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BLACKPOINT
OF
WHEAT

by

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November
1988

CONTENTS

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1. ABSTRACT

Literature on the blackpoint disease of cereals is reviewed from worldwide reports since the end of the nineteenth century. The review covers:

1) History and occurrence of blackpoint

2) The condition and symptoms

3) Causal organisms and host range

4) Effects on grain quality

5) Influence of environmental factors

6) Control measures

The term "blackpoint" is used to describe a darkened embryo end to the grain which can lead to discoloured flour. There are a number of causes, the most important of which are the fungi <u>Alternaria alternata</u> (also known as <u>A. tenuis</u>) and <u>Helminthosporium sativum</u>, although bacterial infection and pest damage have also been implicated. <u>A. alternata</u> is most usually regarded as the causal organism in European

countries, Australia and New Zealand. Both A. alternata and H. sativum cause a similar discoloration of the grain.

H. sativum is well known as a cause of foot rot and leaf disease in warm, arid areas. Alternaria does not cause these symptoms and may even be associated with a bolder grain.

Durum wheat is especially susceptible to blackpoint but bread wheat, barley and triticale are also affected. Grain samples may be rejected by millers owing to discoloration of white flour and several reports show reduction of pasta and bread quality with flour made from affected grain. Blackpoint may also increase protein but decrease Hagberg Falling Number.

The method by which Alternaria infects the ripening grain is not clear. Evidence suggests that the fungus is only able to infect the ears in the period after flowering and before milky ripe.

Alternaria is spread by air-borne spores, which are found in highest concentrations in the air in late July and August. Sequential monitoring of air-borne Alternaria spores during flowering may help in the prediction of subsequent incidence of blackpoint.

Blackpoint is favoured by high atmospheric humidity, rainfall and soil humidity. Frequent rain and warm temperatures during grain ripening are likely to increase blackpoint, although precise conditions necessary for infection have not yet been established. Work in Canada has shown that irrigation during the milky ripe and mid-dough stages results in a higher incidence of blackpoint than irrigation applied before the end of flowering. It would be useful to investigate the effect of different irrigation timings on blackpoint in the field in the U.K.

Studies with fungicide sprays during ripening have not shown any consistent effects on the incidence of blackpoint. Some chemicals (e.g. carbendazim) may actually increase the incidence of Alternaria spp. in the microflora of ripening cereal grains when sprayed as a pre-harvest treatment. Field evaluation of chemicals shown to be effective against Alternaria in vitro and on crops other than wheat (e.g. oilseed rape) is

necessary, as also are detailed studies on spray timing, since the plant growth stage at the time of application may be critical.

Differences in cultivar susceptibility to blackpoint have been reported from several countries, but not from the U.K. There is some evidence that infection is associated with open glumes and that the large-grained cultivars are most commonly attacked. Reports in the U.K. have suggested that Avalon, a long-grained cultivar, may be particularly susceptible but this may simply reflect its popularity as a milling wheat; there has been no experimental work on this subject.

Losses due to blackpoint in the U.K. have been calculated to be as high as £3 million/annum. However, this figure is derived from estimates provided by the major millers and not from precise data. A survey should be made in each of three successive seasons to obtain detailed information on the incidence and severity of blackpoint and to relate this to agronomic and climatic conditions. This would involve examination of samples of grain rejected by millers to assess blackpoint, the identification of the fungi involved and investigation of the history of the parent crop (i.e. location, soil type, cultivar, fungicide programme, husbandry practices and weather conditions during ripening). Grain quality (i.e. specific weight, % protein, Hagberg Falling Number) and processing tests would need to be done to establish threshold levels of blackpoint above which the quality of grain is reduced in terms of flour and breadmaking. The survey would yield valuable information on blackpoint and provide a basis for further research work. It may also identify areas of the U.K. and cultivars at high risk to blackpoint. The aims of further work should be:-

(i)   to predict the likely seasonal occurrence of the disease by relating airspora to weather conditions in crops at high or low risk;

(ii)   to screen cultivars of breadmaking wheats to assess their relative susceptibility to blackpoint;

(iii) to evaluate the effect of fungicide sprays applied during anthesis
and grain ripening on the incidence of blackpoint.

2. GLOSSARY

Agar – gelatinous substance extracted from seaweeds which is used to solidify culture media on which fungi and other micro-organisms are grown.

Airspora – fungal spore component of the air.

Aleurone Layer – single layer of storage cells rich in protein and fat, commonly found underneath the testa in cereal grains.

Bran – comprises aleurone and pericarp cell layers. The bran and germ are separated during milling.

Coleoptile – protective sheath surrounding plumule in cereal and grass seedlings.

Crease – shallow linear depression running length of grain.

Embryo – a young plant in its earliest stage of development.

Embryonic Leaves – initial leaves which are easily discernible in the germ of a mature grain.

Embryonic Roots – initial roots which are easily discernible in the germ of a mature grain.

Endosperm – part of the grain which surrounds and nourishes the embryo and which nourishes the seedling plants.

Epidermis – surface layer of cells.

Germ – a collective name given to the embryonic roots and shoot, and the scutellum tissue of the grain.

Glumes – bracts forming part of flower and covering wheat grain.

Gluten – protein found in wheat grains, which accounts for the elasticity of dough made from the flour, and which remains after the starch has been washed out.

Integument – coat of ovule.

Mycelium – mass of fungal strands.

Mycotoxin – toxin produced by a fungus.

Ovule – structure in the flower which, after fertilization, forms the seed or grain.

Pericarp – comprises several layers of cells. In the mature grain, the innermost layers form a solidified, dense, protective coat which prevents water entering and moulds from infecting the grain. The outer layers form a coarse coat over the grain surface. These outer layers can become infected by moulds such as <u>Alternaria</u> and <u>Cladosporium</u> by harvest time, giving a dull colour to the grain.

Phylloplane – leaf surface.

Rachilla – short stalk attaching grain to the ear stem.

Root Cap – a layer of dead cells overlying and protecting the delicate root apex as it grows.

Saprophyte – an organism using dead organic material as its food source and commonly causing its decay.

Scutellum – effectively the colyledon of a graminaceous embryo, it comprises a layer of specialised cells which absorb and transfer the sugars and nutrients released by enzyme action during germination, to the developing cells of the embryonic shoot and roots.

Shoot Apex – the growing tip of the shoot. The cells of the apex multiply and differentiate as germination proceeds giving rise to further leaves and stem. Later on the apex produces the cells which form the components of the ear.

Spikelets – the structural units of the wheat inflorescence (and all members of the grass family), which after fertilization develops to become the parts of wheat ear bearing individual groups of grain.

Testa – seed coat.

Internal structure of wheat grain

- Aleurone layer
- Endosperm
- Beard hairs
- Pericarp layers
- * areas commonly discoloured by black point
- Kernel crease *
- 'embryo region' *
- Testa
- Endosperm
- Scutellum
- Coleoptile
- Embryonic leaves
- Shoot apex
- Embryonic root
- Root cap
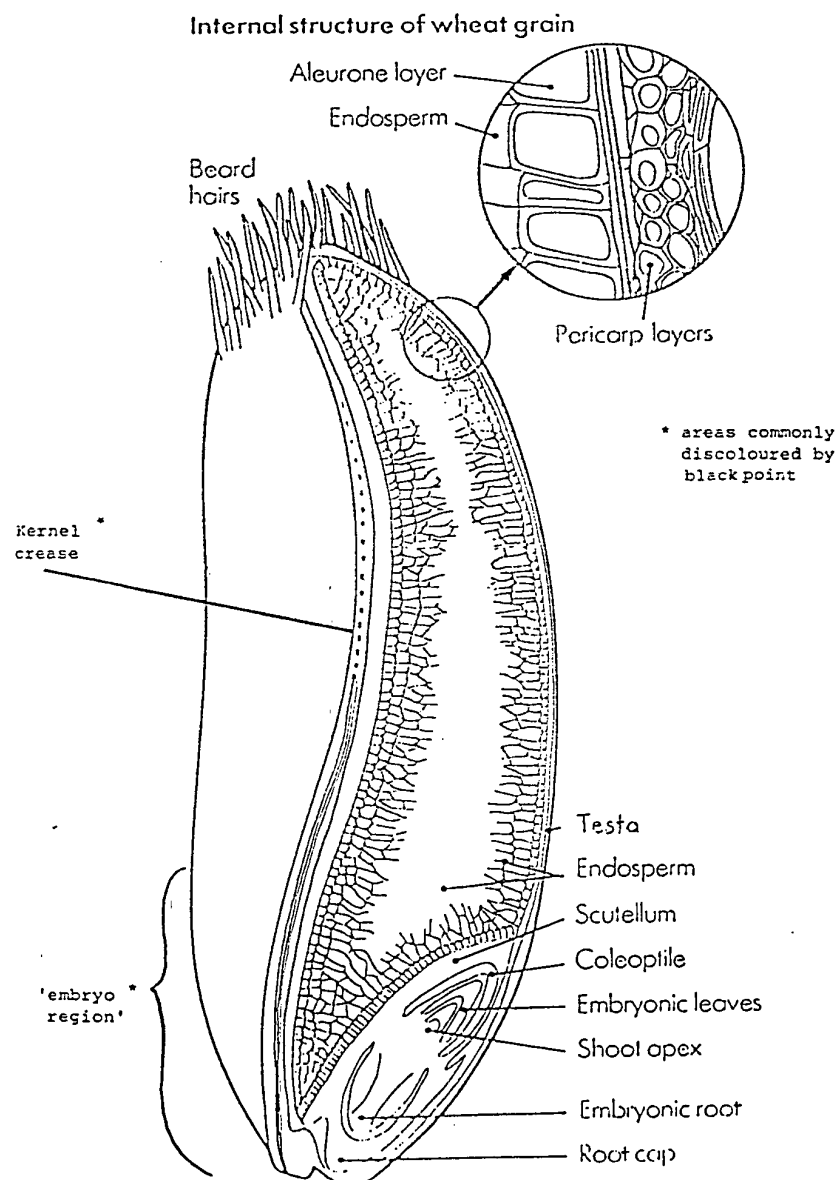
Source: A farmer's guide to wheat quality, NAC Cereals Unit.

3. INTRODUCTION

"Blackpoint" is the name given to a black discoloration of the embryo end of the cereal grain, which sometimes extends into the crease of the kernel. It is most commonly caused by fungal infection, although the symptom has also been associated with bacteria. Generally, the condition does not seriously affect germination, growth or yield of the following crop but it may impair grain and flour colour, reducing grain quality and leading to rejection by millers who refer to it as a cause of "dark gluten". Blackpoint is mainly a problem in durum and breadmaking wheats although it can affect other cereals.

The term "blackpoint" was first used in 1913 by Bolley (Bolley, 1913) to describe a grain discoloration supposedly due to fungal invasion. Previous to this date, however, Zöbl (1892) and Puchner (1897) – both cited by Machacek and Greaney, 1938 – used the adjectives "braunspitzige" and "schwarzspitzige" respectively, in Germany to describe similar symptoms in barley. Other names include "kernel smudge" in Canada (Machacek & Greaney, 1938), "black germ" (Ziling, 1932) or "black radical" (Fomin & Nemlienko, 1940) in Russia, "moucheture" in France (Rosella, 1930b), "mouchetage" (Miège, 1930) in Morocco, and "puntatura" in Italy (Curzi, 1926).

The condition has been reported throughout the world, for example Ziling (1932) stated that blackpoint had occurred in Siberia, at least since 1914, while Evans (1921) recorded the disease in the Upper Mississippi Valley in 1921. It has also been reported in Argentina (Pasinetti, 1931), India (Dastur, 1932), Algeria (Laumont & Murat, 1934), Egypt (El-Helaly, 1947), and Australia in 1948 and 1949 (Adam, 1950).

Most recent work has been undertaken in the U.S.A., Canada, New Zealand, Australia and India. Generally, blackpoint may be found wherever wheat is grown, but durum cultivars are reported to be especially susceptible, although this may be because a high quality white flour is needed for pasta production.

Incidence appears to be affected by climatic conditions, for example

El-Helaly (1947) found that the intensity of the disease was correlated with rainfall. Several workers have found differences in cultivar susceptibility to blackpoint (Conner & Thomas, 1985; Greaney & Wallace, 1943; Southwell et al., 1980b; Statler et al., 1975).

One of the fungi that cause blackpoint, Helminthosporium sativum (conidial state of Cochliobolus sativum), causes more severe symptoms than Alternaria spp., reducing germination and causing foot rot in the following crop (Machacek & Greaney, 1938; Ziling, 1932). Temperate climates appear unfavourable to this fungus, however, and it rarely causes problems in the U.K. Blackpoint caused by Alternaria spp. - of which A. alternata (syn. A. tenuis) is the most commonly reported - does not adversely affect the following crop performance.

In the U.K., growers have become more acutely aware of the condition in recent years as competition to achieve milling quality has increased. Discussions with millers in the U.K. suggest that the area most usually affected is in mid-Bedfordshire, Cambridgeshire, mid-Essex and into mid-Suffolk. The problem was reported as rarely severe south of the Thames. It has been suggested that the condition has become more common as the area of oilseed rape has increased. However, we can find no evidence to substantiate this view which is probably related to climatic factors increasing blackpoint in recent years and increased competition for milling quality samples. In addition, the Alternaria spp. which are pathogenic on oilseed rape have not been recorded on cereals.

ADAS experiments in 1987 showed that, although blackpoint incidence varied from site to site, fungicide treatments used had no consistent effect on the proportion of grains affected.

<u>Objective</u>

The aims of this review are:

1) to outline the history and occurrence of blackpoint;

2) to discuss possible causes and assess their role;

3) to describe the symptoms, including effects on grain quality, and to outline the epidemiology of the main causal fungi, including the influence of environmental factors on disease development;

4) to summarise information on control of blackpoint, including work on variations in cultivar sensitivity;

5) to identify priorities for further research.

The review is based on principal worldwide references since the end of the nineteenth century.

4. SYMPTOMS

Blackpoint is characterised by brown or black discoloration, of varying intensity, usually limited to the embryo region in the grain. There is also often a narrow brown discoloured area extending along the crease. The cause of the discoloration is unknown but may involve the oxidation of phenolic compounds in the bran, possibly as a defence mechanism following fungal penetration of the surface cells.

Ziling (1932) and Fomin and Nemlienko (1940) compared blackpoint symptoms caused by <u>A. alternata</u> with those caused by the less frequently occurring <u>H. sativum</u>. Discoloration and mycelium of <u>Alternaria</u> were limited to the embryo region and the mycelium was usually located in the pericarp, penetrating to the integument (more rarely to the aleurone layer and very exceptionally to the

endosperm), but never entering the germ. By contrast, with H. sativum, the external brown discoloration may extend to other portions of the grain. The mycelium permeates both the pericarp and the endosperm, and sometimes also attacks the embryo which, in severe cases, may be mummified. Bhowmik (1969) noticed dense mycelial mats of Alternaria below the inner epidermal cells. The embryo and starchy endosperm region were neither invaded nor discoloured.

Lorenz (1986) used scanning electron microscopy to study the penetration of Alternaria mycelium. A network of mycelium was seen covering the germ end of the kernel. Only the germ end and the crease region showed black discoloration and he suggested that fungal growth occurs in moisture between the base of the grain and the glumes. Mycelial growth could be seen within the embryo and a micrograph of the crease region showed that the fungus had penetrated the epidermal cells. In agreement with other reports, the starchy region of the endosperm had not been invaded.

Machacek and Greaney (1938) found that blackpoint caused by Alternaria and Helminthosporium was microscopically indistinguishable. However, Adlakha and Joshi (1974) found that it was sometimes possible to distinguish the grains infected by A. tenuis and H. sativum. Agarwal and Verma (1983) reported that seeds infected by blackpoint may be confused with those infected with the more serious Karnal bunt (Neovassia indica), although this disease does not occur in Europe.

4.1 Assessment of symptoms

A variety of methods has been used to assess the extent of blackpoint. These have included both visual assessments and microscopic examination of stained tissue. Huguelet and Kiesling (1973) devised a simple key:

    0 sterile floret.
    1 healthy kernel.
    2 tip or crease of kernel discoloured.
    3 50% of kernel discoloured.

4   75% of kernel discoloured.

5   kernel totally discoloured and generally shrivelled.

A similar scheme was devised by Cromer and Mulholland (1988) who also calculated a numerical index:

0   No symptoms.

1   Slight smudging, especially around the embryo and along the vertical crease.

2   Distinct darkening at embryo end and along the vertical crease.

3   Extensive blackening over at least one third of grain.

The presence of dark brown or black discoloration in the embryo region of the grain was considered a positive symptom by Southwell et al. (1980a). Discoloration of the tissue at the very tip of the embryo region of the grain where it was attached to the rachilla was not considered to be positive.

Some millers examine grain against a white background to assess blackpoint incidence.

Recently, Agarwal et al. (1987) have attempted to use microscopic examination to differentiate between different levels of Alternaria infection of wheat grain. Like Hyde and Galleymore (1951), they found that normal seed had a high level of Alternaria present sub-epidermally. However, they categorised the level of blackpoint infection into four categories:

1   Bold normal seed, mycelium confined to the pericarp.

2   Seeds with a brown discoloration, with infection of pericarp and the embryo end. No hyphae in the aleurone/endosperm layers.

3   Black discoloration of the embryo with heavy infection in the pericarp and necrosis of host cells.

4   Black discoloration with extensive mycelial colonisation of all components of the seed including the embryo.

The extent of fungal mycelium was positively correlated with the intensity of symptoms of blackpoint. However, this method is time-consuming, not suited to field assessments of _Alternaria_, and the results are potentially very difficult to interpret. The conclusions of Agarwal et al. (1987) that _Alternaria_ mycelium may occur in seed not showing symptoms has also been found in barley (Jacobs & Rabie, 1987).

Other qualitative methods have included direct plating of whole or dissected ripening kernels onto agar (Machacek & Greaney, 1938; Hyde & Galleymore, 1951). Comparison of growth on up to 12 different media suggested that 2% malt extract agar allowed easy identification of _Alternaria_ (Hill & Lacey, 1983). This malt extract agar direct plating method has been used to follow colonisation of ripening wheat grain either artificially inoculated with _Alternaria_ spores or with natural infection (Magan, 1982; Magan & Lacey, 1986).

Serial dilution of suspensions of comminuted wheat ears or grain in 0.1% tap water agar to give numbers of colony-forming units per ear consistently detected _Alternaria_ only from about GS 75 (Tottman, 1987) onwards. By contrast, using the direct plating method, _Alternaria_ was consistently detected at GS 50-55 (Magan & Lacey, 1986). A certain threshold level of contamination may perhaps be required before the serial dilution technique allows detection of _Alternaria_. Another method used (Hill & Lacey, 1983) relies on blowing _Alternaria_ spores from samples suspended in a wind tunnel on to agar plates in an Anderson sampler. Specialised equipment is required and the method could not realistically be used routinely.

A direct plating technique may therefore provide the best method of assessing the time at which _Alternaria_ spores are first deposited onto the ripening ears and of following subsequent levels of contamination and infection.

4.2   Host range

Most records of blackpoint are from hard and soft wheats although some of the earliest references to the disease are from barley

(Zöbl,1892; Puchner, 1897 – both cited by Machacek & Greaney, 1938). Stakman (1920) also found blackpoint caused by <u>H. sativum</u> on rye. There are several reports of the disease on triticale in India, e.g. Khetarpal and Agarwal (1979).

Many reports suggest that durum wheat is more commonly infected by blackpoint than hard or soft wheat varieties (Brentzel, 1944; Evans, 1921; Greaney & Wallace, 1943; Ziling, 1932) but it is certainly not rare on varieties of hard wheat (Waldron, 1934) or spring wheat (Conner & Thomas, 1985; King <u>et al</u>., 1981). Miège (1930) reported that hard wheats were more susceptible than soft wheats and that in Morocco, the disease was more widespread on barley than on wheat.

5. CAUSAL ORGANISMS

The precise causes of blackpoint symptoms are unclear, although fungi are consistently associated with the problem which may also be physiological, resulting from adverse environmental conditions (Pasinetti, 1931) or genetic in origin (Rao & Bhardwaj, 1981). Harris <u>et al</u>. (1941) listed a number of possible causes including fungi, bacteria and weathering. Possible involvement of thrips has been suggested in France (L. Lescar, pers. comm.).

5.1 Fungi

The two fungal genera to which the disease is most commonly attributed are <u>Helminthosporium</u> and <u>Alternaria</u>, the most usual cause in the U.K. being <u>Alternaria</u> spp. These are common fungi, some of which (especially <u>A. alternata</u>) are ubiquitous saprophytes (Ellis, 1971) which often occur on dead or dying material of a wide range of plant species. However, many other organisms have been implicated. King <u>et al</u>. (1981) isolated a range of fungi including <u>Alternaria</u> spp., <u>Ascochyta</u> spp., <u>Aspergillus</u> spp., <u>Botrytis cinerea</u>, <u>Cladosporium</u> spp., <u>Fusarium nivale</u>, <u>Helminthosporium</u> spp., <u>Stemphylium</u> spp. and <u>Tricothecium roseum</u>. Symptoms on grains in all of the samples examined were sufficient to justify the term 'blackpoint' although grains with <u>Cladosporium</u> infection had a

bleached appearance unlike grains infected by <u>Alternaria</u> spp. <u>B.</u>
<u>cinerea</u> is not a common pathogen of wheat but since 1974 has been
observed on the leaves and glumes, although not on the kernels.

Hyde and Galleymore (1951) studied the sub-epidermal fungi of cereal
grains and found <u>A. tenuis</u> in two-thirds of the grains. Bacteria
were also common and the fungi <u>Mycogone</u> sp., <u>Cladosporium herbarum</u>,
<u>Pullularia pullulans</u>, <u>Fusarium</u> sp., <u>Botrytis cinerea</u> and <u>Stemphylium</u>
<u>botryosum</u> were also occasionally isolated.

In North America, Luz and Hosford (1980) found that the fungus
<u>Pyrenophora tritici-repentis</u> (<u>P. trichostoma</u>), the cause of wheat tan
spot, can also cause blackpoint symptoms. This disease was recorded
by ADAS on wheat in England in 1987 (Cook & Yarham, 1988).

Machacek and Greaney (1938) reviewed the literature on causal
organisms and concluded that <u>Alternaria</u> and <u>Helminthosporium</u> were the
main fungi responsible. Their eight-year study from 1929 to 1936
showed that the species chiefly associated with blackpoint in
Manitoba were <u>A.tenuis</u>, <u>A. peglioni</u>, <u>H. sativum</u> and <u>H. teres</u>.

In India the two main pathogens causing blackpoint have been
recognised as <u>H. sativum</u> and <u>A. tenuis</u> (Dharam Vir <u>et al</u>., 1968;
Adlakha & Joshi, 1974). <u>Alternaria triticina</u>, a pathogen causing
leaf blight in wheat, may also cause brown to black discoloration of
seeds. However, Bhowmik (1969) reported that although 3% of
blackpoint-affected grains yielded <u>A. triticina</u> the predominant
pathogen was <u>A. tenuis</u>. Ram and Joshi (1979) found that inoculation
with <u>A. triticina</u> caused the symptoms of blackpoint in up to 18% of
grain. This fungus can be distinguished from <u>A. tenuis</u> by colony
morphology on nutrient agar (Prabhu & Prasada, 1967).

In Egypt the disease has been attributed only to <u>Alternaria</u> (El-
Helaly, 1947) and in North America and Australia <u>A. alternata</u> is
considered to be the most common cause of blackpoint (Conner &
Thomas, 1985; Southwell <u>et al</u>., 1980b; Rees <u>et al</u>., 1984).

5.2 Bacteria

There is some evidence from Germany (Poschenrieder, pers. comm.) that
the bacterium Pseudomonas syringae cv. atrofaciens is associated with
the condition although the grain, unlike that affected by Alternaria,
is occasionally shrunken. Stakman (1920), distinguished between
symptoms produced by Helminthosporium and those by Bacterium
atrofaciens, which caused the condition referred to as "black tip".

6. EFFECTS OF BLACKPOINT

6.1 Economic effects in the U.K.

Economic effects are difficult to assess, especially as some millers
often try to divert affected grain into brown flour where the
discoloration is less important. A load with 10-15% blackpoint-
affected grain can be acceptable for white flour. In a bad year
about 15% of loads can be rejected. Assuming a demand for two
million tonnes of home-produced milling wheat with a price premium
averaging about £10/t (Anon, 1987), this could represent an annual
loss of £3 million.

6.2 Germination, crop performance and yield

Most reports show that blackpoint associated with Alternaria has no
adverse effect on germination of affected grain (Brentzel, 1944;
Cromer & Mulholland, 1988; Machacek & Greaney, 1938) whereas with
H. sativum, germination, emergence and yield are reduced and foot rot
often increases in the following crop (Christensen & Stakman, 1935;
Machacek & Greaney, 1938; Stakman, 1920). Rosella (1930b) observed
that Alternaria reduced viability when grains were tested in Petri
dishes, but not when the seeds were sown in sand. In Australia (Rees
et al., 1984), seed germination was unaffected by blackpoint caused
by A. alternata although emergence was marginally reduced. Ziling
(1932) showed that under controlled conditions A. tenuis reduced
germination by 2.4% and H. sativum by 33.3%. Similar effects for
Alternaria were reported by Rana and Gupta (1982). Earlier, Dharam

Vir et al. (1968), had shown no effect of Alternaria blackpoint on germination.

6.3   Grain size and weight

A number of workers have reported that blackpoint-affected seeds are often larger and heavier than unaffected seeds.   It has been suggested that the difference may be due to the fact that the largest grains in the ear force open their covering glumes, allowing spores access, whereas the glumes of small kernels remain closed and exclude the Alternaria spores (Machacek & Greaney 1938).   Waldron (1934) found that within any given grain group on the ear, the affected grains were significantly heavier than the unaffected grains.   Some of the weight differences seem to be caused by the fungus penetrating the ovule and stimulating the development of the endosperm.   They may also be partly attributed to a difference in infection of grains differing normally in size because of their position in the ear. Huguelet and Kiesling (1973) used mixtures of H. sativum and A. alternata, in glasshouse inoculation tests.   Wherever H. sativum predominated in the inoculum mixture, kernel size was reduced - agreeing with Ziling (1932).   A. alternata had no adverse effect on kernel size.   Similarly, Adlakha and Joshi (1974) artificially inoculated A. tenuis and H. sativum onto plants and found that grains infected with A. tenuis were usually larger and heavier than normal grains whereas those infected with H. sativum were lighter and smaller.   Cromer and Mulholland (1988) also found that blackpoint-affected grains were significantly heavier than symptomless grains and that most affected grains were in the central part of the ear.

Lorenz (1986) found that both kernel weight and nitrogen content increased as blackpoint severity increased.   He also found, similarly to Waldron (1934), Machacek and Greaney (1938) and El-Helaly (1947), that the plumpest kernels were worst affected, whereas smaller grains were generally clean.   He suggested that plumper kernels probably mature earlier, on primary tillers, and might have been invaded by the fungus during the wetter part of the growing season.   The smaller grains could be in heads on later maturing tillers and would ripen during a subsequent drier period.   If this explanation is valid however, the reverse situation might be expected in some seasons.

however, the reverse situation might be expected in some seasons.

Similar increases in thousand grain weight have been recorded on triticale in India (Khetarpal & Agarwal, 1979).

6.4   Flour quality

Flour discoloration is the main effect on quality.   Assuming there are no adverse effects on baking quality this would not prevent the use of affected grain for wholemeal bread (Stewart, pers. comm.). Both El-Helaly (1947) and Conner and Thomas (1985) state that blackpoint is a major cause of down-grading wheat.

There are several reports of the disease affecting the cooking quality of pasta from durum wheat.   For example, Harris and Knowles (1943) reported that fungal infections of grain, including <u>Alternaria</u> causing blackpoint, reduced cooked weight and tenderness of macaroni. In earlier studies on North Dakota durum wheat tolerances of infection for semolina and macaroni were quantified by Harris and Sibbitt (1942).   Slightly injured kernels, in which discoloration is confined to the tip, could be tolerated in a proportion up to 10% with good milling durum, while 25% infection did not appreciably impair the colour of macaroni or increase 'speckiness' in semolina. An incidence of 50%, however, would entail a very high risk of problems in the mill mix.   In the case of heavily damaged kernels, however, inclusion in the blend would cause more serious problems and the presence of 5% would significantly increase 'speckiness' in semolina and decrease the macaroni score; 10% would be highly detrimental, and 50% would cause a grade reduction which would cause a serious financial loss to the grower.

Dexter and Matsuo (1982) also studied the effect of blackpoint and other diseases on the quality of durum wheat.   Gluten properties and semolina granulation did not appear to be adversely affected by blackpoint though alpha-amylase activity and protein were both increased.   Spaghetti colour was slightly influenced by blackpoint, diseased samples being slightly duller and browner, and the semolina

contained black specks. Spaghetti cooking quality was not related to blackpoint damage in terms of elasticity or firmness and according to earlier studies by Dick et al. (1974) the increased levels of amylolytic activity observed in the affected samples would not affect spaghetti cooking quality.

In studies on bread wheat, Lorenz (1986) found an increase in protein content in one cultivar. He suggested that this could be of economic importance since some New Zealand mills base their wheat purchasing contracts on protein content. It should be noted that Lorenz was studying a hard wheat, where different milling standards may apply compared with durum wheat. Alpha amylase activity tended to increase with increasing severity of blackpoint, while Hagberg Falling Number decreased accordingly. Two possible explanations were given for this: a) heavy blackpoint infection may contribute to the amylase activity of the infected kernels, or b) the fungus may interfere with metabolic activities of the grain. Proteolytic activity of infected kernels was considerably lower than that of normal kernels; this could be caused either by effects of the fungus on grain metabolism or by the presence of enzyme inhibitors. A similar increase in protein has been recorded in triticale (Khetarpal & Agarwal, 1979).

Rees et al. (1984) studied quality of grain samples with different levels of blackpoint. Hagberg Falling Number, total flour yield and dough stability were highest in low disease grain whereas flour colour grade was increased by blackpoint. The results of baking evaluations were not significantly different, indicating that the use of blackpoint-affected grain is likely to have little effect on breadmaking quality. The overall quality effects of severe blackpoint infection were minor, with the possible exception of flour colour.

In contrast, Lorenz (1986) found overall breadmaking quality decreased with high levels of blackpoint. Bread volume decreased, the grain became more open, and the texture was less silky as the extent of blackpoint infection increased. Blackpoint also caused a darker bread crumb colour.

Goswami and Sehgal (1969) studied the chemical composition of wheat samples prepared with different percentages of infected grains. There was little effect of blackpoint infection on protein, calcium or phosphorous for the three cultivars tested. They also tested aspects of the quality of chapaties including dough and 'puffing' character, flour and dough colour, chapati colour texture, taste and keeping quality. Most characters were unaffected by blackpoint infection, although in two of the three cultivars infection above 50% gave weaker and stickier dough and poorer puffing character.

In England, tests by King et al. (1981) showed that affected grain samples can give rise to discoloured flours which are unsuitable for breadmaking.

Maree and Cairns (1985) found that blackpoint-affected barley kernels were suitable for malting.

6.5 Mycotoxins

Many Alternaria spp. are known to be toxic to animals so that various workers have investigated the toxicity of crude preparations of cultures and infected substrates. Many isolates have been shown to be lethal to chicks, rats and mice (Doupnick & Sobers, 1968; Christensen et al., 1968; Meronuck et al., 1972).

The most important secondary metabolites of known mammalian toxicity produced by A. alternata include altenuene (AE), alternariol (AOH), alternariol monomethyl ether (AME) and tenuazonic acid (TZA) (Harvan & Pero, 1976). Some of these mycotoxins are known to act additively. A. alternata has been demonstrated to produce some or all of these mycotoxins in various concentrations on plants such as tomato (Harwig et al., 1979) or sorghum (Sauer et al., 1978). A. alternata isolates from a variety of small grains (Bruce et al., 1984) and from wheat grain in the U.K. (Magan et al., 1984) were also found to produce these mycotoxins. Watson (1984) suggested that more attention should be directed to major food plants that are susceptible to Alternaria infection, particularly in cases of heavy Alternaria infection.

It has been shown that AE, AME and AOH are all produced _in vitro_ on a wheat extract agar at 5-30°C and at an estimated humidity of 98, 95 and 90%, respectively (Magan _et al._, 1984). Further work on autoclaved grain suggested that at 15°C and 25°C substantial quantities of two or more of these mycotoxins were produced at 26% and 22% water content, while at 19% very little was produced. There are no reports of _Alternaria_ mycotoxins being isolated from ripening wheat grain except for very small traces after boosting _Alternaria_ populations on ears by inoculation at GS 50, GS 60 or GS 70 (Magan, 1982).

It is not known whether _Alternaria_ mycotoxins persist during milling and baking but studies suggest that _Alternaria_ grows well on whole wheat bread and is able to produce some mycotoxins (Reiss, 1983). However, the most toxic metabolite, TZA, was not detected in the bread. More information is, therefore, required on possible mycotoxin production and whether any such mycotoxins are able to survive milling and breadmaking.

7. INFLUENCE OF ENVIRONMENTAL FACTORS

Studies to assess the relationship between blackpoint symptoms and infection by _Alternaria_ have predominantly been carried out under glasshouse conditions, often in high humidity. Bhowmik (1969) dipped spikelets into spore suspensions and then kept them in a high humidity environment for 48h. Similarly, Huguelet and Kiesling (1973) used concentrations of 5-10,000 spores/ml while Adlakha and Joshi (1974) used 10-12,000 spores/ml and high relative humidity for 48h. More elaborate studies were carried out by Southwell _et al._ (1980a) who used a range of spore concentrations and dew periods, spraying spore suspensions in a weak detergent solution onto spikelets using a pressurised atomiser.

All of the glasshouse inoculation experiments reported have resulted in an increase in the percentages of grain with blackpoint symptoms, the largest increase being 42% (Huguelet & Kiesling, 1973).

7.1 Method of infection

There has been considerable debate about the method by which *Alternaria* infects the ripening grain and the assessment of the level of infection (see also 2.1). In early studies, Machacek and Greaney (1938) suggested that infection of wheat grains by *Alternaria* can only occur when the glumes are open and allow entry of air-borne spores. In detailed studies of the sub-epidermal fungal colonisation of wheat, Hyde (1950) and Hyde and Galleymore (1951) suggested that the possible mode of entry was by germination of *Alternaria* spores, deposited onto the outer surface of the immature ripening grains exposed by the opening of the glumes. They suggested that dead floral parts remain closely associated with the developing grain and provide a possible source of infection through the epidermis. This is supported by Prabhu and Prasada (1967) and Bhowmik (1969) who suggested that growth of *Alternaria* occurs in moisture between the base of the grain and the glumes. In studies on the microflora of cereals, Mills and Wallace (1979) suggested that during prolonged wet harvest conditions fungal development (of *Alternaria, Cladosporium* and *H. sativum*) continues to produce excessive blackening of seeds and also chaff.

7.2 Spore production and distribution

Spores of *Alternaria* are abundant in daytime airspora in temperate regions with daily mean concentrations of up to $150/m^3$ (Lacey, 1981). It has also been demonstrated that up to 25% of the airspora released from cereal crops at harvest can be of *Alternaria* spp. (Darke *et al.*, 1976). Furthermore, spore counts of *Alternaria* showed that in both 1981 and 1982 the concentrations above cereal crops increased significantly during ripening with optimum concentrations at harvest (Magan, 1982).

These studies confirm earlier work (Stakman *et al.*, 1922; Machacek & Greaney, 1938) which showed that spores of *Alternaria* could be lifted to considerable heights and dispersed over wide areas, and that they were particularly numerous close to harvest in late July and August.

Studies have, therefore, been carried out to try and relate the concentrations of air-borne spores of Alternaria and Helminthosporium to time of grain infection. For example, El-Helaly (1947) kept immature ears of susceptible plants under controlled conditions to prevent infection from the air. When these grains ripened, they were apparently clean while the rest of the ears, exposed to the air, contained some discoloured grains. A close correlation between wind direction and numbers of air-borne Alternaria spores was found by Machacek and Greaney (1938). They were able to predict a severe occurrence by monitoring the airspora.

7.3 Spore germination conditions

Germination of A. alternata is known to occur over a wide range of temperatures (Dickinson & Bottomley, 1980; Magan & Lacey, 1984a). The range of Equilibrium Relative Humidity (ERH) over which A. alternata spores germinate varies with temperature. Spore germination occurred over the range 100-85% ERH at 25°C but between 100 and 91% at 10°C. Southwell et al. (1980a) have suggested that a minimum dew period of 3-6h is necessary for infection and symptom development. Mycelial growth at 25°C occurred over the range 100-88% (Magan & Lacey, 1984a). A. alternata spores are able to withstand long periods of desiccation (Dickinson, 1981; Park, 1982). The size and morphology of A. alternata conidia have been shown to be affected by temperature, water availability and nutrient status of the substrate (Misaghi et al., 1978). Even in the absence of nutrients, A. alternata spores are able to germinate rapidly on glass slides with optimum mycelial growth between 20 and 25°C (Dickinson & Bottomley, 1980).

A. alternata (and Cladosporium spp.) can penetrate leaves of cereals resulting in death of some host cells in the vicinity of the penetration points, although visible symptoms do not always occur (O'Donnell & Dickinson, 1980; Tolstrup & Smedegaard-Petersen, 1984). It has been suggested that an ERH of 97% is required for A. alternata to penetrate host cells (O'Donnell & Dickinson, 1980). Once established on senescing plant surfaces or on newly-dead plant remains, A. alternata sporulates vigorously, especially in warm, dry weather.

7.4   Weather and soil factors

Other factors that influence the distribution of the disease are atmospheric humidity, rainfall and soil humidity, all of which favour its occurrence (El-Helaly, 1947).   El-Helaly noticed that the incidence of blackpoint was highest where the crop received more than one irrigation.   This effect is also reported from irrigated, soft-white spring wheats in Western Canada (Conner & Thomas, 1985). Later, Conner (1987) demonstrated that blackpoint incidence was significantly increased when crops were irrigated during the milky ripe and mid-dough stages and that incidence was consistently low when the final irrigation was applied before the end of flowering. Similarly, in France blackpoint incidence has been associated with heavy rain during milky ripe (L. Lescar, pers. comm.).

Adlakha and Joshi (1974) reported that near Delhi the disease appears only if there are rains in March/April and humid conditions prevail for some days.

Glasshouse studies with A. alternata by Southwell et al. (1980a) showed that in artificial inoculations the incidence of blackpoint increased with increasing duration of dew period up to 48h.

Huguelet and Kiesling (1973) found that increasing the period of high humidity from 48 to 72h. after inoculation, increased the severity of blackpoint due to H. sativum, but not A. alternata.

7.5   Growth stage and infection

Ram and Joshi (1979) inoculated A. triticina (the cause of leaf blight) at different growth stages.   Blackpoint did not occur in any pre-flowering inoculations, but four successive post-flowering inoculations gave similar blackpoint infections.   Similarly, Adlakha and Joshi (1974), found that maximum blackpoint infection with H. sativum and A. tenuis was obtained when plants were inoculated after anthesis.   Inoculation with H. sativum at GS 45 and GS 59 destroyed the florets but seed set was not affected by A. tenuis. The four different inoculation times tested for A. tenuis gave the

following infection levels: GS 45, 27%; GS 59, 31%; GS 69, 73%; and GS 85, 11%. Southwell et al. (1980a) found that the incidence of blackpoint was linearly related to the stage of grain development, at inoculation between anthesis and late milk (GS 75).

El-Helaly (1947) suggested that incidence of infection depends on the glumes being open, permitting the passage of spores to the grain. Thus, unless the grain is large enough to force open the glumes, infection is unlikely to occur.

Grain moisture is particularly critical and there is a period of 10–20 days between anthesis (GS 61–69) and milky ripe (GS 75) when infection might occur. The moisture content is about 70% at GS 75 and decreases rapidly during dough development as the grain ripens. While the water content is high, the substrate is ideal for spore germination and growth of Alternaria. Hyde and Galleymore (1951) could, however, only find sub-epidermal mycelium when grain moisture was 40% or less. By contrast, Southwell et al. (1980a) found that infection can occur at any time from anthesis onwards depending on the concentration of Alternaria spores. Magan (1982), used an inoculum of 10^5 spores/ml to spray onto wheat ears at GS 50, 60 or 70 and found that blackpoint infection of wheat grain remained below 10% irrespective of the time of application.

More detailed field studies are necessary to determine the effect of rainfall, temperature and humidity on the development of Alternaria under field conditions.

7.6 Interactions between causal fungi

Machacek and Greaney (1938) offered antagonism as an explanation for the predominance of blackpoint caused by H. sativum rather than Alternaria spp. in mixed infections, despite the greater abundance of Alternaria spores in the air. They found that antagonism was present in dual cultures; H. sativum grew without inhibition whereas growth of A. tenuis was much reduced on the side nearest to the H. sativum colony. They suggested that when infection by both fungi more or less simultaneous, H. sativum inhibits the establishment of Alternaria in the kernel.

Contrary to Machacek and Greaney (1938), Huguelet and Kiesling (1973) found that when both organisms were simultaneously inoculated onto agar the growth of both fungi was reduced by about 35%. When A. alternata was the initial inoculum, H. sativum growth was reduced by 44% and A. alternata by 20%; when H. sativum was the initial inoculum, A. alternata growth was reduced by 42% and H. sativum by 26%. This difference can probably be explained either by inter-isolate variation or differences in culture media used. Neither set of workers found evidence of inhibition zones by either fungus. Huguelet and Kiesling (1973) noted that H. sativum had the most rapid growth rate of the two fungi but this cannot really be related to competitive ability in the plant.

Alternaria may also interact with other common phylloplane fungi such as Cladosporium and Fusarium spp. Wallace and Sinha (1981) suggested that Alternaria and Cladosporium may compete for the same niche on the ripening ear. However, in detailed in vitro studies, Alternaria and Cladosporium were found to intermingle freely on wheat extract agar while Fusarium culmorum antagonised Alternaria spp. (Magan & Lacey, 1984b).

It has been suggested that Alternaria may compete with Fusarium for the same ecological niche on ripening ears (Bateman, 1983) and it has been found that in a year with severe Fusarium infection, the populations of Alternaria on ripening ears were decreased (Magan & Lacey, 1986).

8. CONTROL

8.1 Resistant cultivars

There is no information on the relative susceptibility of wheat cultivars to blackpoint in the U.K. Most affected samples received by the Flour Milling and Baking Research Association (B. Stewart, pers. comm.) in 1987 were of Avalon, but this may be a reflection of

the popularity of the cultivar. There have been studies on cultivar susceptibility to blackpoint in other countries. Greaney and Wallace (1943) found that _Triticum durum_ (durum wheat) was more susceptible than _T. vulgare_ – now known as _T. aestivum_ (bread wheat) – and one cultivar of early maturing, hard-red spring wheat, Garnet, was highly resistant.

Some workers (El-Helaly, 1947; Machacek & Greaney, 1938) have shown that diseased grains are invariably larger than apparently healthy ones suggesting that resistant cultivars produce small grains. Their glumes remain closed and do not permit the passage of spores to the grain, avoiding infections. However, small kernels are undesirable in durum wheat (Statler _et al._, 1975). It has been recognised in France that large grain cultivars are more susceptible (L. Lescar, pers. comm.). Interestingly, Avalon does have a long grain (B. Stewart, pers. comm.) so these physical effects may be implicated in reports of blackpoint in this cultivar.

It is possible that late-flowering cultivars are more susceptible owing to the presence of higher levels of _Alternaria_ in the atmosphere later in the season. However, Cromer and Mulholland (1988) have shown there is no relationship between earliness of ripening and blackpoint severity.

Most of the reports on cultivar resistance are on durum wheat. Southwell _et al._ (1980b) suggested that resistant cultivars may be the most effective method of control. Differences in susceptibility between established cultivars and recent introductions – the latter showing higher resistance – led to the suggestion that resistance to _A. alternata_ may be simply inherited and controlled by one or a few major genes.

Conner and Thomas (1985) found large differences in blackpoint incidence between cultivars of soft-white spring wheat (_Triticum aestivum_) grown under irrigation in the field in Western Canada. Cultivars with similar parentage showed similar reaction to blackpoint, supporting the suggestion of Southwell _et al._ (1980b), that the genetics of inheritance is probably fairly simple. Later,

Conner (1987) showed that incidence in a resistant wheat remains relatively stable even when irrigation increases blackpoint on susceptible cultivars.

Resistance to H. sativum has also been studied and Statler et al. (1975) found that a single recessive gene conditioned resistance to the pathogen.

8.2 Seed treatment

Seed treatment for future crops is relatively unimportant as the problem is usually only of significance in milling wheat samples. There are some reports on the effects of seed treatment on blackpoint incidence in the following crop and on the resulting crop performance. However, most of these refer to disease control in seed crops rather than in seed lots for processing (Chaudhary et al., 1984; Fomin & Nemlienko, 1940; Harris et al., 1941; Mishra, 1974; Rosella, 1930b; Sinha et al., 1984).

8.3 Fungicide sprays

Chaudhary et al. (1984) tested a range of fungicide sprays on ears inoculated with a spore suspension of A. alternata. The fungicides tested, benomyl (Benlate), captan, maneb (Dithane), carboxin (Vitavax) and ziram all significantly decreased disease incidence except for captan. All treatments enhanced seed germination and inhibited spore germination.

Hill and Lacey (1983) studied the effect of pre-harvest fungicide application on the microflora of ripening barley. The most common fungus detected was A. alternata. Sprays of benomyl, captafol (e.g. Sanspor), benodanil (Calirus) and tridemorph (e.g. Calixin) applied at GS 61, 77 and 91 did not decrease the incidence of Alternaria, while benomyl significantly increased it. Similarly, Magan and Lacey (1986) found that carbendazim + maneb (e.g. Delsene M), captafol, imazalil (Fungaflor) and prochloraz (Sportak) had no effect on Alternaria on winter and spring wheat.

There are no published reports from the U.K. of a consistent beneficial effect of fungicides on grain at risk from blackpoint. On other arable crops prone to Alternaria diseases, for instance oilseed rape, the most effective fungicide is iprodione (Rovral), and vinclozolin (Ronilan) and prochloraz also give good control (Anon, 1984). In vitro tests on Alternaria sensitivity to fungicides (Mais, 1979) showed that prochloraz gave the greatest reduction of mycelial growth, but did not inhibit spore germination even at concentrations above 10 ppm. Iprodione, captafol and imazalil gave some reduction of mycelial growth above 0.1 ppm. Field evaluation of these chemicals will be important in future studies. However, in an ADAS experiment on five sites in 1987 there was no overall relationship between blackpoint incidence and leaf disease, green leaf area, yield, specific weight or thousand grain weight. A range of products which included iprodione, prochloraz and carbendazim (e.g. Bavistin) were applied at ear emerged or grain watery ripe growth stages (ADAS, unpublished data).

8.4 Husbandry

Fomin and Nemlienko (1940) suggested cultural measures of control for blackpoint, such as regular weeding of wild grasses, immediate drying after harvest and deep ploughing. If blackpoint is due to Alternaria, where infection is non-systemic, restricted to the grain and not carried over to the succeeding crop, removal of trash and deep ploughing will have little effect, whereas blackpoint caused by Helminthosporium, where there is the hazard of associated foot rot, will be discouraged by effective disposal of crop debris.

There is no evidence of blackpoint development on wheat in storage and storage temperature and humidity have been found not to affect blackpoint severity (Cromer & Mulholland, 1988).

9. CONCLUSIONS

The conclusions of the review may be summarised:

1. The disease is widespread, occurring wherever cereals are grown and its incidence has been reported since the end of last century. Although there are records of blackpoint on rye, barley and triticale, the condition is most important where the grain quality is critical, i.e. winter and spring soft and breadmaking wheats.

2. Blackpoint symptoms are usually ascribed to the fungi Alternaria alternata (A. tenuis) and Helminthosporium sativum. A. alternata is the most usual cause in the U.K.

3. A. alternata generally causes blackening at the germ end and in the kernel crease. The mycelium penetrates the embryo and epidermis. H. sativum tends to cause blackening over other parts of the seed and its mycelium penetrates the endosperm.

4. H. sativum reduces germination, emergence and yield of the following crop whereas Alternaria tends to have little or no effect on germination and no adverse effect on emergence or yield.

5. Alternaria has been associated with increased kernel weight, whereas H. sativum can cause shrivelled grain.

6. Blackpoint causes down-grading of grain and rejection by millers. It may affect the colour of pasta from durum wheat and reduce its cooking value. It may increase protein but decrease Hagberg Falling Number. Effects on breadmaking quality are inconsistent. In the U.K., blackpoint can discolour flour.

7. The fungi causing blackpoint are spread by air-borne spores and infection is favoured by humid conditions. Irrigation or heavy rain between anthesis and mid-dough increases the incidence of blackpoint.

8.   Cultivars of wheat vary in their susceptibility to blackpoint;
     early reports suggest that this may be related to morphological
     characters. The resistance factor in durum wheat seems to be
     simply inherited.

9.   There is little evidence that fungicide sprays applied to the
     ear reduce infection by _Alternaria_ spp. and some fungicides
     (e.g. carbendazim) may increase _Alternaria_ infection.

10. RECOMMENDATIONS

Millers regard blackpoint as an important cause of down-grading and rejection of wheat for breadmaking. Losses due to blackpoint have been estimated to be as high as £3 million. There is a paucity of information, however, on the effect of agronomic and climatic factors on the incidence of blackpoint in the U.K.

The priorities for further research are to obtain improved estimates of national losses from blackpoint and to relate incidence to the agronomy of crops and to weather conditions.

Current estimates of losses due to blackpoint are based on the experience of the major millers and not on precise data. A survey should be made in each of three successive seasons to obtain detailed information on the incidence and severity of blackpoint and to relate this to agronomic and climatic conditions. This would involve examination of samples of grain rejected by millers to assess blackpoint, the identification of the fungi involved and investigation of the history of the parent crop (i.e. location, soil type, cultivar, fungicide programme, husbandry practices and weather conditions during ripening). Grain quality (i.e. specific weight, % protein, Hagberg Falling Number) and processing tests should be done to establish threshold levels of blackpoint above which the quality of grain is reduced in terms of flour and breadmaking. The Flour Milling and Baking Research Association have the facilities to assess effects on baking quality. The survey would yield valuable information on blackpoint and provide a basis for further research work. It may also identify areas of the U.K. and cultivars at high risk to blackpoint. The aims of further work should be:-

(i)   to predict the likely seasonal occurrence of the disease by relating airspora to weather conditions in crops at high or low risk;

(ii)   to screen cultivars of breadmaking wheats to assess their relative susceptibility to blackpoint;

(iii) to evaluate the effect on blackpoint of fungicide sprays applied during anthesis and grain ripening on the incidence of blackpoint.

11. ACKNOWLEDGEMENTS

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