dr C.A.Foster, The Welsh Plant Breeding Station; Mr C. Gibson, Simmons Watts Ltd., Miss J.M. Thurston, Rothamsted Experimental Station and Mr L. Nisbet, Imperial College.

I am grateful to Dr D.W. Joyce and to Rothwell Plant Breeders, Ltd., for the provision of male-sterile wheat cultivars, and to the many people who contributed to the nation-wide collection of grass and cereal ergots.

Invaluable technical assistance has been provided by the laboratory and engineering staff of the Biochemistry Department, Imperial College;

also the laboratory, field and Centre Services staff of the L.R.R.C., and the gardening staff of the Chelsea Physic Garden.

My thanks are also due to Mrs H.Mungle and Mrs R. Westcott for typing, to the Reprography Department of Ewell County Technical College for printing and Mr A. Lock for photography.

Finally I should like to take this opportunity to thank Pat, not only for her constant help during the latter two years of this research, but also for her encouragement during, and tolerance of, six months of married life shared with this thesis.