STUDIES ON

ALTERNARIA BRASSICAE AND *ALTERNARIA BRASSICICOLA* INFECTION OF CRUCIFEROUS CROP PLANTS

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Dedicated to my sons,

Sundeep and Naveen, for their patience,

to my wife, Susheela, and my parents,

who contributed in countless ways to making this study

a reality rather than a dream.

DECLARATION

This thesis has been composed by myself and describes experimental work which I carried out between October 1981 and August 1984.

(Kothanur P.R. Prasanna)

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CONTENTS

SUMMARY

In studies on *Alternaria* infection of cruciferous plants, *A. brassicae* and *A. brassicicola* were recognized to be the two main pathogenic species involved. The results of surveys of oilseed rape crops in east Scotland showed *Alternaria* disease to be a major problem, and *A. brassicae* was found to be the predominant pathogen: factors which were associated with a higher incidence included higher rainfall, previous cropping with oilseed rape and luxuriant growth. *A. brassicicola* occurred only occasionally, mainly where vegetable *Brassicas* provided a source of inoculum. From a survey of seed samples of cruciferous plants, *A. brassicae* appeared widespread in oilseed rape and *A. brassicicola* in other crop plant groups. In artificial inoculation studies both fungal species were found to adversely affect seed yield and quality of oilseed rape: *A. brassicae* acted as a more aggressive leaf pathogen, whereas *A. brassicicola* exhibited a capacity for prolific spore production and an ability to rapidly colonise senescing or immature, unprotected tissue. In assessing variation in levels of infection among different oilseed rape cultivars, differences were quantitative rather than qualitative in nature, with no evidence of major gene effects. A leaf disc method of assessment was developed to study host-pathogen interactions covering a wide range of cruciferous plants and different isolates of each pathogen. The patterns of response of the host range to both pathogens tended to be similar and there was no evidence of marked host specificity among isolates. Low infection levels in some host groups was associated with a high degree of waxiness of the cuticle. In further leaf disc studies, increased lesion development was found with increasing atmospheric humidity, while surface tension characteristics

of spore suspension droplets appeared to influence the initiation of infection. The optimum temperature for leaf lesion development was 25°C, but maximum colonisation of inert substrate from infected seed occurred over a wider temperature range. Both pathogens were observed to be seed-borne either superficially or within the embryo. More deep-seated infection was associated with germination failure, while the incidence of seedling mortality was increased in moist germinating conditions. With deep-seated infection, only those treatments which had a penetrative effect substantially controlled transmission.

1. GENERAL INTRODUCTION

Alternaria is a large, universally occurring genus with more than 46 species so far described on various substrates (Ellis, 1971; 1976). Many of these species cause serious plant diseases (Ellis, 1971; 1976), in some of which phytotoxins are involved in pathogenesis (Daly and Knoche, 1982): others are implicated in animal health problems (Lacy, 1983) which may possibly be associated in some cases with mycotoxin production (Rodricks, Hesseltine and Mehlman, 1977). The most predominent species causing disease in *Brassica* crop plants of the family Cruciferae are *Alternaria brassicae* (Berk.) Sacc. and *Alternaria brassicicola* (Schw.) Wiltshire, which can infect together or independently (Channon and Maude, 1971; Dixon, 1981). Although *Alternaria raphani* Groves and Skolko. has been reported to cause disease on *Brassica campestris* L. and *Brassica napus* L. crops (Berkenkamp and Degenhardt, 1974), this fungus is usually most common and serious on radish, *Raphanus sativus* L. (McLean, 1947). *Alternaria cheiranthi* (Lib.) Bolle is common on wallflower, but has only occasionally been recorded on other plants including members of the Cruciferae (Ellis, 1971).

Alternaria disease of plants of Cruciferae is primarily seed borne, but can be seen throughout the growing period and may be dispersed by air-borne spores or persist with infected plant debris in soil (Channon and Maude, 1971; Dixon, 1981). Seed infection may result in seed rot, seedling mortality, wire stem and damping-off in seed beds; in adult plants the disease can give rise to light grey to dark brown circular spots on leaves and, with severe leaf spotting, defoliation and plant death. Infection can spread from leaves to leaf petioles, and later to stems. The fungi are responsible for severe spotting on cauliflower and cabbage heads, as well as causing a slow decay of crop produce, including turnip roots in storage and transport (Nelson, 1927; Chupp,

1935). The disease is destructive on seed pods (Channon and Maude, 1971) and, according to Dixon (1981), the most damaging phase of *Alternaria* infection relates to the inflorescence of *Brassica* seed crops: severe spotting on stems and pods may greatly reduce the photosynthetic activity of green pods and stems which are the most important source of assimilates for the seed (Daniels, Scarisbrick, Mahamud, Chapman and Addo-Quaye, 1982); infected plants reach senescence early giving premature ripening of pods and seeds and a consequent reduction in seed size and seed number. Heavily infected pods split open and seed is thus lost prior to harvest.

In his reference to British parasitic fungi, Moore (1959) refers to *A. brassicicola* as the cause of dark leaf spot of *Brassica* species, along with but more common than *A. brassicae*, although he indicated that the two species are rarely differentiated. He describes *A. brassicicola* as common and widely distributed in Britain on various crops and also a common cause of rotting of cabbage seed pods in Scotland. More recently, *Alternaria* infection has presented a major disease problem in oilseed rape (*Brassica napus*) in England (Evans and Gladders, 1981; Evans and Cox, 1982). In England, until 1976, *A. brassicicola* appeared to be the important pathogen of cabbage seed crops but, by late 1978, *A. brassicae* became more prevalent in vegetable *Brassica* seed crops. This sudden increase in incidence of *A. brassicae* was attributed to the intensification of production of oilseed rape (Humpherson-Jones, 1983).

Early reports of its occurrence in Scotland are given by Foister (1961) who indicated that *A. brassicicola* was found in 1956 on cauliflower causing wire stem in the south-central area: it was suspected that it may be more often associated with this symptom than had been realised. *A. brassicae*

was thought to be the cause of black stem on cauliflower in Ayrshire in 1954. In 1976 (Anon, 1976), *A. brassicicola* was reported on cauliflower curds in south-east Scotland and later in 1977, 1980 and 1981 (Anon, 1977; 1980; 1981). *A. brassicicola* was also recorded on a crop of Dutch cabbage in north Scotland in 1980 (Anon, 1981a). *A. brassicae* was observed in 1981 from south-east Scotland on hedge mustard in Fife and, along with *A. brassicicola*, on a cabbage crop sprayed with tridemorph to control mildew in the Lothian region (Anon, 1981).

Following the trend in England, winter oilseed rape has recently gained popularity in Scotland in a short period of time, increasing in area from 120 ha (Denly, 1983) in 1981 to 1752 ha in 1982 (Gill, 1981). This dramatic increase in area can be largely attributed to the improved financial returns and also to its role as a much needed break crop in cereal production systems (Nystrom, 1979).

The present study has been, in part, stimulated by the increasing practical importance of *Alternaria* infection in *Brassica* crops in Britain as a consequence of the extension in production of oilseed rape. Investigations in three seasons - 1982, 1983 and 1984 - were undertaken to record the distribution, incidence and severity of *Alternaria* infection on *Brassica* crops with special reference to oilseed rape in east Scotland, where 80-90% of the area of oilseed rape in Scotland was concentrated. A survey of the incidence of *Alternaria* in *Brassica* seed stocks in Britain was also undertaken. In addition, factors influencing disease development and the effects of *A. brassicae* and *A. brassicicola* on seed yield and quality in oilseed rape were considered. Other aspects which received attention were host-pathogen interactions using a range of different isolates of *A. brassicae* and *A. brassicicola* on different cruciferous host plants; the effects of seed infection on seed germination and seedling

infection; and measures of controlling *Alternaria* infection, including the use of chemical or heat treatment of seeds.

2. LITERATURE REVIEW

The host plants considered in this study are members of the family Cruciferae and are of economic interest as vegetable or fodder crops, or because of their seeds which provide sources of various oils and condiments. In all cases, they are propagated by seed. The plants of the Cruciferae are characterised by being closely allied, with often a high degree of genetic affinity between taxa: this can at times result in difficulties in classification, while the use of common names can often lead to confusion. In the present work the system of nomenclature listed in Appendix I, based on the recommendations of ISTA* (1982), has been followed as much as possible.

Various species of *Alternaria* have been found associated with members of the Cruciferae, including *A. brassicae* and *A. brassicicola*, which are of primary interest in this work, and others which are listed in Table 2.1 along with reference sources. In the case of *A. brassicae* and *A. brassicicola*, references relating to countries and hosts are listed in Appendices II and III. Of these, *A. raphani*, as already indicated, is mainly a pathogen of radish but also occurs on oilseed rape. *A. alternata* has been regarded as a weak parasite (Neergaard, 1945), although it was reported to cause leaf spots on Polish rape in an inoculation study (Vaartnov and Tewari, 1972): more recently it was reported to cause leaf spotting in radish and internal seed infection (Narayanappa, 1982). Another species, *A. longipes*, has been reported to cause leaf spot in cauliflower (Rao, 1979). *A. cheiranthi* forms yellow spots on wallflower and is occasionally recorded on other plants, especially those in the Cruciferae (Ellis, 1971). The remaining species are reported on seeds of various members of the Cruciferae as saprophytic contaminants (Ellis, 1971; 1976).

*International Seed Testing Association

TABLE 2.1: *Alternaria* species associated with the Cruciferae.

Species	Cruciferous hosts	Reference
A. brassicae (Berk.) Sacc.	Broccoli, Brussels sprouts, cabbage, collards, Chinese cabbage, forage rape, radish, kale, khol rabi, mustard, oilseed rape, swede and turnip.	CMI (1971) Appendix II
A. brassicicola (Schw.) Wilts.	Broccoli, Brussels sprouts, cabbage, cauliflower, Chinese cabbage, kale, oil seed rape, radish and swede.	CMI (1982) Appendix III
A. alternata (Fr.) Keissler	Radish (Weak parasite - Cruciferae	Narayanappa (1982) Neergaard [1945])
A. cheiranthi (Lib.) Bolle	Wallflower and other members of Cruciferae.	Ellis (1971)
A. consortiale (Thum.) Simmons	*Brassica* seeds	Ellis (1976)
A. longipes (Ellis & Everh.)	Cauliflower	Rao (1979)
A. macrospora Zimm	Cauliflower	Rao (1977)
A. raphani Groves & Skolko	Cabbage, cauliflower	Holtzhausen and Knox-Davies (1974)
	Radish	McLean (1947)
	Rape, turnip rape.	Berkenkamp and Degenhardt (1974)

Alternaria is a large genus in the family Dematiaceae which includes fungi of the Hyphomycetes bearing either dark conidia or hyphae or both. It is grouped under the order Moniliales, a very large group consisting of probably over 7000 species, many of which are of immense importance as either plant pathogens, human pathogens or industrial fungi. With members of the genus *Alternaria*, the conidiophores may be simple or branched and are septate and dark; the light or dark brown conidia are obclavate, beaked, usually formed in simple or branched chains and are mainly muriform although longitudinal septa may be absent. The Hyphomycetes represent a class of the Deuteromycotina, an assemblage of fungi in which the perfect or sexual state is absent. Many are now recognized as conidial states relating to the members of the Ascomycotina, Basidiomycotina or Zygomycotina, but the sub-division also denotes fungi whose perfect states are still completely unknown (Alexopoulos and Mims, 1979). Tentatively, it is likely that were perfect states of *Alternaria* to be found, they would be Ascomycotina (class Pyrenomycetes) and belong to the genera *Leptosphaeria, Pleospora* or *Sporodesmium* (Roscoe, 1967).

With reference to the colony growth characteristics of *A. brassicae* and *A. brassicicola*, the following descriptions of the two species are based on the diagnoses of Wiltshire (1947), Neergaard (1945) and Brooks (1953). *A. brassicae* produces usually unbranched conidiophores with 0 to 7 septa and from 6 to 11 µm wide. The conidiophores are geniculate with a prominent scar at each geniculation. The conidia are usually solitary on the natural host, but occur in chains of up to four in culture: they are greyish-olive, obclavate with a conspicuous beak, possess usually 11 to 15 cross septa and 0 to 8 longitudinal ones, and measure 76 to 350 X 11 to 42 µm (with the beak about one-third to one-half the

length). Its larger spores with a prominent beak readily distinguish it from *A. brassicicola*. *A. brassicicola* has, again, usually unbranched conidiophores which are 0 to 3 septate and olivaceous. The conidia may be solitary or in chains of up to 20 or more: they appear light to dark, olivaceous and taper slightly towards the apex or are obclavate in shape, with a beak which is occasionally developed but usually almost non-existent. They have mostly less than six cross septa and few longitudinal ones, with a central pore visible in the cross walls, and measure 18 to 130 X 8 to 30 µm (with the beak, when present, about one-sixth of the length).

A. brassicae was first described by Berkeley in 1836 on decaying cabbage leaves at Kingscliffe, Northamptonshire, England, under the name *Macrosporium brassicae*. Later, Kuhn (1855) reported it from Germany on rape and, in 1882, it was observed on radish in the USA (Ellis and Martin, 1882). Other countries from which it was noted in the last century include Italy (Peglion, 1894) and Sweden (Eliasson, 1897): during the early part of this century, *A. brassicae* was reported from Holland (Ritzemabos, 1902), Brazil (Puttemans, 1907), Russia (Estifeyeff, 1925), China (Porter, 1926), India (Mason, 1928) and Japan (Yoshii, 1933). *A. brassicae* is now recognized to have a world wide distribution (Figure 2.1) and is a major pathogen of *Brassica* crops in temperate regions of the world. All kinds of crops in the family Cruciferae may be heavily infected by the fungus with large reductions in yield. Although less common and less severe on cabbage and cauliflower seed crops in humid regions, it is common on other Cruciferae seed crops, *B. napus*, *B. campestris*, *B. nigra* and radish (Neergaard, 1979). Reference to the countries from which it has been reported along with their host plants are given in Appendix II.

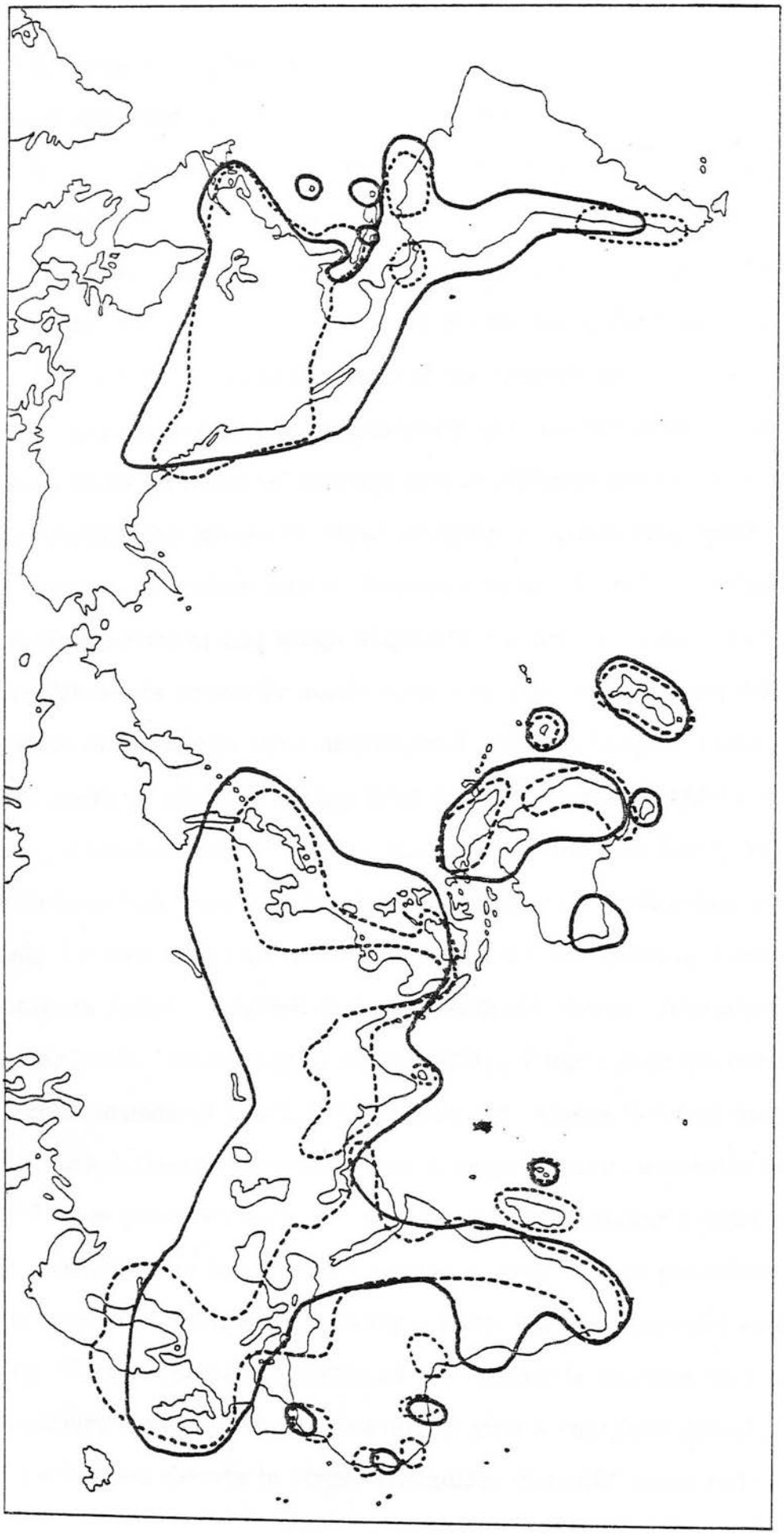


FIGURE 2.1: World distribution of *A. brassicae* (———) and *A. brassicicola* (- - - - -) on Cruciferous crops.

A. brassicicola has, also, a wide geographical distribution and has been reported on cruciferous crops from many parts of the world (Figure 2.1, Appendix III). The first reference to *A. brassicicola* was in 1832 in the USA under the name *Helminthosporium brassicicola*. A later reference is given by Berkeley (1875) under the name *Macrosporium cheiranthi* var. *citcimans*. It is now recognized as a major pathogen on seed crops of members of the Cruciferae in temperate parts, substantially lowering yield qualitatively and quantitatively. High infection rates on seeds of cabbage and cauliflower are common, but it is probably less severe on other cruciferous seeds (Neergaard, 1979).

Both *A. brassicae* and *A. brassicicola* are capable of infecting susceptible plants at any stage of growth (Milbraith, 1922; Rangel, 1945). They are primarily seed-borne and infected seed may fail to germinate or seedlings show damping-off effects (Rangel, 1945) and severe seedling blight in forage *Brassicas* (Vanterpool, 1949); the seedling mortality may be 100% in mustard (Chahal and Kang, 1979). *A. brassicae* has been associated with damping-off of cabbage seedlings, causing 80-100% losses (Govshkov, 1976). On the growing plants, infection is usually confined to lower, matured leaves, inflorescences and seed pods. According to Dixon (1981), foliar symptoms consist of circular lesions of 0.5-2.5 cm in diameter, having distinct margins with a sunken centre surrounded by a bright yellow, chlorotic halo. *A. brassicae* produces light brown orange spores giving a light to dark grey appearance to the lesion, whereas *A. brassicicola* produces dark brown spores in abundance, giving a sooty black appearance to the lesion. Host tissue at the centre of each lesion is reduced to a thin, dry, papery texture and may fall out to give a shot-hole effect. Cohesion of these lesions results in large, irregular, necrotic areas and premature

defoliation. Trails of secondary lesions run in dark streaks from the site of primary infection. Lesions are not usually found in juvenile foliage, although on young leaves symptoms may be seen in the form of small specks or dark streaks. Descriptions of cruciferous leaf spot symptoms for the two fungi are given by Ellis (1971): *A. brassicae* produces circular, zonate, light brown to greyish or dark brown spots from less than 0.5 to 12 mm in diameter, sometimes coalescing; on the midribs of the leaves, the spots are oblong or linear and sunken; *A. brassicicola* gives rise to dark brown to almost black, circular, zonate spots, 1-10 mm in diameter.

Considerable damage is caused to cruciferous crops intended for direct human or animal consumption and also to seed crops by *A. brassicae* and *A. brassicicola*. Dixon (1981) observed dark brown to black spots on Brussels sprouts caused by *A. brassicicola* and similar lesions were seen with dark green margins with *A. brassicae* with decaying cauliflower curds. It was also found associated with soft rot of turnip and swede bulbs (Ogilvie, 1954). Severe spotting on cauliflower and broccoli heads by *A. brassicae* were also reported by Higgins (1917). Considerable damage to cabbage and cauliflower heads during transit by *A. brassicicola* has been observed (Pound, Cheo, Calvert and Raabe, 1951), thus making these heads worthless for the market (Weimer, 1924).

The major damage caused by these two pathogens is to the seed crops: when the weather is damp and warm the fungi spread rapidly from lower leaves to stems and onto the ripening pods. In a few days of favourable weather conditions the appearance of the whole seed crop may be changed from green to brown or almost black (Gram and Weber, 1952). Dark necrotic lesions are produced on inflorescence branches; irregular necrotic streaks are also found on infected sepals and on pods.

Infected pods split open prematurely, shedding the seeds to give a reduced seed yield. The mycelium on pods grows through the wall into the seed, either killing the embryo or establishing itself in a dormant condition in the seed. Severely infected pods shrival and die. Infected seeds are light in weight and produce weak seedlings which are soon killed by the fungus (Rangel, 1945).

In Holland, van Schreven (1953) reported a loss of 1070 pounds of turnip rape per acre and seed losses worth 250,000 dollars on 4446 acres: *A. brassicae* was the dominant fungus. Whereas in Germany, Domsch (1957) reported a loss of rape seed up to 77%: *A. brassicicola* was considered to be the most damaging fungus. Chupp (1923), in an early report, recorded a 2% crop loss in Long Island in a cabbage seed crop due to *A. brassicicola*. Degenhardt *et al*. (1974), in artificial inoculation studies, recorded a 42-63% seed yield reduction in rape due to *A. brassicae*. In Denmark, Neergaard (1979) reported a 17-42% seed loss due to *A. brassicicola* in cabbage seed crops. In the UK, it is reported that seed losses up to 80% have occurred in vegetable *Brassicas* due to *A. brassicicola* (Maude and Humpherson-Jones, 1980). In more recent reports, the oilseed rape yields were about 20% lower in 1981 than in 1980 in south-east England and *Alternaria* possibly accounted for a 10% seed loss (Evans and Cox, 1982). In oilseed rape, a significant reduction in oil and protein content was observed due to *A. brassicae* infection (Degenhardt, Skoropad and Kondra, 1974). Studies in USA indicated that infection of *A. brassicicola* at any stage of seed development will cause a reduction in seed quality (Chirco and Harman, 1979). According to Jorgensen (1967), *A. brassicae* seed infection lowers seed germination, seed weight and free fatty acid content of seeds. Neergaard (1979) reported the occurrence of reduced seed size and

shrunken seeds in cabbage due to *A. brassicicola* infection. Neergaard (1979) concluded that *Alternaria* may cause heavy losses of seed both qualitatively and quantitatively in cruciferous seed crops, making seed production unprofitable in Scotland.

Alternaria diseases are predominantly epidemic in a definite period during the growing season: they do not usually reach severe proportions throughout the growing season, but are severe either at the seedling stage, causing damping-off, or when host plants are weakened, particularly at senescence (Neergaard, 1979). According to Harter and Jones (1918), infections are most common and very severe on older leaves in a field. Changsri and Weber (1963) reported that *Brassica* plants with thin leaves such as mustard, turnip and radish, showed greater infection with *A. brassicae* than thick leaved varieties like cabbage, cauliflower, collards and kale which are, however, readily infected by *A. brassicicola*.

The importance of the disease problem depends on temperature and, particularly, humidity (Neergaard, 1945; Louvet, 1958). The disease is often most destructive on cruciferous seed pods and infection develops rapidly under moist, warm conditions during their maturation (Raabe, 1939; Gram and Weber, 1952; Mcdonald, 1959; Jorgenson, 1967; Dixon, 1981). Extensive studies carried out in Denmark showed that with cabbage seed, early maturing varieties harvested during the midsummer months under fairly dry conditions escaped infection by *A. brassicicola*, whereas late maturing varieties were severely attacked due to the high humidity during harvest (Neergaard, 1979). Workers in Canada reported that *A. brassicae* can reduce seed yield by 20%, but the incidence and severity can vary annually depending mainly on weather (Downey, Klassen and McAnsh, 1974).

Excessive early vegetative growth caused by high rates of seed bed nitrogen application has invariably shown greater susceptibility to *A. brassicae* (Hughes, 1975). This may relate in part, however, to a humid microclimate within the crop. Neergaard (1979) suggests that wider row spacing in *Brassica* seed crops, to keep plants drier and to allow maximum sunlight to reach the lower leaves, reduces the risk of heavy infection of *Alternaria*. Further, he also suggests that wind exposed, open uplands are better suited for *Brassica* seed crops than low-lying and closed locations, where stagnant damp air promotes disease development more quickly.

Alternaria thrives well in plant debris in soil and may be found in Virgin soils (Clayton, 1924). It has been reported that to a very limited extent slugs can spread the disease by ingesting spores which remain viable in the alimentary canal and can infect plants when excreted (Hasan and Vago, 1966). It is readily spread by wind and rain (Clayton, 1924). Wind-borne spores are carried up to 1000 metres and the spore load in air is as high as 3200 spores/cubic metre at the time of combining (Maude, 1977). These spores may act as a secondary source of infection to other *Brassica* crops grown in adjacent fields. Seed-borne transmission forms a major source of infection. Both *A. brassicae* and *A. brassicicola* are internally seed-borne: *A. brassicicola* persisted in cabbage seed up to 7 years and 8 months in normal storage (Neergaard, 1969) or 12 years in controlled storage (Maude and Humpherson-Jones, 1980). On the other hand, *A. brassicae* persists in cabbage seed up to 1 year 2 months (Neergaard, 1969). However, Chahal (1981) reported that *A. brassicae* infected mustard seed samples (84-92%) were completely free from infection after storing for 6 months at room temperature and suggested that seed treatment is not necessary.

Control of plant diseases caused by parasites is based largely on prevention rather than cure and intelligent control of plant disease depends on a thorough knowledge of the pathogen, host plant and their interaction. The methods must be simple, economic and safe to apply. Van der Plank (1963) pointed out that most control measures reduce either the initial inoculum or the rate of spread of the pathogen or both. Sowing disease-free seeds or planting material, crop rotation, field sanitation, growing resistant varieties, destruction of the pathogen on or in the planting material by chemical or heat treatment, soil fumigation and host isolation, all reduce the initial inoculum. Several workers have studied the effect of hot water treatment to reduce *Alternaria* infection in *Brassica* seeds, and have successfully reduced the internal seed infection (Walker, 1922; Chupp, 1923; Schimmer, 1953; Baker, 1969a). Maude (1967), however, concluded that seed treatment alone is not enough to control *Alternaria* disease and that each source of the pathogen must be identified and eliminated. Baker (1969) successfully eliminated *A. brassicae* seed infection with an aerated steam treatment at 50°C for 30 minutes. Harman and Nash (1978) observed that the fungicide benomyl or PCNB or thiram soak lessened *A. brassicicola* in cabbage but did not eradicate it. *A. brassicae* was reported to be best controlled by organomercurials in rape (Darpoux *et al.*, 1957). Vanterpool (1949) noted the increased germination percentage of infected seeds treated with Ceresan in glasshouse conditions. Richardson (1970) observed that captan seed treatment was successful in reducing *A. brassicicola* but the levels of *A. brassicae* were not significantly reduced by the fungicide. Kanwar and Khanna (1979), however, reported that thiram and difolatan gave complete eradication of *A. brassicae* from infected mustard seed.

Among cultural measures to reduce infection, Maude (1977) recommended sufficient isolation of *Brassica* vegetable crops from oilseed rape.

Measures to prevent the spread of the disease include application of protective fungicides and growing resistant varieties. Atlhough Tewari and Skoropad (1976) reported that there are no rape seed lines immune to *A. brassicae*, and Dixon (1975) concluded that there has been so far little evidence of a range of cultivar resistance to *A. brassicicola* in oilseed rape, some sources of resistance to *A. brassicae* were reported by Husain and Thakur (1963) and Bhander and Maini (1965) in *Brassica napus* and *Brassica alba* cultivars, while Braverman (1971) showed that four cauliflower introductions and three Brussels sprout introductions (Braverman, 1977) were resistant to *A. brassicicola*. Skoropad and Tewari (1977) showed that the variation in the epicuticular wax in *B. napus* and *B. campestris* was linked to variation in *Alternaria* leaf spot caused by *A. brassicae*.

Much work has been carried out on the control of *Alternaria* in *Brassica* seed crops by fungicide sprays. An increase in seed yield has been noted and reduced seed infection with a Bordeaux mixture spray (Nielson, 1933; Green, 1947). Effective control of *A. brassicae* was obtained with three sprays of Bordeaux mixture on a radish crop (Chand and Jatian, 1969). It has been reported that (Anon, 1970.) Bordeaux mixture was found to be effective against *Alternaria* pod spotting. Seed yield losses in mustard were negligible when intensity of infection with *A. brassicae* on pods was reduced to 10.7% with six sprays of Bordeaux mixture (Chahal and Kang, 1979). In a cauliflower crop six to eight sprays of thiram and benomyl alternating with Mancozeb + insecticide at 10 day intervals increased seed yield by 2-22 times that of the control (Kudela, Novak and Skorpik, 1978). Neergaard (1979)

observed that two fungicide sprays after flowering or three - one before and two after flowering - gave adequate protection against *Alternaria* species such as *A. brassicae* and *A. brassicicola* under weather conditions which prevailed in Denmark. Humpherson-Jones, Maude and Ainsworth (1980) reported that an oil-based iprodione formulation was an effective treatment to reduce the incidence of *Alternaria* in cabbage seed crops. An increased seed yield up to 20% in winter oilseed rape has been recorded with a single spray application of iprodione at the early pod stage (Cox, Swash and Paviot, 1981).

3. EXPERIMENTAL STUDIES

3.1 SURVEY STUDIES ON THE INCIDENCE OF *ALTERNARIA*

Introduction

A. brassicae and *A. brassicicola* are recognized to be the dominant seed-borne pathogens of cruciferous crops. Until recently, *A. brassicicola* was the most serious and frequently reported on *B. oleracea* seed crops in England (Maude and Humpherson-Jones, 1980). However, in 1980, *A. brassicae* was found causing damage in cabbage and kale seed crops: until then this species had not been regarded as a problem on *B. oleracea* seed crops in England. Furthermore, with the extension in oilseed rape production, *A. brassicae* was reported as the dominant species on oilseed rape in east and south-east England (Evans and Gladders, 1981): the increased incidence of *A. brassicae* on horticultural *Brassica* seed crops may, thus, be attributed to the increased oilseed rape crop, which is considered to act as a reservoir for *A. brassicae* and as a potential source to horticultural *Brassica* seed crops (Humpherson-Jones, 1983).

In Scotland, oilseed rape increased in area of production from 120 ha in 1981 (Denly, 1983) to 1752 ha in 1982 (Gill, 1981). This dramatic increase can be largely explained by the improved financial returns compared with winter wheat and barley (Anon, 1982; 1983), as shown in Table 3.1.1. It is also recognized as providing a much needed break crop in cereal production systems to control grass weeds and cereal diseases (Nystrom, 1979). In considering the increasing area under oilseed rape in Scotland against the background and experience of an earlier increase in England, where a widespread oilseed rape problem due to *Alternaria* infection emerged with implications for vegetable production, it was decided to monitor the incidence of *Alternaria*

TABLE 3.1.1: Gross margins (£/ha) of winter oilseed rape and winter cereals, 1982 and 1983.

Year	Crops		
	Winter barley	Winter wheat	Oilseed rape
1982	455	491	595
1983	466	516	601

(Source: Anon, 1982; 1983)

and its distribution in east Scotland. Crops of oilseed rape were sampled over three growing seasons along with seed produce. In addition, a small number of observations were made on vegetable *Brassica* crops in one growing season and on overwintering oilseed rape crops in one year.

A further phase of the survey work was concerned with testing cruciferous seed samples from various sources for the presence of *Alternaria* species and other fungi: the purpose of these tests was to establish the relative distribution of different *Alternaria* species and their relationships with different crop plants, as well as their relative importance in comparison with other fungal species. *Alternaria* pathogens are carried on seed as fungal spores and also as a mycelium in the seed coat. With *A. brassicicola*, up to 500 spores per seed have been recorded on cabbage seed (Maude and Humpherson-Jones, 1980). Schimmer, in 1953, recorded levels of *A. brassicicola* infection up to 100% in seed lots of cabbage in England. Maude and Humpherson-Jones (1980), from a survey in England for the years 1976-1978, reported that 96% of commercial seed samples of *Brassica oleracea* were infected with *A. brassicicola* and 24% of all *Brassica* types contained *A. brassicae*: in basic seed

samples the infection levels were 64% and 16%, respectively. Most reports would seem to indicate that *A. brassicicola* is the predominant species in *Brassica* vegetable seed, but that the growing of oilseed rape has increased the incidence of *A. brassicae*.

The experimental studies in the section may be considered under the following headings.

3.1a Incidence of *Alternaria* infection in oilseed rape crops in east Scotland, 1982-84.

3.1b Incidence of *Alternaria* infection in overwintering oilseed rape crops in east Scotland, 1982-83.

3.1c Incidence of *Alternaria* infection in vegetable or root *Brassica* crops in east Scotland, 1983.

3.1d Incidence of *Alternaria* in seed lots of *Brassica* crop plants from different sources, 1980-1983.

Materials and Methods

Experiment 3.1a

Observations were made on winter oilseed rape crops grown in east Scotland in three seasons, 1982, 1983 and 1984, by sampling about 10 fields in each of four areas, the Borders Region, the Lothians Region, Fife and Perth, Kinross and Angus. The most common cultivar was Rafal, followed by Jet Neuf. In 1982, a few fields of Brutor (2)* and Elvera (2) were also included along with Rafal (22) and Jet Neuf (14), whereas in 1983 all the fields sampled were either Rafal (30) or to a lesser extent, Jet Neuf (15). In 1984, Rafal was again the dominant

*( ) = No. of infected fields

cultivar along with very few fields of Jet Neuf and the new cultivars, Bienvenu and Fiona. The first sampling was done at the time of flowering, late May to early June, when the disease normally starts spreading to the upper leaves and inflorescences. The second sampling was done at the time of seed maturation just before harvest, late-July to early-August, when the disease generally reaches its maximum severity. A total of 25 plants were either pulled with roots intact or cut at the base from five to eight points at random across each field. The plants were collected in polythene bags and held in a cold store at 5°C, then examined within 2 days of collection. All leaves including the lower, old yellowing leaves, were examined for *Alternaria* spots with the aid of a stereobinocular microscope with a magnification up to X80. The disease severity on leaves was scored at the first sampling each year, using the following 0-5 scale, to give a leaf disease index based on the assessment method followed by ADAS (1982), reflecting the general levels of infection throughout the crops:

0 = Healthy, without *Alternaria* spots.

1 = Scattered minute specks without any chlorotic haloes; less than 10% leaf area infected.

2 = Small scattered spots without distinct chlorotic haloes; infected area 11-25%.

3 = Several distinct well developed spots with clear chlorotic haloes; infected area 26-50%.

4 = Many large spots; infected area 51-75%.

5 = Many large scattered spots often coalescing to form large shot-holes with considerable yellowing; infected area 75%.

Plate 3.1.1 illustrates some representative leaf symptoms caused by both *A. brassicae* and *A. brassicicola*.

PLATE 3.1.1: Leaf symptoms of *Alternaria* infection.

(a) Leaf spots caused by *A. brassicae* (A), and *A. brassicicola* (B), on oilseed rape from artificial inoculation.

(b) Field symptoms of natural infection by *A. brassicae*.

Samples without clear symptoms were surface sterilized with 1% sodium hypochlorite and incubated on moist blotters at 20 ± 2°C for further examination after 3-5 days.

The samples collected at the seed maturation stage were scored for stem and pod spotting, using the following 0-5 scale for level of stem and pod disease (ADAS, 1982) to reflect again the general level of infection throughout the crop:

0 = No *Alternaria* infection symptoms.

1 = Scattered minute lesions less than 1 mm in diameter; 1% pod or stem area infected (trace).

2 = Distinct spots of 1 mm or above; 1-10% area infected (slight).

3 = Many scattered spots 2-3 mm in diameter; 10-25% area infected (moderate).

4 = Large spots up to 3-4 mm; 25-50% area infected (severe).

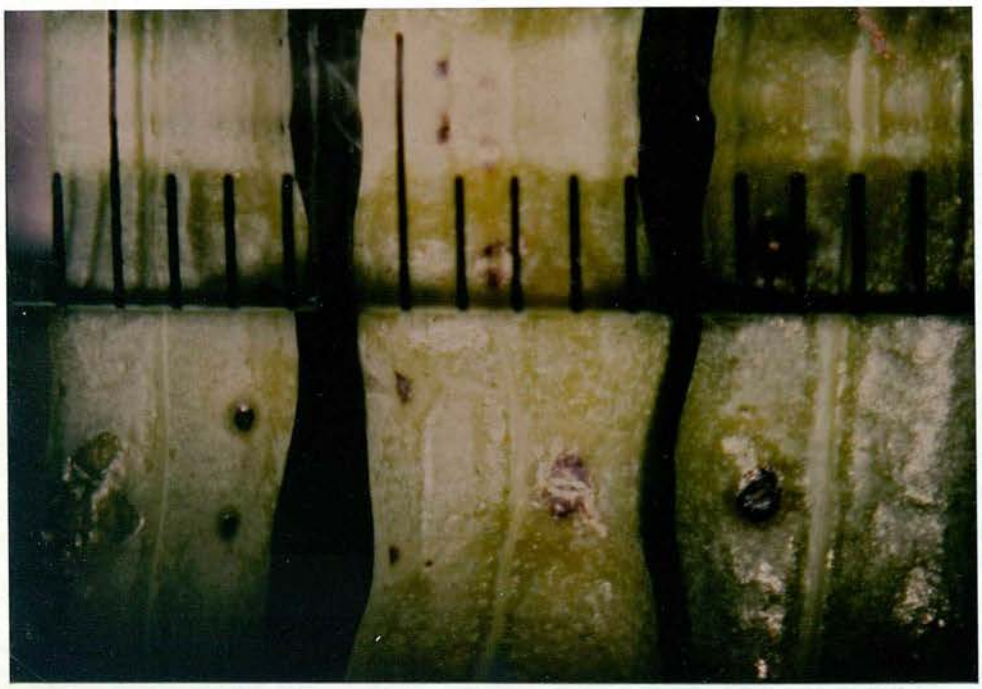
5 = Spots coalesced, general blackening; more than 50% pod or stem area affected (very severe).

Plate 3.1.2 illustrates some categories of infection caused by *A. brassicae*.

Distinguishing between *A. brassicae* and *A. brassicicola* was attempted visually but, in case of doubt, diagnosis was confirmed by taking 25 leaves or pods at random from each sample. The material was then dipped in 1% sodium hypochlorite solution, incubated in plastic humid chambers at 20 ± 2°C for 3-5 days and examined for characteristic spores (Ellis, 1971). The spore types are illustrated in Plate 3.1.3.

The pods in each sample were dried and the seeds were extracted and examined for seed infection. From each sample, 200 seeds were

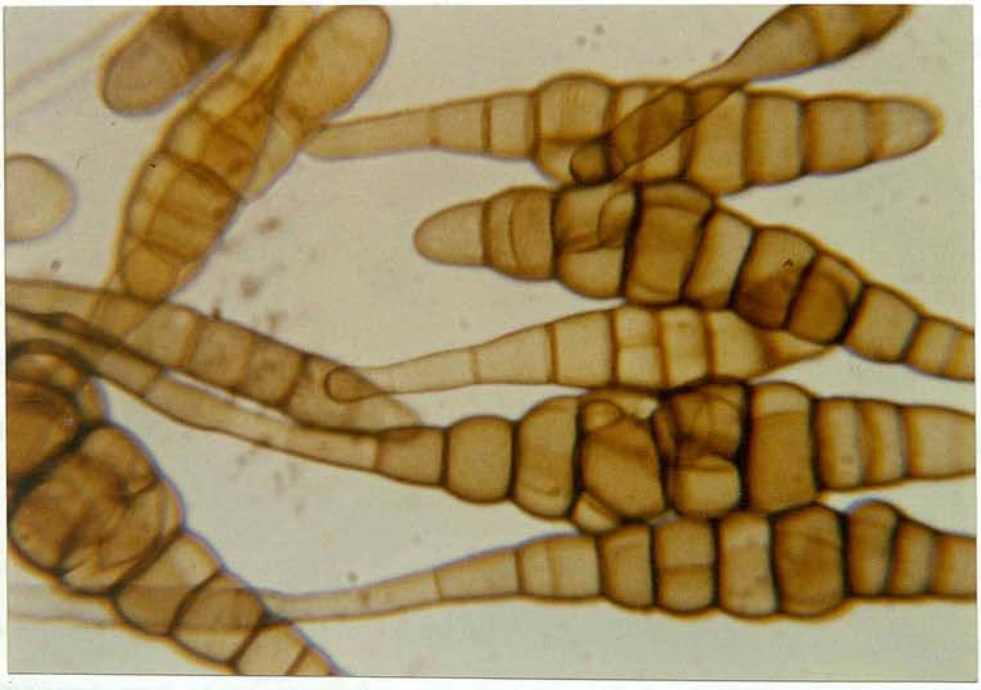
PLATE 3.1.2: Categories of pod infection initiated by *A. brassicae* on oilseed rape.



(a) Trace - slight symptoms from artificial inoculation (millimetre scale).

(b) Severe - very severe symptoms from artificial inoculation.

PLATE 3.1.3: *Alternaria* conidia.



(a) *A. brassicae* spores from oilseed rape seed after 7 days of incubation.

(b) *A. brassicicola* spores from oil seed rape seed after 7 days of incubation.

tested, incubating on V-8 agar (Petrie, 1974a). The seed samples were sub-divided into four lots of 50 seeds, which were each transferred on to the V-8 agar in 10 cm square petri dishes, using a vacuum seed-counting head. The seeds were surface sterilized with 1% sodium hypochlorite solution for 1 minute before placement and the seed counting head was disinfected with alcohol between each sample. The plates were incubated at 20 ± 2°C in NUV light for 12 hours, alternating with 12 hours darkness. The plates were examined for *Alternaria* infection after 3 days of incubation and after a further 2 and 4 days, with the aid of a stereobinocular and a compound microscope. Seed lots infected with fast growing fungi, such as *Botrytis*, *Sclerotinia* and *Rhizopus*, were retested and samples with heavy bacterial infection were also re-tested using an antibiotic in the medium. A record was made of *Alternaria* species present and also of other fungal species observed on the samples.

Experiment 3. 1b

Observations were made on 13 overwintering oilseed rape samples collected from the Borders region during November-December, 1982. The plants were examined at the vegetative growth stage before stem elongation. Each sample comprised about 20 plants, taken at random, and these were examined for leaf spots and also for sporulation, after incubating the lower leaves in the moist chambers as described earlier.

Experiment 3. 1c

Seven vegetable or root *Brassica* crops comprising five Brussels sprouts, one cauliflower and one swede were sampled from the Borders region in August 1983, two to four of the bottom leaves being removed at random from each of 25 plants and examined for *Alternaria* infection.

Experiment 3.1d

The distribution of *Alternaria* species in various *Brassica* seeds was recorded in an examination of 267 seed samples from cultivars of *B. oleracea*, *B. campestris* and *B. napus* species. Seventy-eight *B. oleracea* seed samples, including cabbage, cauliflower, Brussels sprouts, broccoli and kale; eight seed lots of turnip and six swede samples were collected from seed merchants and private seed companies. These along with 85 rape samples from seed production areas in England, received from the National Institute of Agricultural Botany, Cambridge, and a further five seed samples comprising two pre-basic, one basic and two certified seed lots, from the National Seed Development Organisation, Cambridge, were tested for *Alternaria* infection using V-8 agar as described in experiment 3.1a.

The seeds were examined for *Alternaria* infection and, also, observations were recorded on other fungi associated with the seeds. Identification of the fungi was carried out on the basis of the description of Ellis (1971; 1976). Cultures were also sent to the Commonwealth Mycological Institute for confirming identification. Seeds treated with chemicals were washed in running water and later in sterile distilled water, then incubated for seed health testing as described above, to study the infection levels in treated seed samples. In one supplementary study, the results of testing surface sterilised seed and non-surface sterilised seed were compared.

PLATE 3.1.4: Field infections by *A. brassicae* (1982).

(a) Effects at seed maturation stage.

(b) Close-up of pod spotting symptoms.

Results

EXPERIMENT 3.1a: Incidence of Alternaria infection in oilseed rape crops in east Scotland, 1982-1984

The results of assessments of the incidence and severity of *Alternaria* infection on winter oilseed rape crops grown during 1982, 1983 and 1984 in east Scotland are summarised in Table 3.1.2.

TABLE 3.1.2: Incidence and severity of *Alternaria* infection in winter oilseed rape crops grown in 1982, 1983 and 1984 in east Scotland.

Year	No. of crops examined	*A. brassicae* I. sampling	*A. brassicae* II. sampling	*A. brassicicola* I. sampling	*A. brassicicola* II. sampling
% of crops infected		leaf spot	pod spot	leaf spot	pod spot
1982	40	25	80	5	8
1983	45	73	60	16	11
1984	51	14	37	2	0
Mean disease index		leaf	stem/pod	leaf	stem/pod
1982	40	1.2	1.5	<1	<1
1983	45	1.3	2.1	<1	<1
1984	51	1.0	1.0	<1	0

The incidence of *A. brassicae* reached its highest level in the mid-summer of 1982, when 80% of crops showed infection although at the first sampling the incidence was relatively low (25%). In most instances *A. brassicae* alone was present but in a few cases *A. brassicicola* was also recorded, 5% at the flowering stage and 8% at seed maturation: *A. brassicae* was invariably associated with more severe infection (Plate 3.1.4).

In 1983, *A. brassicae* occurred frequently in the early sampling period, with up to 73% of crops infected, but the incidence was down to

60% at seed maturation. The severity of infection in the infected fields, however, remained relatively high in 1983. The incidence and severity was observed to be greatest in dense crops, particularly in crops where the disease was seen early and where crop lodging encouraged the spread of the disease to the flowering stems and pods (Plate 3.1.5). *A. brassicicola* occurred along with *A. brassicae* more frequently than in the previous year but always at a trace level of severity.

During 1984, the number of crops infected with *A. brassicae* were relatively small at both sampling dates and disease severity indices were also relatively low. *A. brassicicola* was recorded in only 2% of crops in early summer and none was seen at the second sampling.

In general, *A. brassicicola* was recorded mostly on incubated material and it was difficult to distinguish the symptoms caused by this fungus: the severity of *A. brassicicola* infection was rated as a trace.

With regard to other diseases, light leaf spot (*Pyrenopeziza brassicae*) and grey mould (*Botrytis cinerea*) were widespread, occurring in most crops in the 3 years, but generally infection was slight. Very occasionally stem canker (*Phoma lingam*) and white leaf spot (*Pseudocercosporella capsellae*) were also observed.

The incidence of *Alternaria* in different areas of east Scotland is presented in Table 3.1.3. *A. brassicae* occurred more frequently in all regions than *A. brassicicola*. The incidence of *A. brassicae* over the 3 years followed more or less the same pattern for different areas as the general trend, i.e. less disease occurring in 1984 than the previous years, and the highest incidence at the first sampling occurring in 1983 and at the second sampling, with one exception, in 1982. In comparing different areas, the highest incidence and severity of *A. brassicae* was invariably found in the Borders region, while the Lothians region showed

PLATE 3.1.5: Field symptoms by *A. brassicae* (1983).

(a) General view of lodging in oilseed rape crop.

(b) Close up of stem and pod spotting associated with lodging.

TABLE 3.1.3: Regional distribution of *Alternaria* on oilseed rape crops grown in 1982, 1983 and 1984 in east Scotland.

Region	Year	No. of samples	*A. brassicae* I sampling	*A. brassicae* II sampling	*A. brassicicola* I sampling	*A. brassicicola* II sampling
			Percentage crops infected			
Borders	1982	16	38	100	0	13
	1983	13	85	85	15	7
	1984	14	29	71	0	0
Lothians	1982	9	22	77	11	11
	1983	17	70	64	17	11
	1984	11	18	36	9	0
Fife	1982	7	14	71	14	0
	1983	9	67	22	7	7
	1984	14	7	21	0	0
Perth, Kinross & Angus	1982	8	13	50	0	0
	1983	6	67	50	17	17
	1984	12	0	16	0	0
			Mean disease index			
Borders	1982	16	1.5	1.7	0	<1.0
	1983	13	1.0	2.3	<1.0	<1.0
	1984	14	1.5	<1.0	0	0
Lothians	1982	9	1.0	1.2	<1.0	<1.0
	1983	17	1.5	2.0	<1.0	<1.0
	1984	11	1.0	<1.0	<1.0	0
Fife	1982	7	1.0	1.2	<1.0	0
	1983	9	1.0	1.5	<1.0	<1.0
	1984	14	1.0	<1.0	0	0
Perth, Kinross & Angus	1982	8	1.0	1.5	0	0
	1983	6	1.0	1.3	<1.0	<1.0
	1984	12	0	<1.0	0	0

more infection than other areas: no consistent differences were found in comparing Fife with Perth, Kinross and Angus. With respect to *A. brassicicola*, the pathogen occurred most frequently in either 1982 or 1983, depending on the area. In 1984, it was observed in the Lothians region only.

The results of the seed tests on harvested samples, averaged for each of the 2 years in which it was recorded, are given in Table 3.1.4. (No infection of seed by either species of *Alternaria* was observed in 1984.) The incidence of *A. brassicae* was higher in 1982 than in 1983. However, the extent of infection within infected samples was, on average, similar for the 2 years, although the range was wider in 1982. *A. brassicicola* occurred more frequently in 1983 than in 1982, but in both years it was less prevalent than *A. brassicae*.

In considering the infection levels in seed samples in relation to area of production (Table 3.1.5), it may be seen that the results reflect those for the growing crop survey for *A. brassicae*: infection was most prevalent and severe in the Borders region while samples from the Lothians were more frequently and severely infected than those from the other areas. The levels of infection were less in 1983 than in 1982 in all areas. With respect to *A. brassicicola*, the Lothians region showed more infection than other areas in both 1982 and 1983, infection levels generally being higher in 1983.

EXPERIMENT 3.1b: Incidence of Alternaria infection in over-wintering oilseed rape crops in east Scotland, 1982-1983

The incidence of *Alternaria* in a small number of over-wintering oilseed rape crops sampled in November-December, 1982, is shown in Table 3.1.6. *A. brassicae* infection was observed in 46% of samples: the symptoms of infection were seen as small minute spots which produced

TABLE 3.1.4: *Alternaria* seed infection in oilseed rape samples produced in 1982 and 1983 in east Scotland.

Year	No. of samples tested	*A. brassicae*			*A. brassicicola*		
		% samples infected	mean % infection in infected samples	% infection range	% samples infected	mean % infection in infected samples	% infection range
1982	40	80	5.3	0.5 - 49.0	13	0.7	0.5 - 1.0
1983	45	47	6.3	0.5 - 29.0	36	1.6	0.5 - 12.0

TABLE 3.1.5: *Alternaria* seed infection levels in oilseed rape samples produced in different regions of east Scotland in 1982 and 1983.

Year	Regions	No. of samples tested	*A. brassicae*			*A. brassicicola*		
			% samples infected	mean % infection in infected samples	% infection range	% samples infected	mean % infection in infected samples	% infection range
1982	Borders	16	100	7.5	0.5 - 49.0	13	0.5	0.5
	Lothians	9	77	2.3	0.5 - 6.0	22	0.7	0.5 - 1.0
	Fife	7	71	4.0	1.0 - 6.5	14	0.5	0.5
	Perth, Kinross and Angus	8	50	3.0	1.0 - 7.0	0	0	0
1983	Borders	13	56	14	0.5 - 29.0	23	0.6	0.5 - 1.0
	Lothians	17	41	1.1	0.5 - 3.0	47	2.6	0.5 - 12.0
	Fife	9	22	0.5	0.5	33	0.6	0.5 - 1.0
	Perth, Kinross and Angus	6	50	0.5	0.5	33	0.7	0.5 - 1.0

spores on incubation. The symptoms were confined to the older, yellowing leaves which were in contact with soil; spots were seen at a trace level only. None of the samples were found to be infected with *A. brassicicola*.

TABLE 3.1.6: *Alternaria* incidence on over-wintering oilseed rape crops sampled in the Borders region, 1982.

No. of crops examined	% Crops infected	
	A. brassicae	*A. brassicicola*
13	46	0

EXPERIMENT 3.1c: Incidence of Alternaria infection in vegetable or root Brassica crops in east Scotland, 1983

The incidence of *Alternaria* on vegetable or root *Brassica* crops from the Borders region examined in the summer 1983 is given in Table 3.1.7. *A. brassicae* infection was found in all samples tested, while *A. brassicicola* was observed in 43% of the samples. Only trace levels of infection were recorded in cauliflower, turnip and three of the fields of Brussels sprouts: in the remaining fields of Brussels sprouts leaf spotting was moderate in one and severe in another. With the severely infected crop, Brussels sprout crop buttons close to the ground showed minute dark spots.

TABLE 3.1.7: *Alternaria* incidence in vegetable or root *Brassica* crops sampled in summer, 1983, in the Borders region.

No. of crops examined	% Crops infected	
	A. brassicae	*A. brassicicola*
7	100	43

EXPERIMENT 3.1d: Incidence of Alternaria in seed lots of Brassica crop plants from different sources, 1982-1983.

The results of *Brassica* seed health tests carried out on seed lots from various sources are presented in Table 3.1.8. *A. brassicicola* was found to be the most generally prevalent species: the percentage of samples infected and the average level of infection within infected samples was high in cabbage seed while the fungus was present in some seed samples of all crop plants examined except swede, which was free of both *Alternaria* species. *A. brassicae* was confined to two crop plants only, oilseed rape and kale.

In oilseed rape samples, *A. brassicae* was more frequently recorded than *A. brassicicola*. In comparing the seeds from the results of the survey in east Scotland (Experiment 3.1a) with the commercial seed samples in this investigation, *A. brassicae* was more frequent in the material taken direct from the field in the survey study, while *A. brassicicola* was more or less similar in both sets of data.

Fungi associated with disease, other than *A. brassicae* and *A. brassicicola*, found in the samples are listed in Table 3.1.9. Of the other known pathogens or weak pathogens associated with *Brassica* seeds, *Alternaria alternata* was the most prevalent species and was present in all the *Brassica* groups tested except turnip. The maximum percentage of samples infected and most severe infections occurred with oilseed rape samples from the field survey, followed by cabbage, commercial oilseed rape, kale and broccoli: the least infection was observed in Brussels sprouts and swede. Infection within infected samples was relatively severe in commercial oilseed rape. *Fusarium* species were found in cabbage, cauliflower, kale and oilseed rape samples. *Phoma* was seen in cauliflower, kale and oilseed rape, and *Botrytis* was associated

TABLE 3.1.8: Seed infection levels of *A. brassicae* and *A. brassicicola* in *Brassica* seed samples, 1982 and 1983.

Crop species	Crops	No. of samples tested	*A. brassicae*			*A. brassicicola*		
			% seed lots infected	mean % infection in infected samples	% infection range	% samples infected	mean % infection in infected samples	% infection range
B. oleracea	Cabbage	25	-	-	-	88	12.4	0.5 - 35.0
	Cauliflower	16	-	-	-	75	2.5	0.5 - 5.0
	Brussels sprouts	6	-	-	-	67	1.7	0.5 - 3.0
	Broccoli	12	-	-	-	17	7.0	0.5 - 14.0
	Kale	19	11	1.0	0.5 - 2.0	63	6.5	0.5 - 20.0
B. campestris	Turnip	8	-	-	-	13	1.0	1.0
B. napus	Swede	6	-	-	-	-	-	-
	Oilseed rape:							
(a)	commercial seeds	90	25	1.7	0.5 - 60.0	31	2.6	0.5 - 13.0
(b)	survey seed samples (Expt 3.1a)	85	62	5.6	0.5 - 49.0	25	1.4	0.5 - 12.0

TABLE 3.1.9: Pathogenic/semi-pathogenic fungi other than *A. brassicae* and *A. brassicicola* associated with *Brassica* seed samples, 1982 and 1983.

Crop species	*Alternaria alternata*			*Botrytis cinerea*			*Phoma lingam*			*Sclerotinia sclerotiorum*			*Alternaria raphani*			*Fusarium* spp.			*Rhizoctonia solani*		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Cabbage	70	3	<1-4	-	-	-	-	-	-	-	-	-	-	-	-	4	<1	1	-	-	-
Cauliflower	38	1	<1-3	-	-	-	13	1	1-2	-	-	-	-	-	-	19	<1	1	-	-	-
Brussels sprouts	16	<1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Broccoli	58	2	<1-4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Kale	58	2	<1-10	37	<1	<1-3	26	<1	<1-2	-	-	-	-	-	-	11	1	<1-2	-	-	-
Swede	16	<1	<1	16	<1	<1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Oilseed rape																					
(a) commercial seed samples	65	5	<1-25	17	1	<1-2	34	2	<1-14	3	<1	1	16	1	1-2	4	1	<1-1	4	1	1-3
(b) survey seed samples (Expt 3.1a)	98	14	<1-53	45	3	<1-19	26	4	<1-27	8	1	1-2	20	2	<1-4	20	1	<1-7	6	<1	<1-1

A = percentage samples infected; B = mean percentage seed infection; C = percentage seed infection range.

with kale, swede and oilseed rape. *A. raphani*, *Sclerotinia* and *Rhizoctonia* were confined to oilseed rape. Turnip seed lots were free from all fungi other than *A. brassicicola*. A greater range of fungi were found associated with oilseed rape than with any other of the crops examined, while infection tended to be more severe and often more frequent in the field survey samples than in the commercial lots of oilseed rape.

The saprophytic fungi found in samples are listed in Table 3.1.10. Fungi in this group were absent from turnip and Brussels sprouts which are excluded from the table. The most common species were *Cladosporium* spp followed by *A. tenuissima*, then *Penicillium* and *Stemphylium*. The widest range of contaminants were associated with oilseed rape.

A. brassicicola was found in most samples of cabbage and cauliflower seed known to have been imported into the UK market (Table 3.1.11). Cabbage seed samples received from Denmark, Holland, Japan and Germany were heavily infected with *A. brassicicola*. Cauliflower seed samples produced in Australia, Holland, Denmark and Japan showed *A. brassicicola* infection but the level of infection was lower than in cabbage seeds. One cabbage seed sample from USA and one cauliflower seed sample from Israel were found free from infection.

The incidence of *A. brassicicola* in cabbage seeds known to have been treated with chemicals is given in Table 3.1.12. *A. brassicicola* was recovered in six out of seven samples with infection percentages up to 14%. All the samples known to have been produced in England and the two from Holland were infected: one sample among the treated seeds received from USA was found free from *Alternaria* infection.

When the seed samples of *Brassica* seeds infected with *A. brassicicola* were surface sterilized with 1% sodium hypochlorite for 1 minute and tested for the presence of *Alternaria brassicicola* (Table 3.1.13), the

TABLE 3.1.10: Saprophytic fungi associated with *Brassica* seed samples, 1982 and 1983.

Crop species	*Alternaria tenuissima*			*Cladosporium* spp.			*Stemphylium* spp.			*Ulocladium* spp.			*Epiccocum* spp.			*Arthrinium* spp.			*Penicillium* spp.			*Aspergillus* spp.			*Rhizopus* spp.		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Cabbage	12	<1	<1-1	-	-	-	14	<1	<1-2	-	-	-	-	-	-	-	-	-	12	3	<1-7	-	-	-	-	-	-
Cauliflower	31	1	<1-2	62	<1	2-3	13	<1	<1-1	18	<1	1	-	-	-	-	-	-	13	<1	1	-	-	-	-	-	-
Broccoli	-	-	-	-	-	-	-	-	-	25	<1	<1-1	-	-	-	-	-	-	-	-	-	16	<1	1-3	-	-	-
Kale	10	<1	1	26	7	1-14	16	1	<1-2	5	<1	1	10	<1	<1-1	-	-	-	26	4	<1-6	5	<1	1	-	-	-
Swede	-	-	-	13	<1	1	8	<1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Oilseed rape																											
(a) commercial seed samples	30	1	<1-4	28	2	<1-1	11	<1	1	8	<1	<1-1	6	<1	<1-1	15	<1	<1-3	51	4	<1-26	8	2	<1-9	6	1	1-2
(b) survey seed samples (Expt 3.1a)	45	2	5-10	70	3	<1-20	30	3	<1-14	7	<1	<1-4	9	<1	<1-1	20	1	1-3	17	3	1-16	6	5	<1-22	2	<1	<1-1

A = percentage of samples infected; B = mean percentage infection; C = infection range.

TABLE 3.1.11: Incidence of *A. brassicicola* in cabbage and cauliflower seeds imported into UK market, 1982 and 1983.

Crop plant	Country of origin	No. of seed lots	Mean % infection
Cabbage	Denmark	2	30.0
	Japan	2	26.3
	Germany	1	20.0
	Holland	3	9.5
	USA	1	0
Cauliflower	Australia	5	1.1
	Holland	6	3.0
	Denmark	2	1.7
	Japan	1	1.0
	Israel	1	0

TABLE 3.1.12: Incidence of *A. brassicicola* in chemically treated commercial cabbage seed samples.

Seed lot	Cultivar	Origin	Infection %
1	Bartalo	Holland	14.0
2	Alpna	England	10.2
3	Durbeyday	England	9.2
4	Utility	England	5.0
5	Clipper	England	4.5
6	Langedijk	Holland	3.0
7	Market price	USA	0

level of infection was at least halved by surface disinfection in most instances, suggesting that the infection was superficial. However, in the most extensively infected seed lot there was no reduction in infection counts, indicating deep-seated infection.

TABLE 3.1.13: Effect of surface sterilization on infection counts of *A. brassicicola* in *B. oleracea* seeds.

	Crop	% Infection in untreated seeds	% Infection in treated seeds
1	Cabbage	100	100
2	Cabbage	90	35
3	Cabbage	87	32
4	Cabbage	45	20
5	Cabbage	23	3
6	Cabbage	17	0
7	Savoy cabbage	20	4
8	Cauliflower	38	2
9	Kale	24	7
10	Kale	19	8

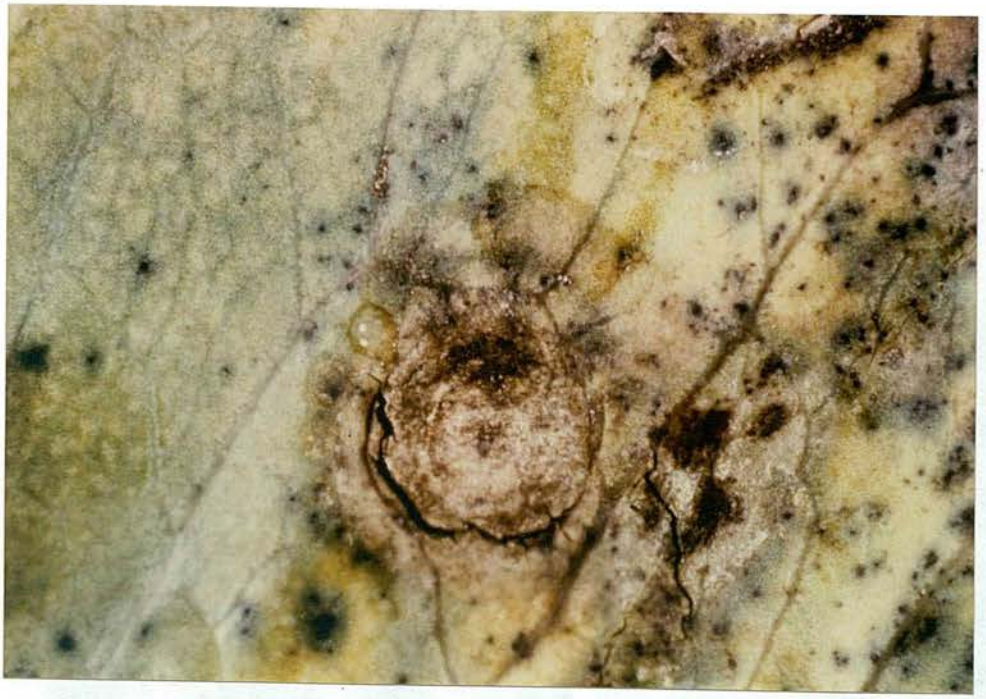
Discussion

Within the last decade, earlier reports (Anon, 1976, 1977, 1980, 1981) on the incidence of *Alternaria* infection in *Brassica* crops in east Scotland made reference to the presence of *A. brassicicola* in caulfilower and, more recently, cabbage (Anon, 1981), whereas later reports (Anon, 1981) have referred to the occurrence of *A. brassicae* in oilseed rape and cabbage. From the present studies

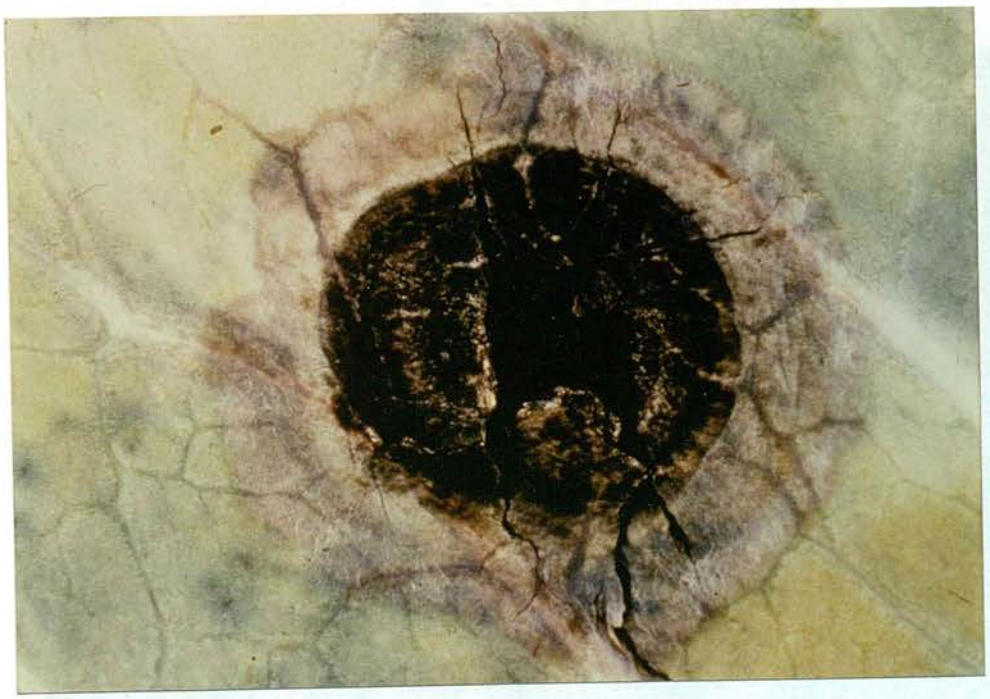
for the years 1982, 1983 and 1984, *A. brassicae* was found to be the predominent pathogen in an extensive survey of oilseed rape crops in east Scotland and occurred more frequently than *A. brassicicola* in a small number of vegetable or root crops of the *Cruciferae* which were sampled in 1983. This trend is in keeping with the findings from England where the increased area of production of oilseed rape has been associated with the emergence of *A. brassicae* as a major pathogen of the crop (Long, 1982; Evans and Cox, 1982), and also been linked with the spread of the pathogen into *Brassica* vegetables (Humpherson-Jones, 1983).

The symptoms of infection by *A. brassicae* appear in the form of grey areas which may be readily distinguished from the darker lesions of *A. brassicicola*, as exemplified in the typical symptoms on cabbage (Plate 3.1.6). On oilseed rape, symptoms on the leaves appeared as dark spots or lesions in the case of *A. brassicae* (Plate 3.1.7a) whereas *A. brassicicola* (Plate 3.1.7b) tends to show indeterminate symptoms and the presence of the fungus required verification by incubation tests, when the spores could be readily distinguished from those of *A. brassicae* (Plate 3.1.3). Several other pathogens causing leaf spotting were observed: of those, *Perenopeziza brassicae* (light leaf spot) and *Botrytis cinerea* (grey mould) were widespread but gave, generally, only slight infections. *P. brassicae* infection is characterised by the production of light coloured blotches surrounded by white spore masses (Plate 3.1.8), while *B. cinerea* produces decayed areas with the presence of the characteristic grey mould growth (Plate 3.1.9). Occasionally, *Pseudocercosporella capsellae* (white leaf spot) and *Phoma* leaf spot were observed: *Phoma* is readily recognized by the presence of black pycnidia (Plate 3.1.10), while leaf spot due to

PLATE 3.1.6: Leaf spot symptoms of field infections of cabbage by *Alternaria*.

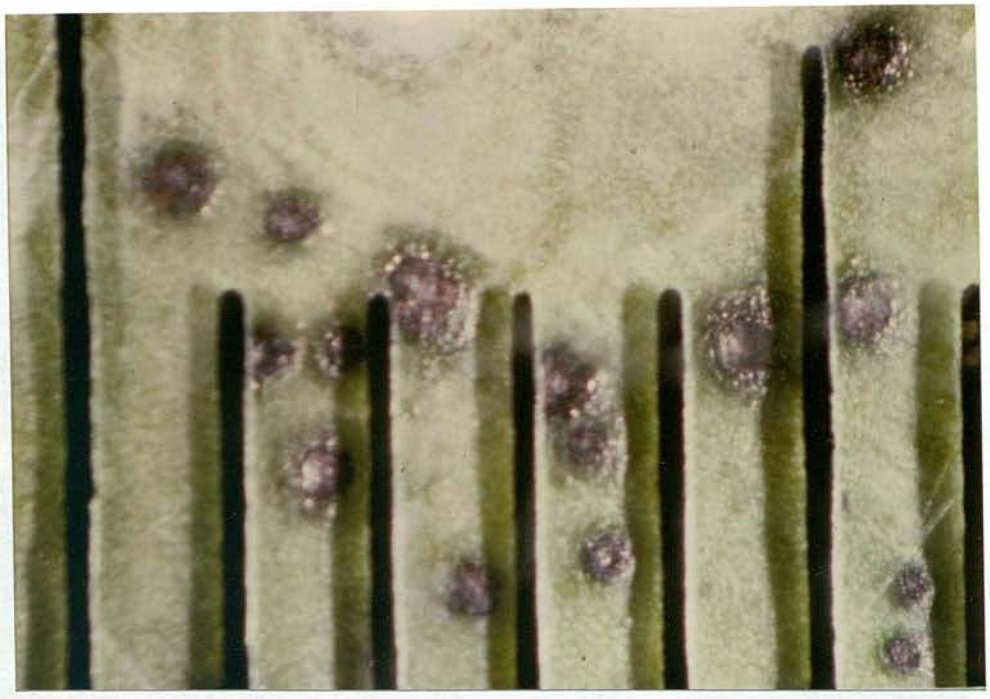


(a) *A. brassicae* (grey spot).

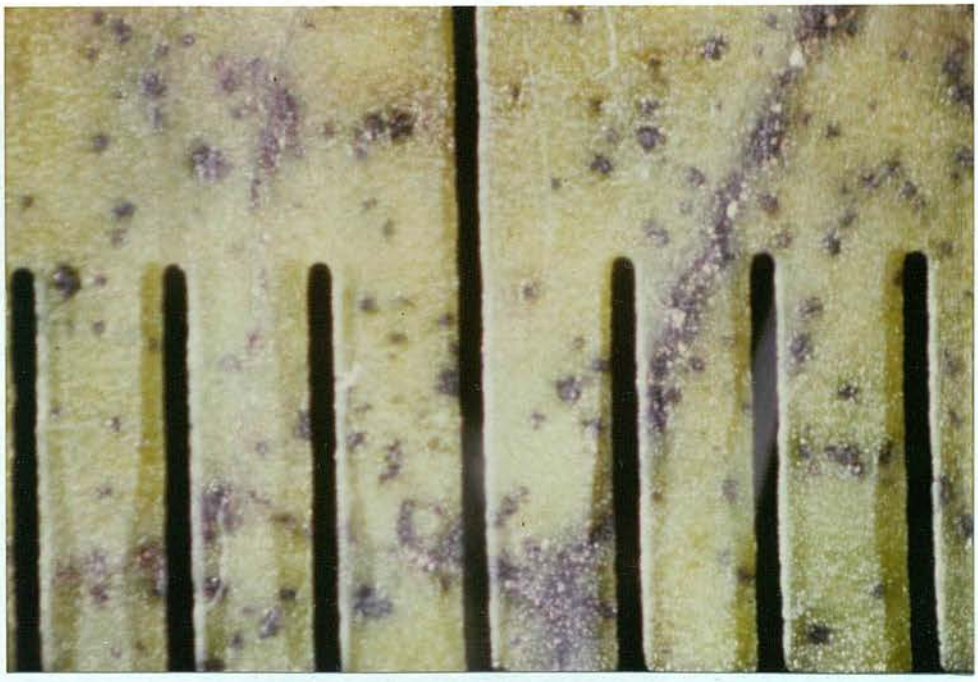


(b) *A. brassicicola* (dark spot).

PLATE 3.1.7: Symptoms of oilseed rape leaf infection by *Alternaria* following artificial inoculation (millimetre scale).



(a) *A. brassicae* on upper young leaf.



(b) *A. brassicicola* on lower aged leaf.

PLATE 3.1.8: Field symptoms of light leaf spot (*Perenopeziza brassicae*) on an oilseed rape leaf.

PLATE 3.1.9: Field symptoms of grey mould (*Botrytis cinerea*) on oilseed rape leaves.

PLATE 3.1.10: Field symptoms of *Phoma* leaf spot (*Phoma lingam*) on an oilseed rape leaf.

PLATE 3.1.11: Field symptoms of white leaf spot (*Pseudocercosporella capsellae*) on an oilseed rape leaf.

P. capsellae forms small white spots (1-5 mm in diameter) which later enlarge and become grey in the centre (Plate 3.1.11).

In considering the variation in incidence of *A. brassicae* infection over the 3 years of the survey (Figure 3.1.1a-c), there were fewer crops infected in 1984 and no crops showed seed infection in 1984 in contrast to the previous 2 years, when the incidences of seed infection were 80% and 47% in 1982 and 1983 respectively. The absence of seed infection in 1984 may be related to generally lower rainfall in that year (Figure 3.1.2a). Humpherson-Jones and Hocart (1983) indicated that *Alternaria* infection requires free water, while a number of workers from different parts of the world reported that *Alternaria* diseases cause severe damage in wet summers. These include Ferraris (1928) from Italy, Fajardo and Palo (1934) from Phillipines, Raabe (1939) from Germany, Green (1947) from England, Dey (1948) from India, Louvet (1958) from France, McDonald (1959) from Canada and Neergaard (1979) from Denmark. Temperature data over the early part of the growing season showed only small differences between years (Figure 3.1.2a). With reference to 1982 and 1983, the pattern of development varied between years: at the first sampling the incidence was much higher in 1983, 73% compared with 25% in 1982 (Figure 3.1.1a), but at seed maturation stage and from seed infection tests more frequent infection was found in 1982, 80% pod spotting compared with 60% in 1983 (Figure 3.1.1b-c). The variation may again be related, at least in part, to the influence of rainfall: although the rainfall was high in the earlier part of both growing seasons, particularly in 1983, the weather during July and August, at seed maturation, was much drier in 1983 than in 1982 (Figure 3.1.2a). A further factor, however, which would account for a lower infection level in 1983 was

FIGURE 3.1.1a: Incidence of *A. brassicae* leaf spotting at the flowering stage of oilseed rape from different sites in east Scotland, 1982-1984.

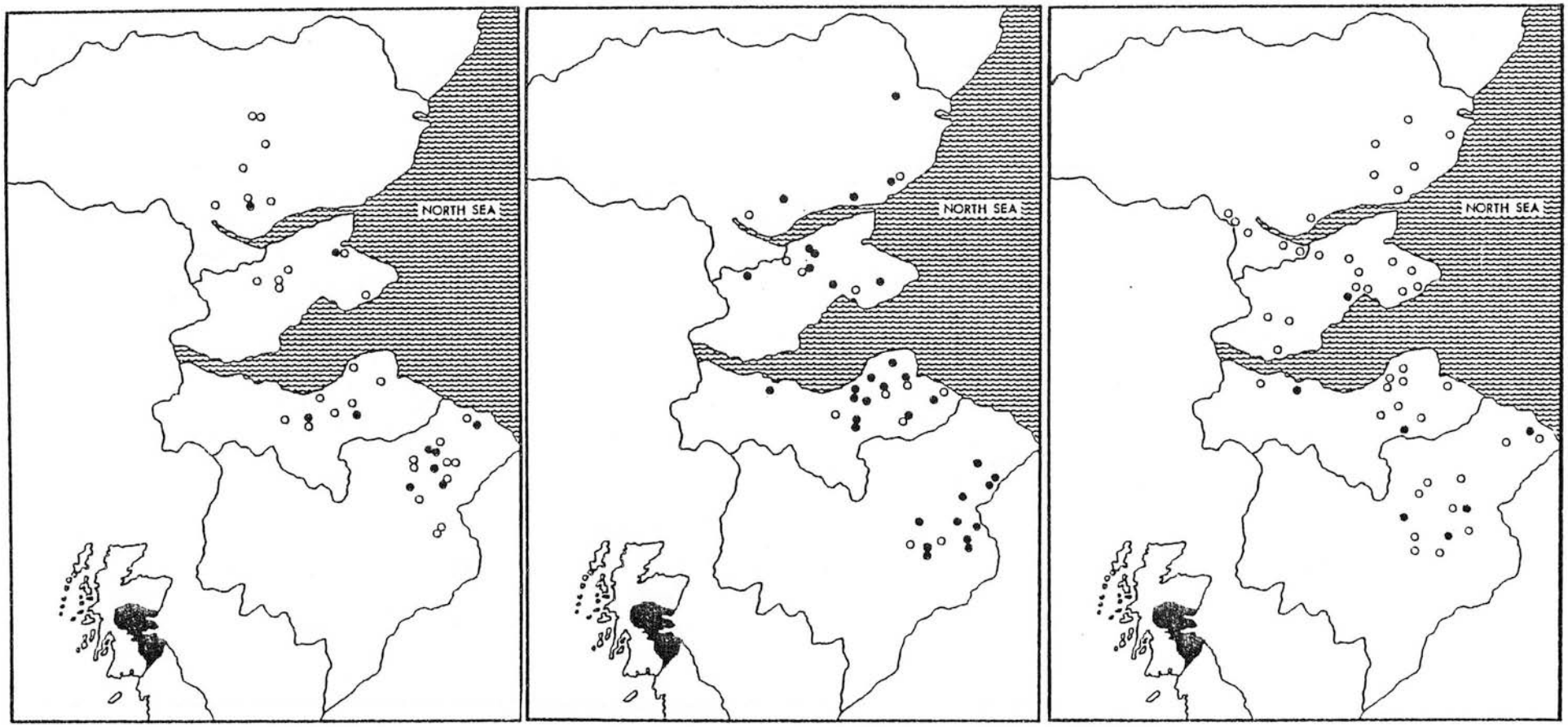


FIGURE 3.1.1b: Incidence of *A. brassicae* pod spotting at the seed maturation stage of oilseed rape from different sites in east Scotland, 1982-1984.

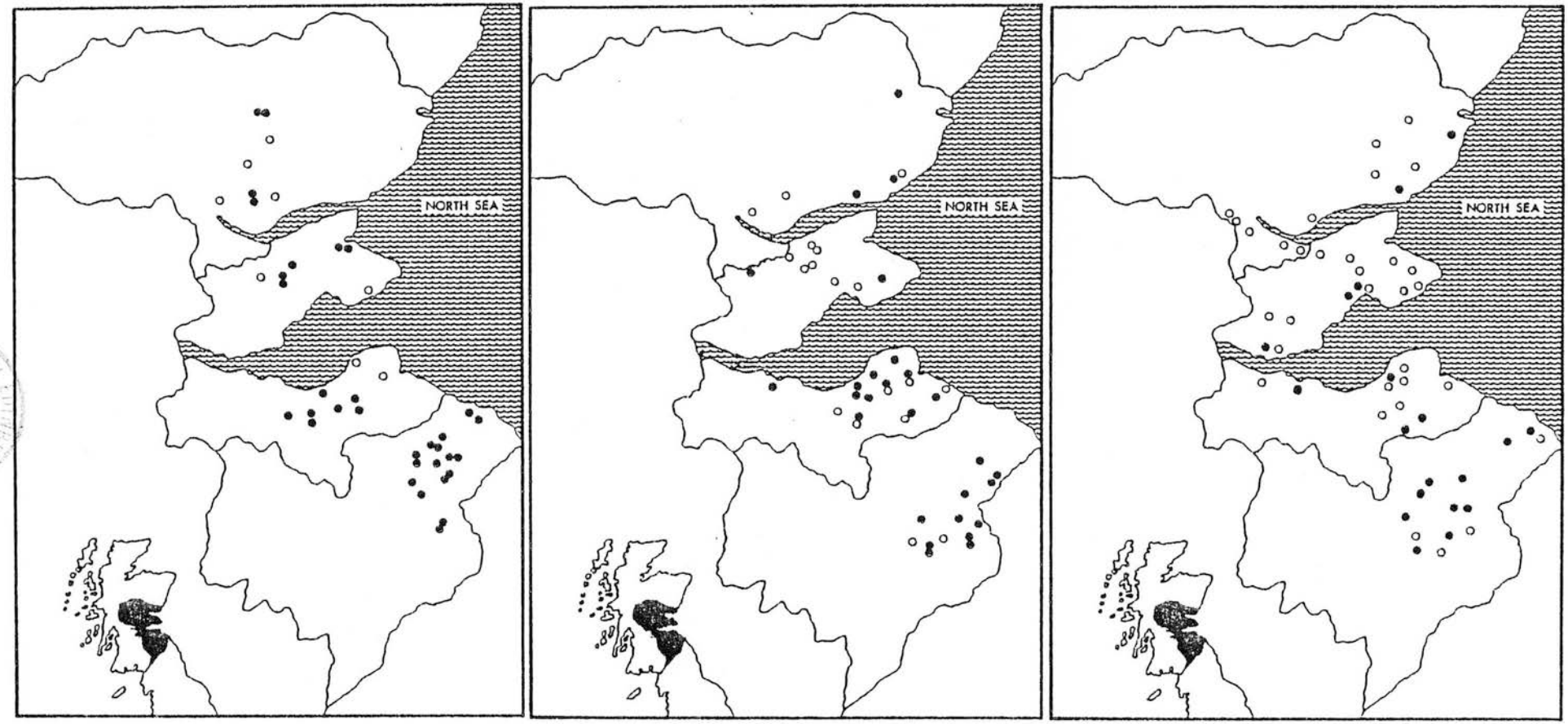


FIGURE 3.1.1c: Incidence of *A. brassicae* infection in seed samples from different sites in east Scotland, 1982-1984.

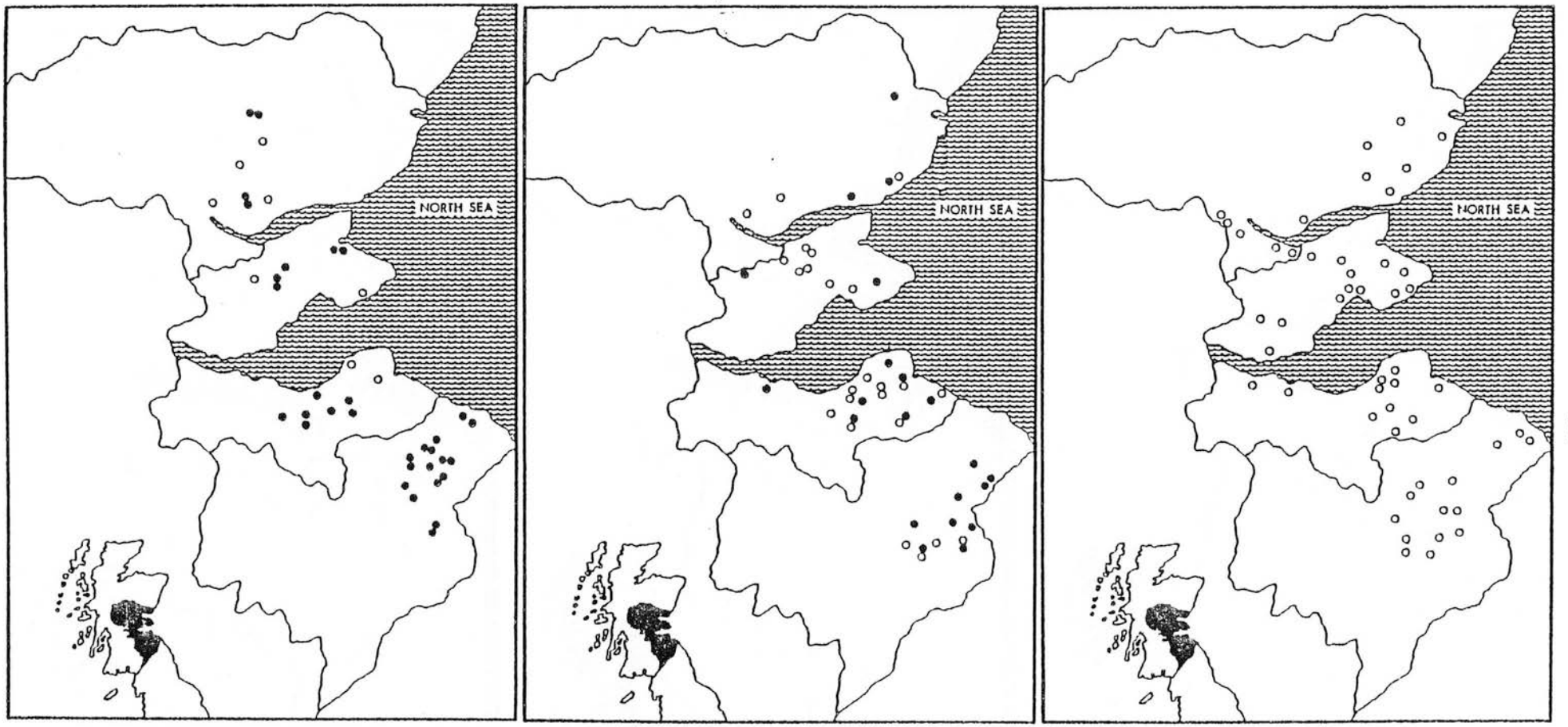


FIGURE 3.1.1d: Incidence of *A. brassicicola* leaf spotting at the flowering stage of oilseed rape from different sites in east Scotland, 1982-1984.

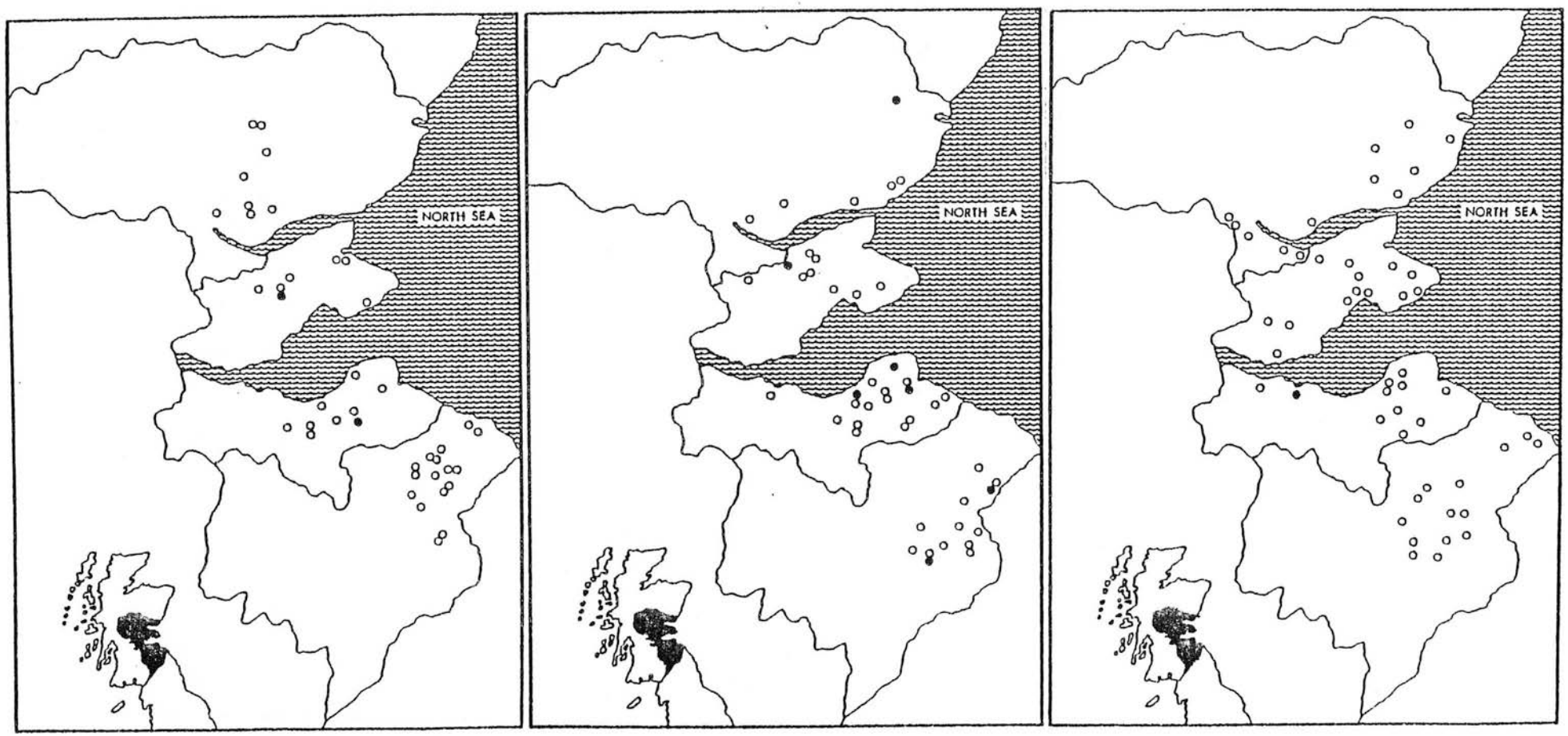


FIGURE 3.1.1e: Incidence of *A. brassicicola* pod spotting at the seed maturation stage of oilseed rape from different sites in east Scotland, 1982-1984.

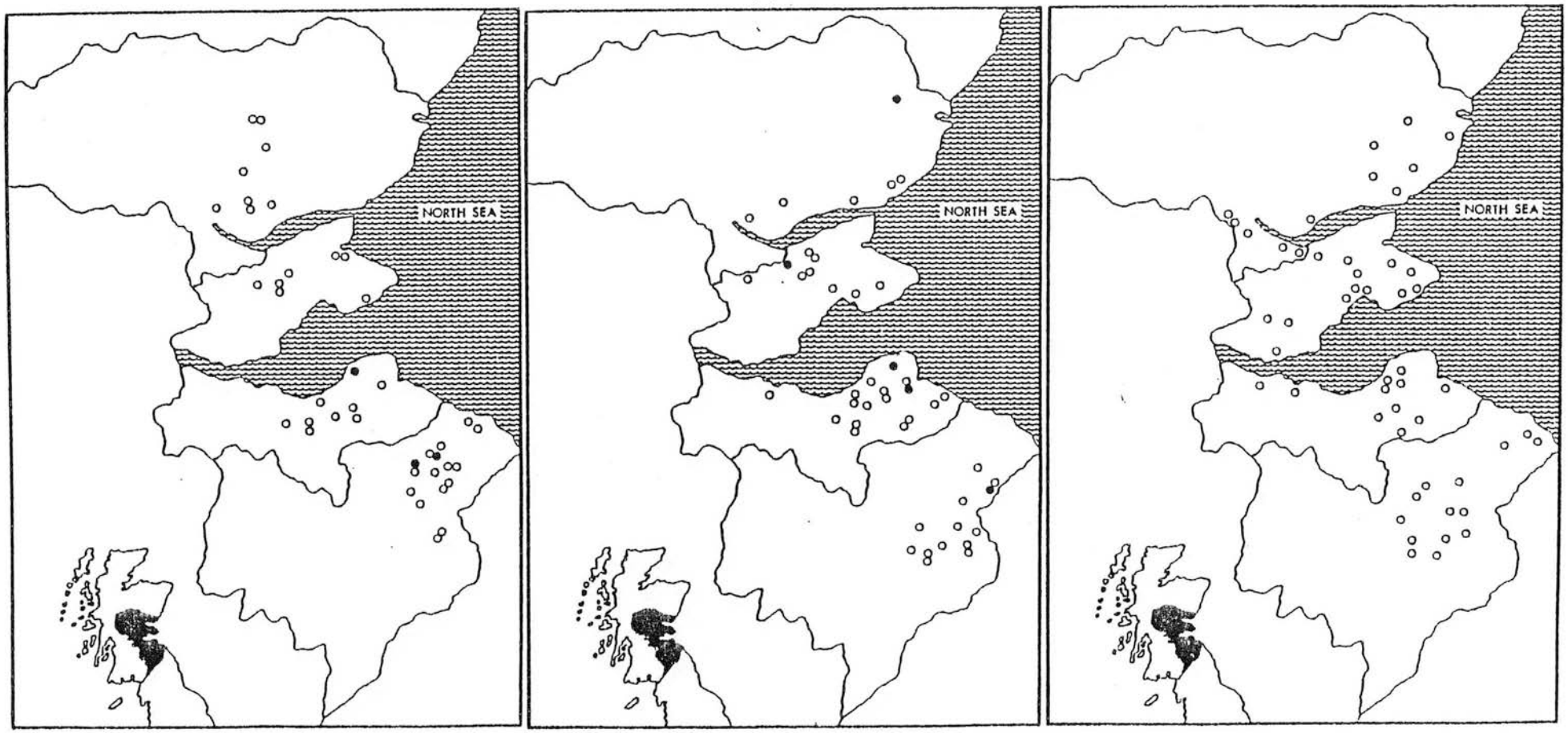
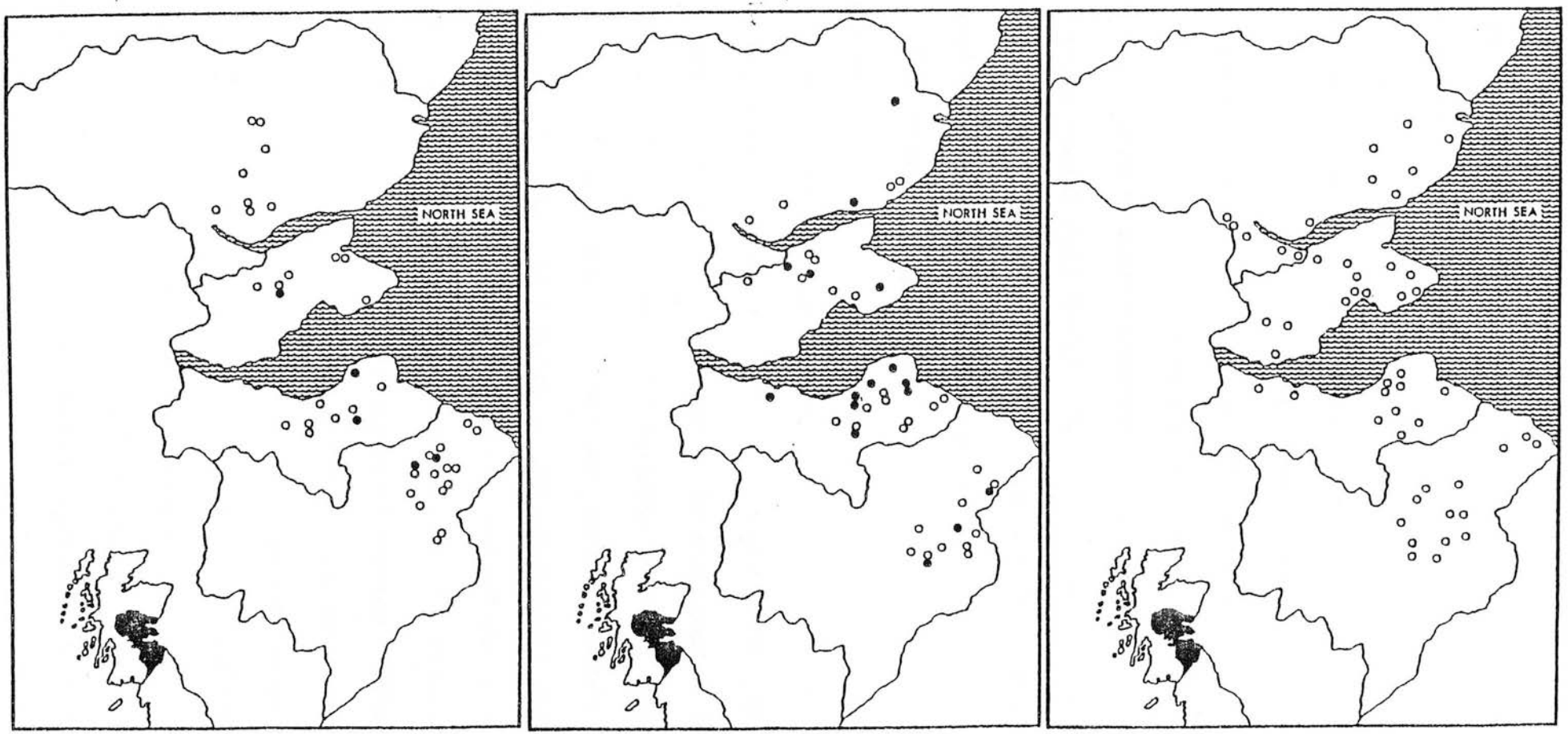


FIGURE 3.1.1f: Incidence of *A. brassicicola* infection in seed samples from different sites in east Scotland, 1982-1984.



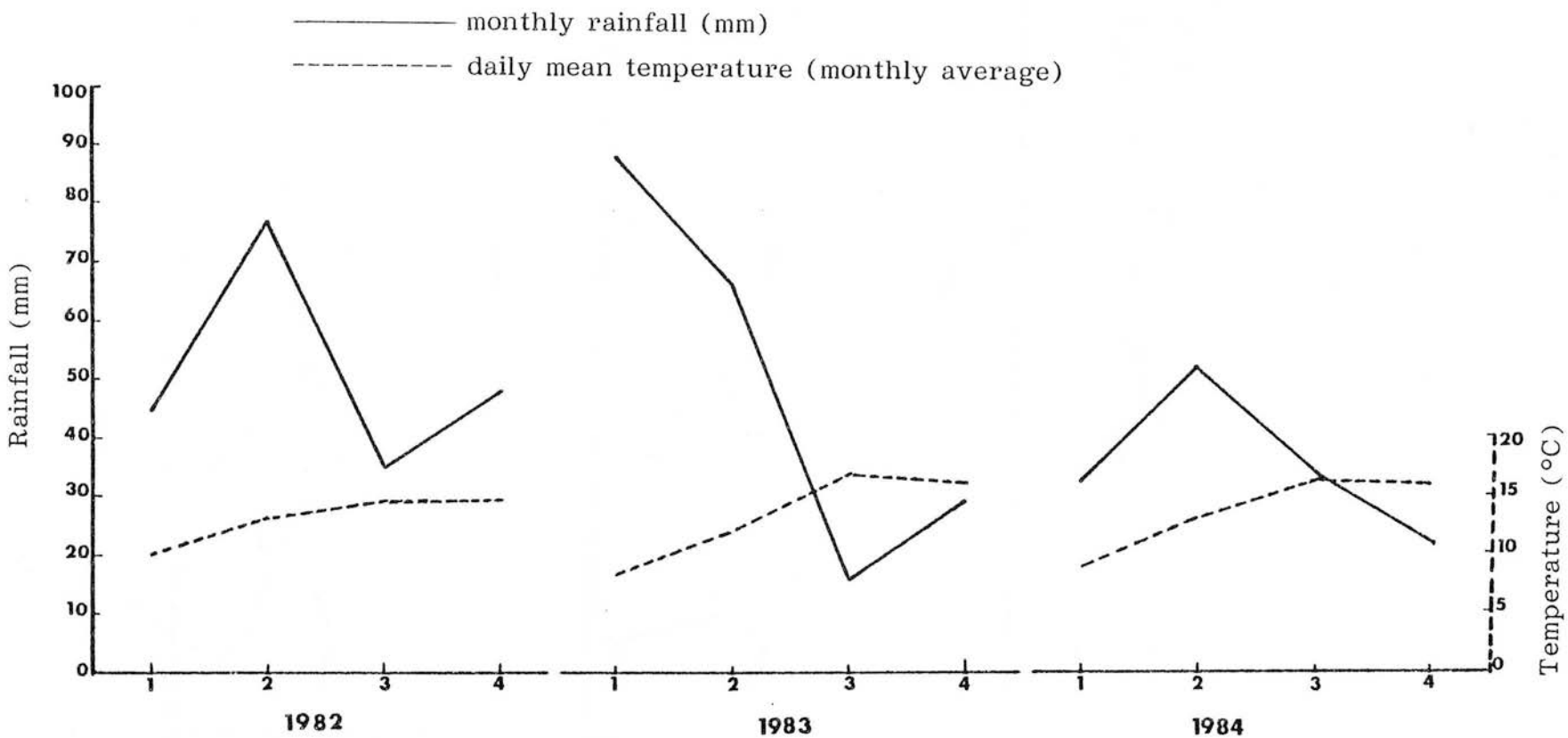
the widespread use of iprodione as an aerial spray: 36 of the 45 fields sampled were known to have been treated. However, of crops known to be unsprayed, three samples were free from seed infection and only one showed severe infection (29%). With spray application, seven of the 36 samples still showed over 5% seed infection. Very few crops were sprayed in 1984 but the disease levels were generally very low. It was not possible to gain accurate records of the extent of seed treatment to control *Alternaria*, but this should be considered as a further complicating factor. However, in some cases where seed dressings, such as iprodione, had been known to have been applied, the level of control was poor, as reflected in disease in the subsequent crop. The evidence suggests that seed treatment did not give prolonged protection.

A. brassicae infection was greatest in dense crops with vigorous vegetative growth: it spread quickly in lodged crops from infected lower leaves to stems and pods, due to the close contact between infected and healthy plant parts and to the humidity build-up caused by the compactness of the vegetation. In a few fields the entire crop appearance was changed in 3 to 4 weeks by the development of heavy infection during wet weather conditions. Where severe infections were observed in crops sprayed with iprodione, this could be due to lack of penetration of the spray into the bottom layers of infected crops. Neergaard (1979) stressed the need for proper crop ventilation in *Brassica* seed crops: ample width of row spacing in cabbage seed crops substantially reduced attack by *A. brassicicola* and other *Alternaria* species. Further, he reported that closed localities with wind breaks should be avoided for *Brassica* seed production. In this investigation, crops grown in similar, enclosed conditions were heavily spotted with *A. brassicae*.

With respect to *A. brassicicola* (Figure 3.1.1d-f), a low incidence of infection by this fungus in 1984 crops and its absence from the seed produce may again be associated with the prevalence of dry conditions, while the somewhat higher incidence of the fungus in 1983 than in 1982 may be possibly correlated with the slightly higher summer temperatures (Figure 3.1.2a): Degenhardt, Petrie and Morrall (1982) indicated that *A. brassicicola* has a high temperature requirement and Humpherson-Jones and Hocart (1983), working with cabbage, indicated an optimum temperature of 25°C compared with 15°C for *A. brassicae*. It may be noted from Figure 3.1.1 e and f that there was generally a higher incidence of *A. brassicicola* seed infection than that observed in the crop at the seed maturation stage: this may reflect, in part, the progress of spread of this species during the growing season as temperatures increased, but, also, the difficulty of detecting visual symptoms of infection by this fungus on oilseed rape.

The highest incidence and most severe *A. brassicae* infections were observed in the Borders region followed by the Lothians region and levels tended to decline northwards (Figures 3.1.1a-c). This might be associated with the trend towards wetter conditions in the south for parts of the main growing season, at least in 1982 and 1983 (Figure 3.1.2b). A further contributory factor might be the longer history of oilseed rape production in the Borders region, along with the greater intensity of production, accounting for greater residual sources of infection. With individual farms, it was observed that the disease was more frequent where oilseed rape had been grown intensively for several years. The more widespread incidence of *A. brassicicola* in the Lothians region, compared with other regions (Figure 3.1.1d-f), may relate to the presence of more market gardens

FIGURE 3.1.2a: Rainfall and temperature in east Scotland (average for four regions in May - August, 1982 - 1984).

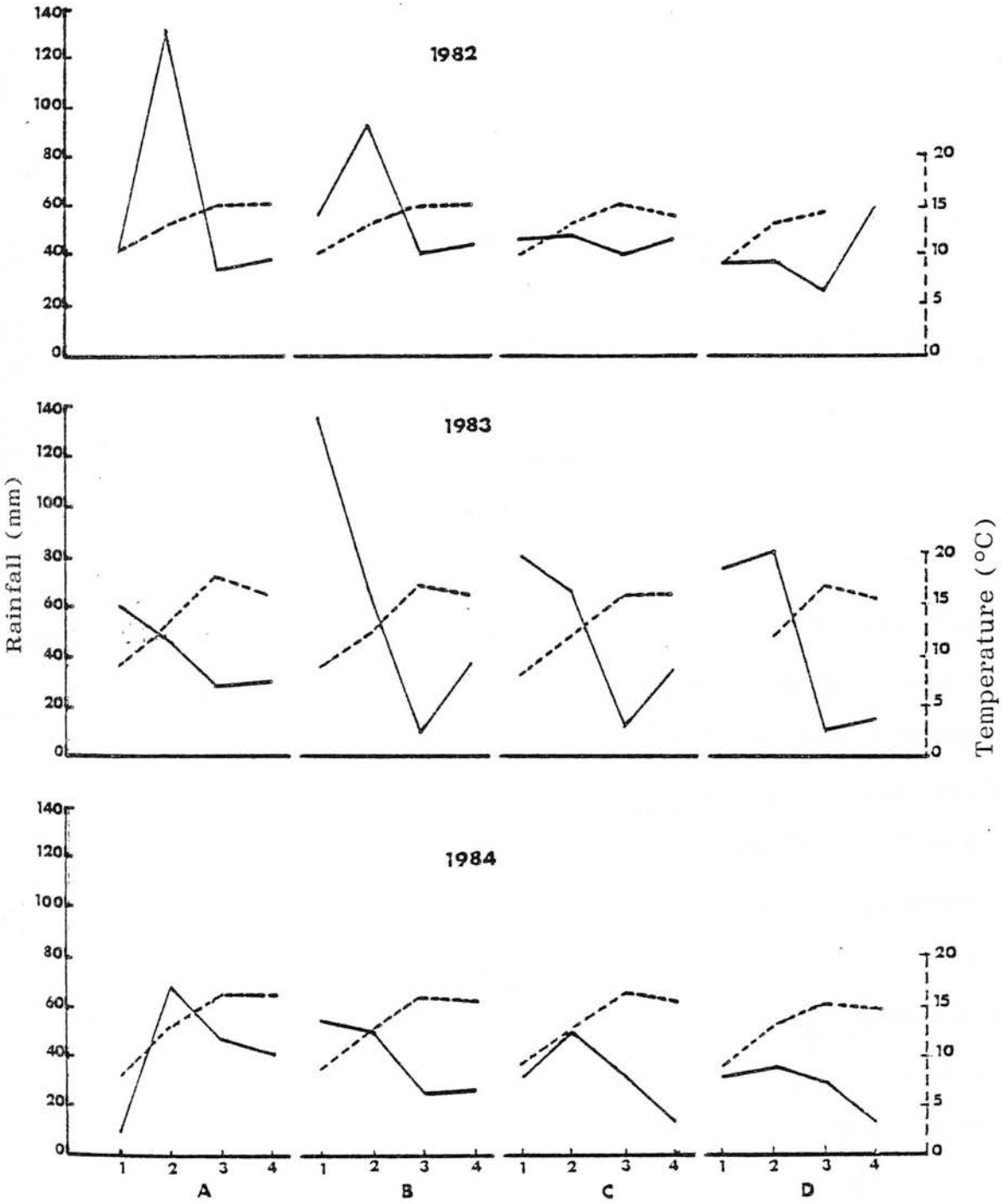


1 = May, 2 = June, 3 = July, 4 = August

FIGURE 3.1.2b: Rainfall and temperature in four regions of east Scotland in May-August, 1982-1984.

——— monthly rainfall

------- daily mean temperature (monthly average)



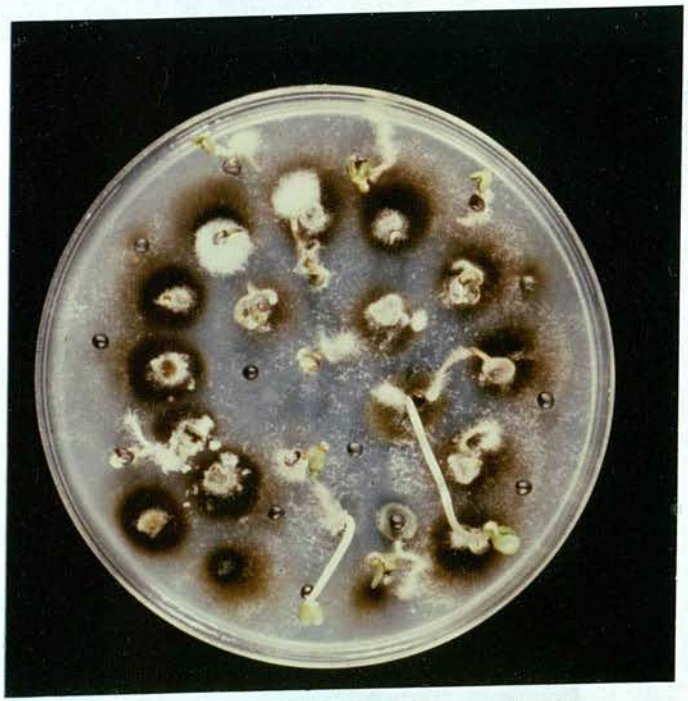
1 = May, 2 = June, 3 = July, 4 = August.

A = Border, B = Lothian, C = Fife, D = Perth.

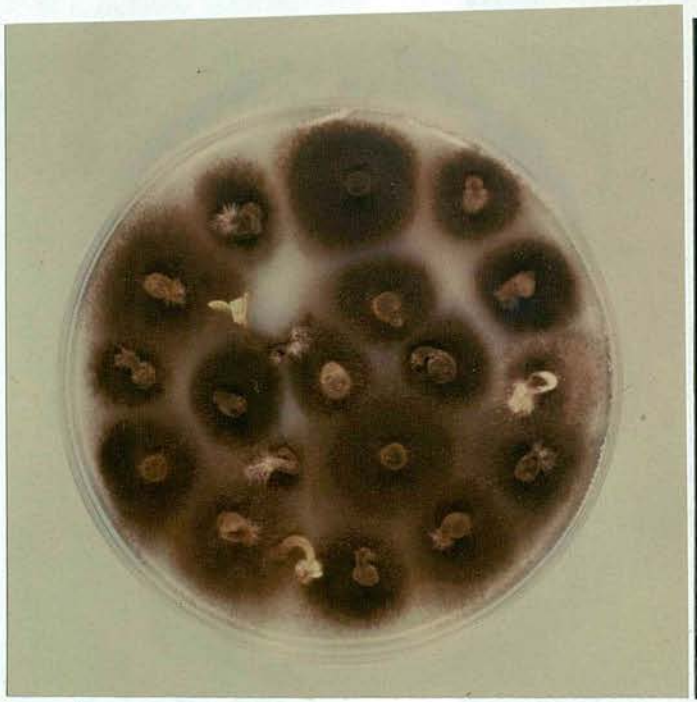
and, thus, more *Brassica* vegetable crops in that area. According to Neergaard (1979), oilseed rape is less affected by *A. brassicicola* than cruciferous vegetables such as cabbage and cauliflower. However, the large presence of these may provide a source of inoculum of *A. brassicicola* for the infection of oilseed rape. The results of the survey point to the implications of the extension in production of oilseed rape with respect to *Alternaria* infection. The production of the *Brassica* crops alongside vegetable crops in the same area provided susceptible material throughout the year. *A. brassicae* was observed in over-wintering *Brassica* crops in December. Degenhardt *et al.* (1982) have noted the ability of *A. brassicae* to infect the host over a wide range of temperatures. There is also circumstantial evidence of cross infection of *A. brassicae* from oilseed rape to vegetable crops (Humpherson-Jones, 1983).

The results of seed health tests of a wide range of *Brassica* crop plants showed that *A. brassicicola* was the most common pathogen on vegetables, whereas *A. brassicae* was most prevalent fungus in oilseed rape samples. Richardson (1970) reported that seed samples infected with *A. brassicae* were also infected, usually to a greater extent, with *A. brassicicola*. The two fungi can be readily distinguished from each other and from other *Alternaria* species by their colony growth characteristics (Plate 3.1.12), and from each other by their forms of spore production (Plate 3.1.13). The distribution of the species *A. brassicae* and *A. brassicicola* was in keeping with the findings of survey reports of a range of workers (Petrie, 1974a; Tahvonen, 1979; Richardson, 1970; Holtzhausen and Knox-Davies, 1974; Neergaard, 1979). It was observed that *A. brassicae* infection was more frequently associated with dead seeds than was *A. brassicicola* infection, where, even with heavy infection, germination still occurred (Plate 3.1.14). Similar observations were recorded by Richardson (1970).

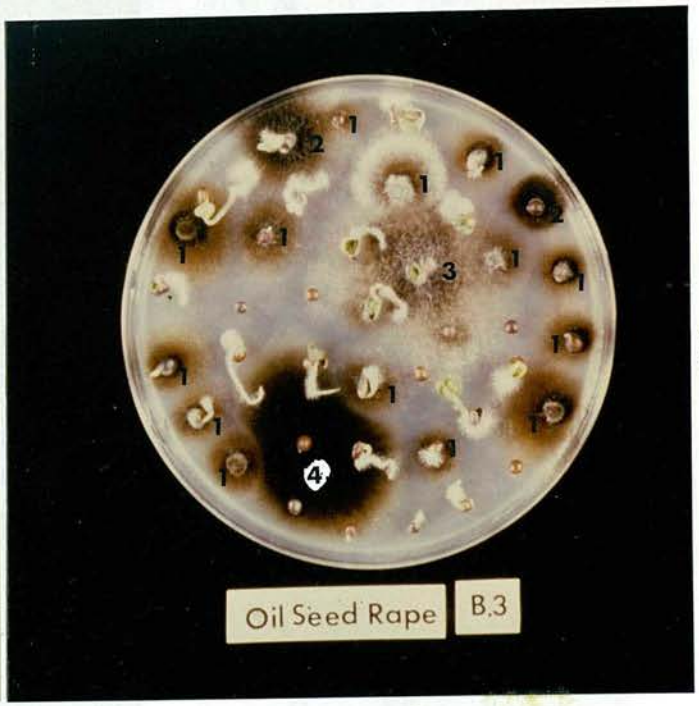
PLATE 3.1.12: Colonies of *Alternaria* species from *Brassica* seeds incubated on V-8 agar under 12 hours NUV alternating with 12 hours darkness in 24-hour cycles.



(a) *A. brassicae* from oilseed rape.



(b) *A. brassicicola* from cabbage.

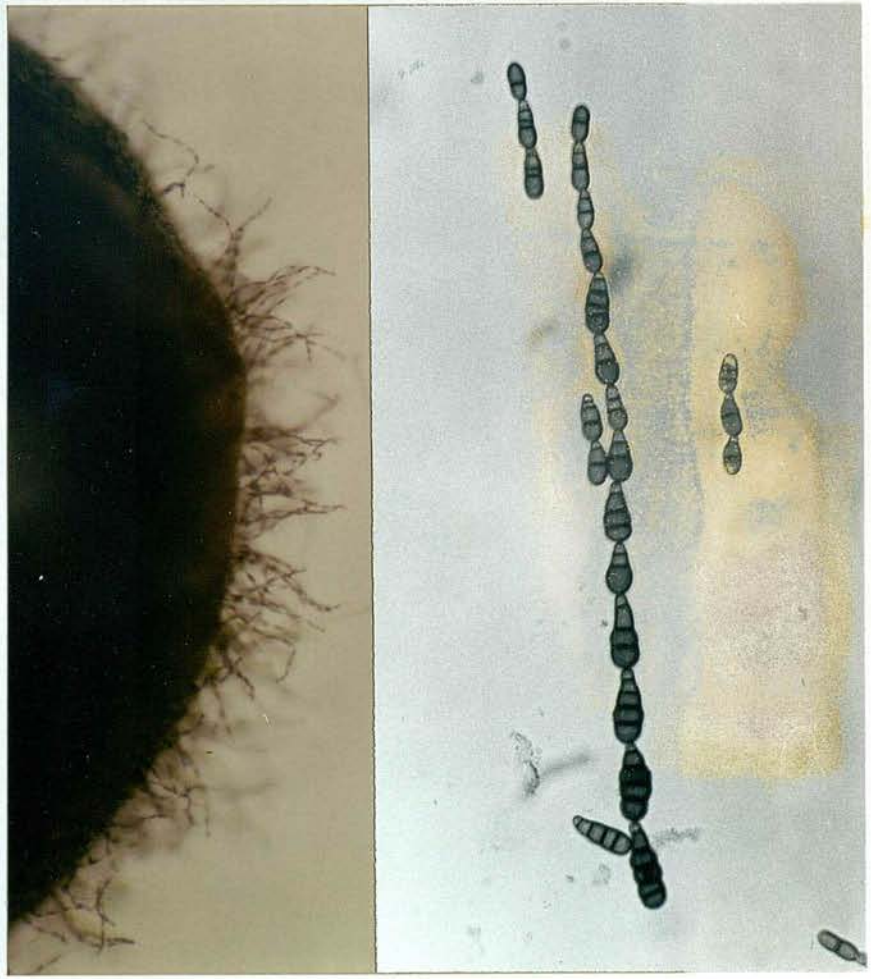


(c) *Alternaria* species from oilseed rape.

1. *A. brassicae*
2. *A. brassicicola*
3. *A. alternata*
4. *A. tenuissima*

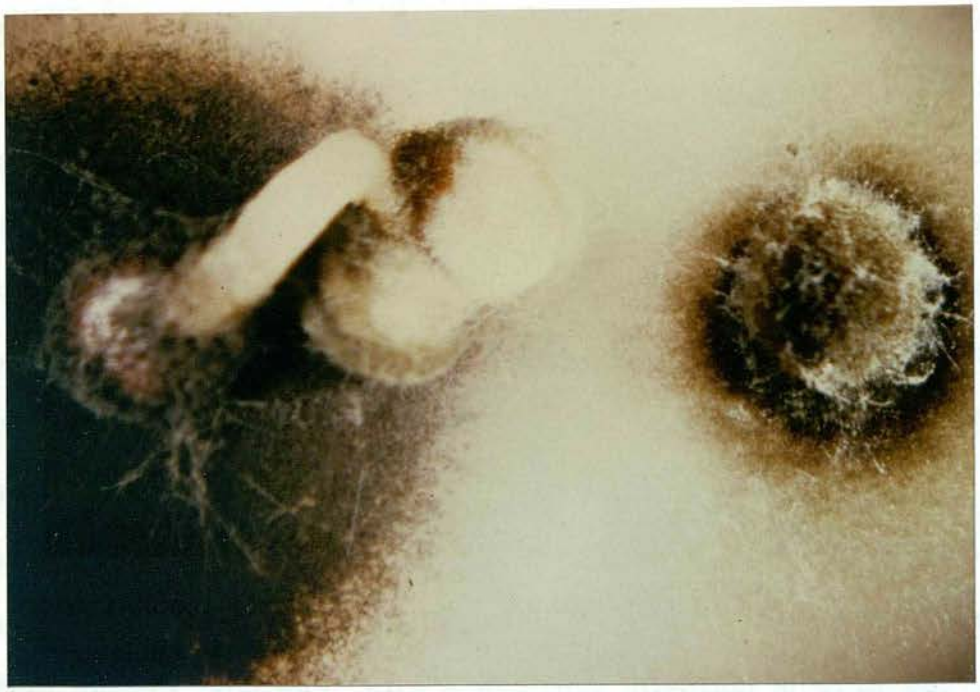
PLATE 3.1.13: Spore production by *Alternaria* species.

(a) *A. brassicae* spores produced singly or in chains up to 4.

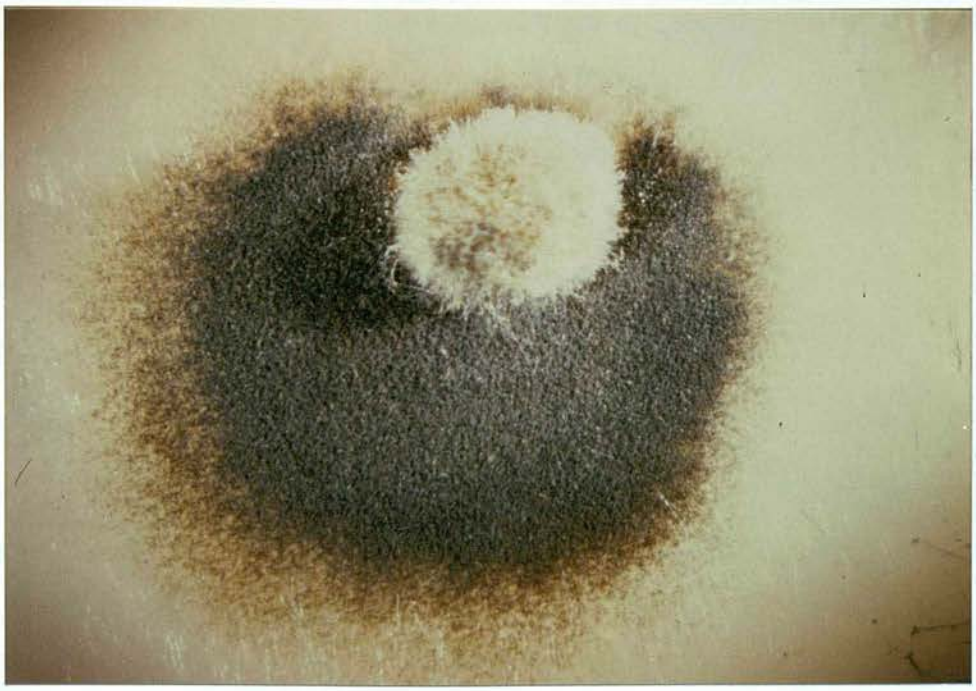


(b) *A. brassicicola* spores produced in long chains up to 25.

PLATE 3.1.14: Seed infection of *A. brassicae* and *A. brassicicola*.



(a) *A. brassicicola* (left) - seedling growth present.
A. brassicae (right) - seed dead.



(b) Mixed infection - seed dead.

Of the other *Alternaria* species, *A. alternata* was frequently found in seeds of most crops with the exception of turnip. The incidence was particularly high on oilseed rape, where 65% to 98% (from field survey) of samples were infected with infection levels up to 53%. This species is described as frequently seed-borne on a wide range of plants but is usually a saprophyte (Ellis, 1971), although it may act as weak pathogen (Neergaard, 1945) and produce leaf spots in rape (Vaartnou and Tewari, 1972). More recently, it was reported to cause heavy infection in a radish seed crop (Narayanappa, 1982). *A. raphani* was not found in seed lots of any of the crop plants except oilseed rape. This species is mainly associated with radish (McDonald, 1947), although Berkenkamp and Degenhardt (1974) have indicated its widespread, destructive nature on *B. napus* and *B. campestris* in Canada. In this survey it was found in 16-20% of oilseed rape lots, with a maximum infection level of 4%. *A. tenuissima*, a saprophytic organism (Ellis, 1971), was commonly found in seed stocks from the survey.

Phoma infection (Plate 3.1.15) was detected in seed lots of cauliflower (13%), kale (26%) and oilseed rape (26-34%) with maximum infection levels of 2, 2 and 14-17%, respectively. In many countries, *Phoma lingam* is considered as an important pathogen (Henderson, 1918; Nielson, 1932; Bontea, 1953; Lloyd, 1959; Schneider, 1960; Van Kampen, 1964; Giessmann and Daebeler, 1973; Tahvonen, 1979) and seed-borne infection is considered to be the most important source of this fungus (Pound, Cheo, Calvert and Raabe, 1951; Giessman and Daebeler, 1973). This fungus can attack all parts of the plant at any stage of development. Its distribution, however, in the present survey was much less than that of *Alternaria* pathogens.

PLATE 3.1.15: *Phoma* infection of oilseed rape seed showing presence of black pycnidia on dead seed.

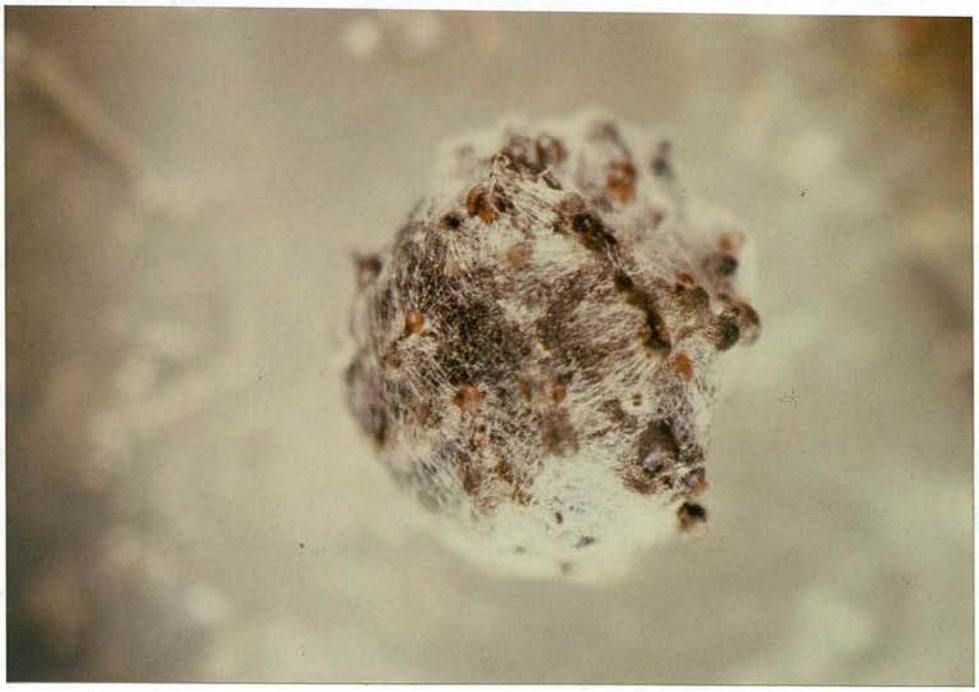


PLATE 3.1.16: *Botrytis* infection of oilseed rape seed showing characteristic conidiophores, conidia and sclerotium on dead seed.

Botrytis, notably *B. cinerea* (Plate 3.1.16), is a ubiquitous fungus, grey mould occurring on a very wide range of plants. In the present study it was found in swede (16% of samples), oilseed rape (17-45%) and kale (37%), the highest levels of infection within samples, up to 19%, occurring in field samples of oilseed rape. It is considered to be an important secondary pathogen of cruciferous oil plants, causing damage to the siliqua, although it may occur as a primary pathogen of rape (Tahvonen, 1979). It is less important as a seed-borne pathogen, and Linnasalmi (1952) has indicated that it is rarely implicated in damping-off in cabbage and cauliflower.

Although Tahvonen (1979) observed that *Fusarium* species were not mentioned in the literature as important seed-borne fungi of cruciferous plants, he found that they occurred in commercial seed lots of a wide range of cruciferous plants in Finland, apart from kale and broccoli: between 19.0 and 66% of white cabbage, cauliflower, red cabbage, Brussels sprouts, rape, radish and turnip rape lots were infected. The *Fusarium* contents of infected seed lots were generally less than 5% but in some of the white cabbage, Brussels sprouts, rape and turnip rape lots the fungus content was higher than 30%. Petrie (1974a) observed *Fusarium* infection in 42% and 78% of *B. napus* and *B. campestris* seed lots with maximum seed infection levels within lots of 0.5 and 17%, respectively. In the present study, *Fusarium* was found in cabbage, cauliflower, kale and oilseed rape (Plate 3.1.17): the percentage of infected lots range from 4% (cabbage) to 19% (cauliflower), while the maximum infection levels were 2% or less, except in oilseed rape where it was up to 7% in field samples.

According to Neergaard (1979), seed transmission of *Rhizoctonia solani* and *Sclerotinia* has been overlooked or under-estimated because of

PLATE 3.1.17: *Fusarium* infection of oilseed rape seed showing white mycelial growth and conidial ooze from dead seed.

PLATE 3.1.18: *Sclerotinia* infection of oilseed rape seed showing white mycelial growth and a dark sclerotium.

the predominance of soil-borne transmission. *Rhizoctonia* is not a commonly recognized seed-borne pathogen of plants of the Cruciferae and in this study it was confined to only about 5% of oilseed rape seed lots. *Sclerotinia* (Plate 3.1.18) was again found only on oilseed rape with a similar frequency as with *Rhizoctonia*.

A range of saprophytic organisms was found in seed samples, the more prevalent being species of *Cladosporium*, *Stemphylium* and *Penicillium* as well as *Alternaria tenuissima*.

In considering the range of contamination by fungi in seeds of different crops, oilseed rape showed the greatest range of organisms, but, as already indicated, commercial seed samples of this crop showed lower infection levels than samples taken directly from the field. This may relate in part to the use of fungicides in seed crops and possibly to a processing operation which would remove smaller seed more likely to be infected.

Seed samples imported into the UK market showed a higher incidence of *A. brassicicola*. Holtzhausen and Knox-Davies (1974) observed that the samples imported from Denmark, Japan and Italy were heavily contaminated with *A. brassicicola* and samples from Holland, USA, UK and Australia were low, possibly associated with fungicide treatment which might have depressed the pathogen counts. Where seed known to have been chemically treated was tested in this study, some infection with *A. brassicicola* was detected in all but one case. The most generally used chemical treatment was probably γ-HCH and captan for the control of flea beetle and soil-borne diseases. Petrie (1974b) reported that *A. brassicicola* was the major pathogen on imported garden cruciferous seeds and was most common in *B. oleracea* var. *capitata*.

Surface sterilization reduced *A. brassicicola* infection counts to a varying extent in most infected samples tested but in one it did not. This might be due to deep-seated infection of the seed. Petrie (1974b) observed that, following surface disinfestation of seed, a considerable amount of infection of *A. brassicicola* remained. Knox-Davies (1979) reported that *A. brassicicola* is carried over mainly in the hilum area and that this region of the seed is not readily wetted: this should be taken into account in studying seed treatment. It should also be noted that *A. brassicae* is shorter-lived in seed than *A. brassicicola*, surviving for up to 14 months compared with 7 years 8 months for *A. brassicicola* in normal storage (Neergaard, 1969).

3.2 STUDIES ON THE EFFECTS OF INOCULATION OF OILSEED RAPE PLANTS WITH *A. BRASSICAE* AND *A. BRASSICICOLA* ON DISEASE DEVELOPMENT, CROP GROWTH AND SEED YIELD

Introduction

Alternaria infection of Cruciferous plants has proved to have a potentially damaging effect on seed yield: infection on stems, branches of inflorescences and maturing seed pods have been long recognized to reduce yield and seed quality (Neergaard, 1945). Although Neergaard (1979) recognized that there were no precise reports on yield losses due to *Alternaria* on oilseed rape, Domsch (1957), from Germany, attributed rapeseed losses up to 75% to *A. brassicicola*. Downey, Klassen and McAnsh (1974), from Canada, reported rapeseed losses up to 20% due to *A. brassicae*. In further studies in Canada, Degenhardt, Skoropad and Kondra (1974) observed that *A. brassicae*, when inoculated onto 36 days old rape plants, caused a seed yield reduction of 63%. From India, Chahal and Kang (1979a) recorded a 45% yield loss due to *A. brassicae* in a naturally infected mustard crop, compared with a crop sprayed with six applications of Bordeaux mixture. In the present studies, carried out in three successive years, plants grown outside were inoculated with *A. brassicae* or *A. brassicicola* or treated with fungicide and the relative progress of disease was observed. In the first experiment, carried out in 1982, seed yield responses were also assessed in addition to seed quality, *viz.* seed viability, seed weight and level of *Alternaria* infection. In the second and third year, weather conditions giving poor plant establishment in 1983 and little disease development in 1984 accounted for yield data not being recorded. The experiments are considered under the following headings:

3.2a The effect of time of *Alternaria* inoculation and iprodione treatment of oilseed rape of different cultivars on disease development, seed yield and quality, 1982.

3.2b The effect of time of *Alternaria* inoculation and iprodione treatment of oilseed rape of different cultivars on disease development, 1983.

3.2c The effect of time of *Alternaria* inoculation and iprodione treatment of oilseed rape of different cultivars on disease development, 1984.

Materials and Methods

Experiment 3.2a

In this investigation seeds of oilseed rape cultivars, Jet Neuf (winter), Rafal (winter), Olga (spring) and Line (spring) were sown in 18 cm plastic pots on 12 February 1982 in an outside area protected by wire netting (Plate 3.2.1). The seedlings were thinned to one per pot. When the plants reached their flowering stage, pots of each cultivar were grouped into lots of 10 and different lots were inoculated with either *A. brassicae* or *A. brassicicola* at the following four stages of the reproductive phase (Harper and Berkenkamp, 1975):

Stage 3.3, lower buds on the terminal receme of the main stem starting to yellow;

Stage 4.2, many flowers opened, lower buds elongating;

Stage 4.4, flowering complete, seeds enlarging in lower pods;

Stage 5.3, seeds in lower pods showing green-brown mottle.

PLATE 3.2.1: Oilseed rape experiment, 1982, showing plants growing outside in pots in protected cage area.

Extra plants had been grown to allow the necessary number at each appropriate growing stage to be selected. The inoculum was prepared by suspending spores in sterile distilled water containing 0.1% Tween-21. The spores were obtained from 2 week-old cultures which had been incubated at 20 ± 2°C on V-8 agar under 12 hours NUV light alternating with 12 hours darkness. The spore load was adjusted to 50,000 spores/ml.

The inoculum was sprayed onto the flower buds/pods until run-off using a pressure sprayer. The 10 pots of each cultivar of each inoculation treatment were held in a glasshouse compartment fitted with an automatic misting system for 48 hours after inoculation. At the end of the 48 hours incubation the pots were returned to the outside caged area until harvest. In addition to the inoculation treatments, three fungicide treatments were also included to study the effects of iprodione (Rovral Flow) in controlling *Alternaria* disease. The first fungicide spray was given at stage 3.3, as flower buds developed, the second spray was given after the end of flowering and in a third spray treatment fungicide was applied twice, before and after flowering. Control plants in each cultivar were sprayed with water and Tween-21. The treatments for lots of ten of each cultivar are summarised below:

1. *A. brassicae* inoculated at stage 3.3
2. *A. brassicae* inoculated at stage 4.2
3. *A. brassicae* inoculated at stage 4.4
4. *A. brassicae* inoculated at stage 5.3
5. *A. brassicicola* inoculated at stage 3.3
6. *A. brassicicola* inoculated at stage 4.2
7. *A. brassicicola* inoculated at stage 4.4
8. *A. brassicicola* inoculated at stage 5.3
9. Iprodione sprayed at stage 3.3
10. Iprodione sprayed at stage 4.4
11. Iprodione sprayed at stage 3.3 and 4.4
12. Control (sprayed with water with Tween-21 0.1%)

The experiment was arranged in a randomised block lay-out with 10 replicates. A split-plot design was used with treatments forming main plots and cultivars sub-plots, each pot containing one plant representing one cultivar of one treatment. The extent of pod spotting before harvest was scored using a 0-5 pod spotting scale as described in Experiment 3.1a. The pods were harvested from each individual plant and dried at room temperature. Seeds were extracted manually and cleaned, using sieves. Seeds retained on a 1 mm sieve were collected and weighed for seed yield. The test (1000 seed) weight was determined by counting 1000 seeds using a vacuum seed counting head. Samples of 200 seeds from each plant were tested for seed infection by incubating on V-8 agar (Experiment 3.1a) and 400 seeds from each plant were assessed for seed germination in rolled paper towels (ISTA, 1976).

Experiment 3.2b

Four oilseed rape cultivars, Rafal, Jet Neuf, Olga and Line were sown on 28 March, 1983, within field blocks (24 x 14 m) replicated four times. Seeds of each cultivar were drilled in parallel plots of six rows, at random in each block. The following six treatments were applied at random to sub-plots across the drills of the plots in each block (sub-plot size, 6 x 2 m):

1. *A. brassicae* inoculated at stage 4.2
2. *A. brassicae* inoculated at stage 5.2
3. *A. brassicicola* inoculated at stage 4.2
4. *A. brassicicola* inoculated at stage 5.2
5. Iprodione sprayed at stage 4.2 and 5.2
6. Control, sprayed with water with Tween-21.

The inoculum was prepared in the same way as described in Experiment 3.2a. Polythene sheeting was used as a screen to prevent the drift of

fungal suspension or fungicide from plot to plot. The disease was scored using a pod spotting, 0-5 disease scale (Experiment 3.1a), taking 20 plants in the middle two rows in each sub-plot before harvest. Due to poor establishment, yield assessments were not carried out.

Experiment 3.2c

During 1984, two oilseed rape cultivars, Jet Neuf and Rafal were sown on 20 October, 1983, in 30 cm pots, and then thinned at the seedling stage to give one plant per pot. When the plants began flowering they were inoculated with *A. brassicae* and *A. brassicicola* or sprayed with fungicide at stages 4.2 and 5.3 according to the following 10 treatments.

1. *A. brassicae* inoculated at stage 4.2
2. *A. brassicicola* inoculated at stage 4.2
3. *A. brassicae* and *A. brassicicola* (50 : 50) inoculated at stage 4.2
4. *A. brassicae* inoculated at stage 5.3
5. *A. brassicicola* inoculated at stage 5.3
6. *A. brassicae* and *A. brassicicola* (50 : 50) inoculated at stage 5.3
7. Iprodione (Rovral Flow) sprayed at stage 4.2
8. Iprodione (Rovral Flow) sprayed at stage 5.3
9. Iprodione (Rovral Flow) sprayed at stage 4.2 and 5.3
10. Control, sprayed with water and Tween-21.

Five replicate pots for each treatment were arranged in a fully randomised block layout. In this study, plants were covered with large polythene bags just prior to spraying with spore suspension, prepared as in Experiment 3.2a, or fungicide. The bags were kept over treated plants for 48 hours after inoculation. Control plants were sprayed with water and Tween-21. Observations were recorded on disease development

using the pod spotting, 0-5 disease scale, as explained in Experiment 3.1a. Due to very little disease development, no yield assessments were made.

Results

EXPERIMENT 3.2a: The effect of time of Alternaria infection and iprodione treatment of oilseed rape of different cultivars on disease development, seed yield and quality, 1982

In the analyses of variance of the data, in the case of the pod spotting disease index, the percentage seed infection and the percentage seed germination assessments, the transformations $\sqrt{x+\frac{1}{2}}$ and angle are used for small whole numbers and percentages respectively, as recommended by Cochrane (1938). From the analyses of variance of the results, treatment relating to inoculation/fungicide had a significant effect on all variates, while significant differences between cultivars occurred in all cases except that of the level of *A. brassicae* infection of seed. There were, however, significant interactions between the two factors in most cases, the exceptions relating to disease index and yield. From the general observations made following inoculation and fungicide application, disease and crop growth responses to treatments were evident: inoculated plants tended to show obvious disease symptoms and restricted growth in contrast to those receiving fungicide treatment. The results of assessments of the effect of the various treatments on pod spotting, seed yield, test weight, seed infection and seed germination are summarized in Table 3.2.1.

The levels of *Alternaria* pod spotting were generally significantly higher in inoculated plants compared with controls, while infection was

TABLE 3.2.1: Effects of inoculation with *A. brassicae* and *A. brassicicola* and fungicide spray treatment on pod spotting, seed yield, test weight, seed infection and seed germination (mean of four cultivars).

Inoculation/fungicide treatment	Pod spotting index 0-5 scale[1]	Seed yield (g/plant)	Test weight (g)	% Seed infected[2] *A. brassicae*	% Seed infected[2] *A. brassicicola*	% Seed germinated[2]
A. brassicae at stage 3.3	1.51	15.1	3.0	23	15	66
A. brassicae at stage 4.2	1.74	14.8	3.0	30	22	67
A. brassicae at stage 4.4	1.76	11.1	3.6	29	17	59
A. brassicae at stage 5.3	1.81	9.4	3.2	32	15	67
A. brassicicola at stage 3.3	1.07	15.0	3.4	1	64	68
A. brassicicola at stage 4.2	1.02	13.1	3.7	3	72	67
A. brassicicola at stage 4.4	1.55	11.4	4.1	2	60	67
A. brassicicola at stage 5.3	1.62	10.2	3.4	3	58	71
Iprodione at stage 3.3	1.82	20.5	3.3	1	13	77
Iprodione at stage 4.4	1.68	21.9	3.5	0	10	80
Iprodione at stage 3.3 and 4.4	0.85	22.7	4.4	0	6	82
Control	1.40	17.5	3.7	5	23	76
SED± (DF = 324)	0.04	0.2	0.1	1.9	4.2	2.1

[1] Transformation $\sqrt{x+\frac{1}{2}}$ where x = disease index on a scale of 0-5.

[2] Angular transformation.

lowest in plants sprayed with iprodione. *A. brassicae* and *A. brassicicola* gave similar disease index levels. Only slight differences tended to be associated with time of inoculation, although inoculation at early flowering (Stage 3.3) gave somewhat less infection than inoculation at later stages.

Seed yield was reduced by inoculation with both *Alternaria* species compared with controls, particularly with inoculation at later times of pod development. A significant increase in seed yield, compared with controls, occurred in plants sprayed with iprodione, maximum seed yield being recorded in plants treated twice. The 1000 seed weight reflected a less consistent response to treatments: the highest test weight was obtained in plants twice sprayed with iprodione while the lowest were associated with early or late inoculation treatments with *A. brassicae* or early fungicide applications.

The percentage seed infection was greatest in plants inoculated with *A. brassicicola* and there was a lower seed infection rate from inoculation with *A. brassicae*. Some degree of cross infection occurred, particularly with *A. brassicicola*, which was seen in moderate amounts in seeds from plants inoculated with *A. brassicae* as well as controls. The incidence of cross infection with *A. brassicae* was much smaller. Complete control of seed infection by *A. brassicae* was obtained in plants sprayed late with iprodione, but some *A. brassicicola* infection occurred in seed from plants of all fungicide treatments. *A. brassicicola* seed infection was greatest when plants were inoculated at about the mid-flowering stage and declined in plants inoculated later. *A. brassicae* did not show this decline. Inoculation with both *Alternaria* species reduced the rate of seed germination compared with seed from control and fungicide treated plants. The lowest percentages of germination tended to be associated with *A. brassicae* infection.

In comparing different oilseed rape cultivars (Table 3.2.2), significant differences were seen in pod spotting, seed yield, test weight, seed infection levels of *A. brassicicola* and seed germination. Cultivars did not exhibit significant differences in levels of *A. brassicae* infection of seed. Only minor differences occurred in pod spotting, with the two winter cultivars showing slightly less disease. Seed yield was relatively low in Line compared with other cultivars and both spring cultivars showed low 1000 seed weights. Olga and Line showed higher levels of *A. brassicicola* infection and the seed germination percentage was low in Line compared with other cultivars.

With respect to the interactions between treatment and cultivar, for the test weight (Figure 3.2.1a), the average weights were similar for Jet Neuf and Rafal, but the results for Rafal were relatively low with early inoculation treatments of both fungi. In the case of seed infection with *A. brassicae* (Figure 3.2.1b), although the different cultivars showed similar levels of infection on average, Jet Neuf and Rafal gave higher incidences than Olga and Line with later inoculation treatments. Figure 3.2.1b also shows the levels of seed infection with *A. brassicicola*: the greater levels of infection associated with Olga and Line were more especially linked with the early inoculation treatments with the fungus. There was also a slight divergence in the responses of Jet Neuf and Rafal to time of inoculation treatment: Jet Neuf showed less seed infection with *A. brassicicola* from early inoculation treatment and Rafal showed less infection with inoculation at the end of flowering. Seed germination percentages were usually relatively low with Line compared with other cultivars, but this feature was less evident with late applications of fungicide and with early inoculation with *A. brassicae* (Figure 3.2.1c).

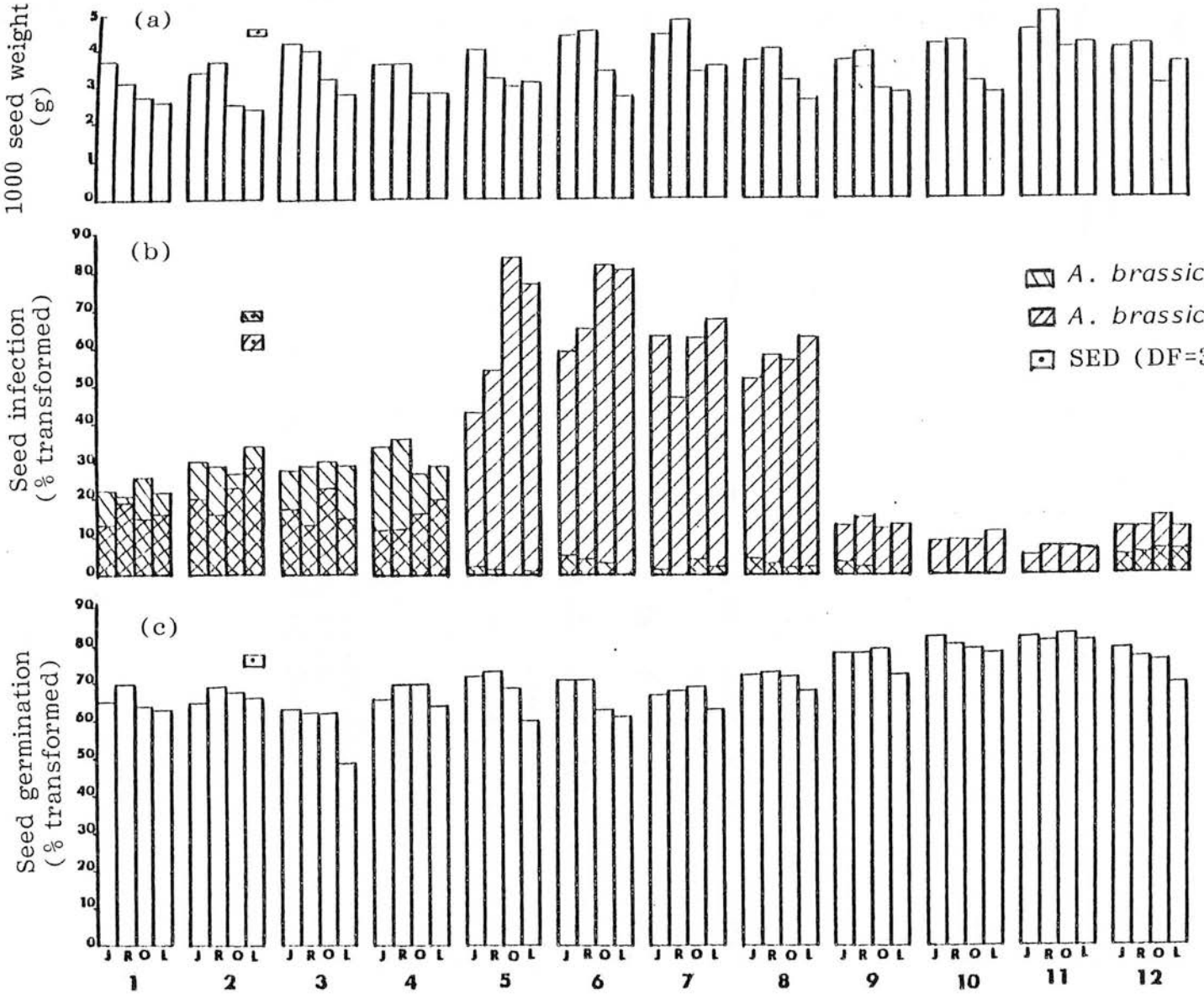
TABLE 3.2.2: Pod spotting, seed yield, test weight, seed infection and seed germination of different oilseed rape cultivars (mean of different *Alternaria* inoculation and fungicide treatments).

Cultivar	Pod spotting index 0-5 scale [1]	Seed yield (g/plant)	Test weight (g)	% Seed infection [2]		% Seed germination [2]
				A. brassicae	*A. brassicicola*	
Jet Neuf	1.46	16.2	3.9	11	27	72
Rafal	1.48	16.2	4.0	11	28	73
Olga	1.51	16.5	3.1	11	35	71
Line	1.52	12.2	3.0	10	35	66
SED± (DF = 324)	0.02	0.5	0.1	0.6	1.2	0.6

[1] Transformation $\sqrt{x+\frac{1}{2}}$ where x = disease index on a scale of 0-5.

[2] Angular transformation.

FIGURE 3.2.1: Test weight, seed infection and germination in oilseed rape cultivars inoculated with *A. brassicae* and *A. brassicicola* fungicide treatment at different times.(1982)



J Jet Neuf
R Rafal
O Olga
L Line

A. brassicae

1 at stage 3.3
2 at stage 4.2
3 at stage 4.4
4 at stage 5.3

A. brassicicola

5 at stage 3.3
6 at stage 4.2
7 at stage 4.4
8 at stage 5.3

Iprodione

9 at stage 3.3
10 at stage 4.4
11 at stage 3.3 & 4.4
12 untreated control

EXPERIMENT 3.2b: The effect of time of Alternaria inoculation and iprodione treatment of oilseed rape of different cultivars on disease development, 1983

The results of the 1983 field trial on disease development in relation to cultivar, inoculation and fungicide treatment are presented in Table 3.2.3.

TABLE 3.2.3: The effect of time of *Alternaria* inoculation and iprodione treatment of oilseed rape of different cultivars on disease development, 1983.

Treatments	Cultivars				
	Jet Neuf	Rafal	Olga	Line	Mean
	(Pod spotting index[1])				
1. *A. brassicae* at stage 4.2	0.95	0.96	0.96	0.92	0.95
2. *A. brassicae* at stage 5.2	1.02	0.93	0.92	0.97	0.96
3. *A. brassicicola* at stage 4.2	0.84	0.88	0.87	0.82	0.85
4. *A. brassicicola* at stage 5.2	0.81	0.80	0.83	0.83	0.82
5. Iprodione at stage 4.2 and 5.2	0.70	0.70	0.70	0.70	0.70
6. Control	0.72	0.75	0.77	0.76	0.75
Mean	0.84	0.84	0.84	0.83	

[1] Transformation $\sqrt{x+\frac{1}{2}}$ where x = disease index on scale 0-5.

SED treatment mean = ± 0.01 (DF=60)
cultivar mean = ± 0.01 (DF= 9)
treatment x cultivar interaction = ± 0.03 (DF=60)

Disease levels were generally low, but there were significant differences between inoculated plants compared with control plants, while fungicide sprayed plants showed no disease. Disease indices produced by *A. brassicae* were slightly greater than those produced by *A. brassicicola*. No significant differences were found between cultivars, on average, but where *A. brassicae* inoculation was carried out during pod development Jet Neuf showed slightly more infection relative to other cultivars.

EXPERIMENT 3.2c: The effect of time of Alternaria inoculation and iprodione treatment of oilseed rape of different cultivars on disease development, 1984

The results of the 1984 pot experiment to study the effect of time of *Alternaria* inoculation and fungicide treatment of oilseed rate on pod spotting are presented in Table 3.2.4. *A. brassicae* showed more distinct symptoms on inoculated plants than *A. brassicicola* but in most cases disease was at a trace level and no disease was recorded on the mixed inoculated treatments and any of the fungicide treated plants.

Discussion

From the results of the 3 years of investigations, substantial levels of disease establishment from artificial inoculation were found only in 1982. The small response to inoculation in 1983 and 1984 may be attributed to the low rainfall during the critical period of July and August, when plants were inoculated (Table 3.2.5). Moreover, in 1983 there was an open crop structure due to poor establishment, which again would discourage disease development (Neergaard, 1979). Neergaard indicated

TABLE 3.2.4: The effect of *Alternaria* infection and iprodione treatment of oilseed rape of different cultivars on disease development, 1984.

Treatment	Cultivar		Mean
	Jet Neuf	Ratal	
	(pod spotting index[1])		
A. brassicae at stage 4.2	1.18	1.18	1.18
A. brassicicola at stage 4.2	0.98	0.92	0.95
A. brassicae] (50:50) *A. brassicicola*] at stage 4.2	0.70	0.70	0.70
A. brassicae at stage 5.3	1.20	1.25	1.22
A. brassicicola at stage 5.3	1.03	1.03	1.03
A. brassicae] (50:50) *A. brassicicola*] at stage 5.3	0.70	0.70	0.70
Iprodione at stage 4.2	0.70	0.70	0.70
Iprodione at stage 5.3	0.70	0.70	0.70
Iprodione at stage 4.2 and 5.3	0.70	0.70	0.70
Control	0.86	0.86	0.86
Mean	0.86	0.86	

[1] Transformation $\sqrt{x+\frac{1}{2}}$ where x = disease index on scale 0-5.

SED treatment mean = ± 0.05 (DF=76)
cultivar mean = ± 0.02 (DF=76)
treatment x cultivar mean = ± 0.08 (DF=76)

TABLE 3.2.5: Weather data at experimental site (Bush Estate), 1982, 1983 and 1984.

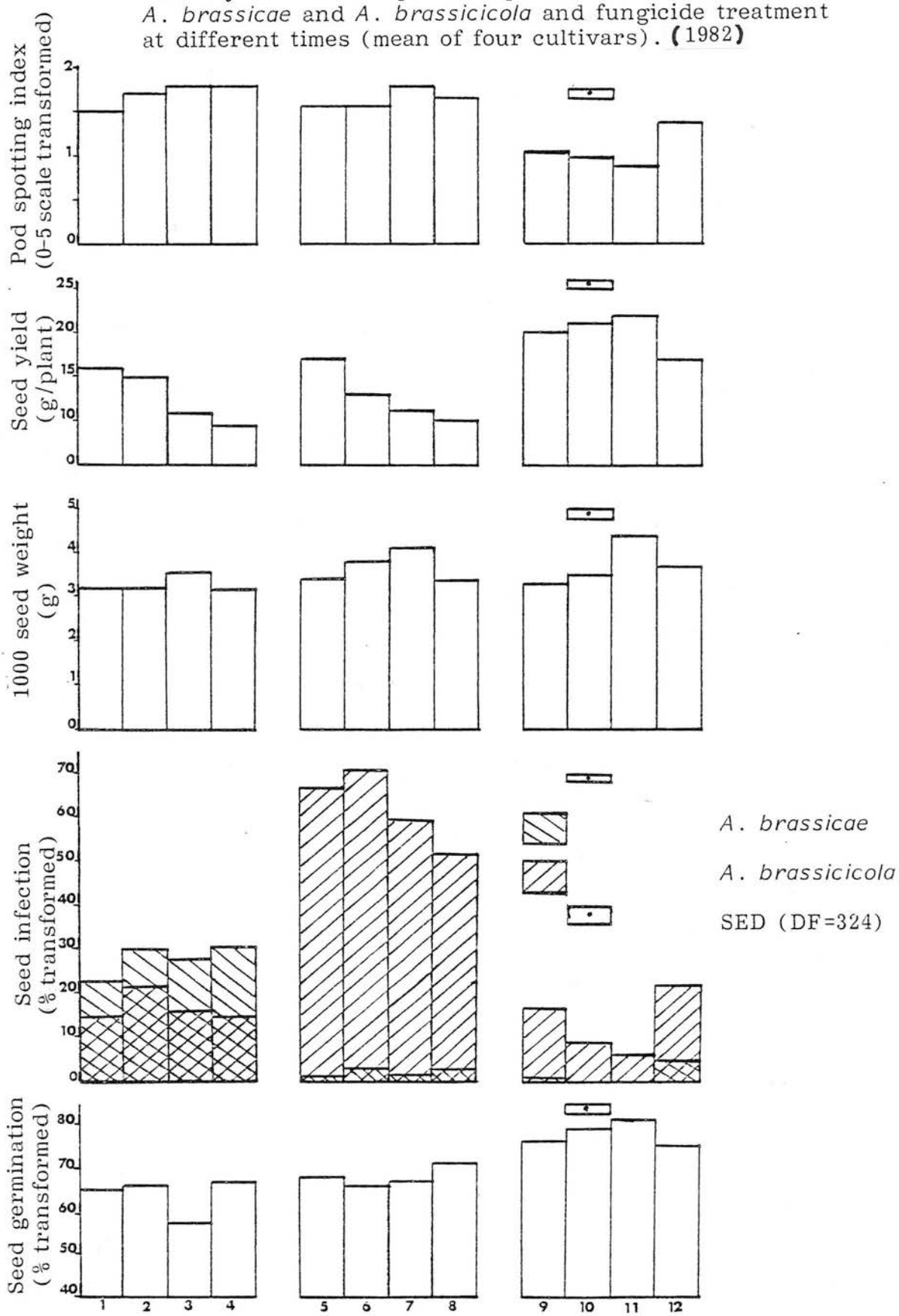
Year	Rainfall (mm)			Temperature (°C)		
	June	July	August	June	July	August
1982	136	45	62	12	14	14
1983	69	10	37	12	16	15
1984	59	17	19	9	12	15

that the development of *Alternaria* in *Brassica* seed crops depends on humidity at the flowering and seed maturation stage, while Humpherson-Jones Maude and Ainsworth (1980) observed that exceptionally wet weather during the seed maturation stage is favourable for infection. The importance of high humidity for infection has already been indicated (Section 3.1a).

In 1982, when relatively high rainfall figures were experienced over the flowering and pod setting period, inoculation with *Alternaria* induced severe pod spotting, particularly when inoculations were carried out at later growth stages (young pod stage). The higher incidence of pod spotting associated with later times of inoculation was more or less correlated with a lower seed yield (Figure 3.2.2). Part of the yield loss may be attributed to pod splitting which was associated with severe infection in this study and has been observed by several workers (Maude and Humpherson-Jones, 1980; Evans and Cox, 1982; Long, 1982).

The levels of pod spotting and yield reductions were generally similar from *A. brassicae* and *A. brassicicola* inoculation but, whereas *A. brassicae* tended to reduce 1000 seed weight relative to controls, *A. brassicicola* did not. This may be related to the more superficial

FIGURE 3.2.2: Disease severity, seed yield, test weight, health and viability in oilseed rape in response to inoculation with *A. brassicae* and *A. brassicicola* and fungicide treatment at different times (mean of four cultivars). (1982)



A. brassicae

1 at stage 3.3
2 at stage 4.2
3 at stage 4.4
4 at stage 5.3

A. brassicicola

5 at stage 3.3
6 at stage 4.2
7 at stage 4.4
8 at stage 5.3

Iprodione

9 at stage 3.3
10 at stage 4.4
11 at stage 3.3 & 4.4
12 untreated control

infection of *A. brassicicola* which, although occurring extensively, appears to cause less damage to at least more mature tissues. Flowers, which are relatively unprotected plant organs were extensively attacked by *A. brassicicola* resulting in reduced seed yields, but there may be some compensatory seed size effect relating to the reduced number of surviving flowers being subjected to less intra-plant competition and giving larger seed. *A. brassicae*, although spreading less, may be associated with a greater degree of pod damage accounting for smaller seed. This aspect concerning the relative rates of spread and degree of tissue damage caused by the two fungi is further considered later, in relation to seed infection and seed germination. The 1000 seed weight appears to be a consequence of the balance of factors relating to fungal damage to pods and the extent of intra-plant competition as determined by established pod number. The 1000 seed weight was generally less in the cultivars Olga and Line, compared with Jet Neuf and Rafal, but the interaction between cultivar and treatment suggest that the spring cultivars showed response to the application of 2 fungicidal sprays in giving an improved seed weight (Figure 3.2.1).

The greatest seed infection with *A. brassicicola*, 72%, was observed in plants inoculated with *A. brassicicola* at the flowering stages, whereas the highest seed infection with *A. brassicae*, 30-31%, occurred from plants inoculated with the fungus either at the flowering or seed maturation stage. High seed infection counts of *A. brassicicola* of 95-100% have been reported in cauliflower (Schimmer, 1953) and cabbage (Vannacci, 1981; Tahvonen, 1979). Seed infection levels reported for *A. brassicae* have tended to be lower, 17% in rape (McDonald, 1959), 18% in rape and 25% in turnip rape (Petrie, 1974a), 59% in mustard (Chahal and Kang, 1979), and 13% in cabbage (Maude and Humpherson-Jones, 1980). Seed infection

with *A. brassicae* showed little variation between cultivars, apart from the slightly greater infection levels on Jet Neuf and Ratal from late inoculations (Figure 3.2.1). On the other hand, the spring cultivars tended to show greater *A. brassicicola* infection more especially associated with early inoculation treatments (Figure 3.2.1). The greater rate of seed infection associated with *A. brassicicola* may be related to its more prolific spore production: the spores were sufficiently numerous to give a black cover over the pods (Plate 3.2.2) and accounted for a much greater amount of cross infection compared with *A. brassicae*.

The maximum reduction in seed germination occurred with *A. brassicae* when inoculated at the early pod stage (Figure 3.2.2). Chirco and Harman (1979) reported a drastic reduction in the germination of cauliflower and broccoli seeds harvested from *A. brassicicola* inoculated plants where pods were kept in polythene bags for several days after inoculation, but Richardson (1970) and Vannacci (1981) observed no influence of *A. brassicicola* on seed germination in standard germination tests. This may be due to a superficial association of fungus with seed which may not initiate severe infection on germinating seed within 6-7 days of the test period. Chahal and Kang (1979) reported that *A. brassicae* caused a severe seed rot and seedling mortality (20-52%) in mustard seeds in a rolled paper towel test. In the present investigations a general impression was that *A. brassicicola* was more prolific but less damaging than *A. brassicae*. Thus, *A. brassicicola* caused a high incidence of seed infection but seed size seemed to be less reduced than with *A. brassicae*, which also caused a greater reduction in seed germination than *A. brassicicola* (Plate 3.1.14). The lowest germination rates were found with Line (Table 3.2.2), particularly when inoculated with *A. brassicae* at the end of flowering (Figure 3.2.1). However, when late

PLATE 3.2.2: Symptoms of pod infection with *A. brassicae* (left) and *A. brassicicola* (right).
(Note black spore cover associated with *A. brassicicola*.)

application of iprodione were made the germination performance of this cultivar was improved to about the level of other cultivars.

In general, spray application of iprodione at the late flowering stage and more especially, a double spray application at early and then late flowering, reduced pod spotting and improved yield and 1000 seed weight: these treatments also reduced seed infection substantially and improved seed germination.

3.3 HOST RELATIONSHIPS OF *ALTERNARIA BRASSICAE* AND *ALTERNARIA BRASSICICOLA*

Introduction

Although *Alternaria* disease of *Brassica* crops is often applied as a general expression to describe the result of infection by *Alternaria* species, many reports suggest that *A. brassicicola* is very common on cabbage and cauliflower and less widespread on other *Brassica* crops, whereas *A. brassicae* is less common on cabbage and cauliflower, but occurs frequently on other *Brassica* hosts including *B. campestris* and *B. napus* (Neergaard, 1979). Further, *A. brassicicola* is considered to cause a more severe disease in seed crops than *A. brassicae* (Ellis, 1971). Plants with thin leaves, such as mustard, turnip and radish, have been reported to show more leaf infection with *A. brassicae* than thick-leaved plants like cabbage, collards and kale which, however, are readily infected by *A. brassicicola* (Changsri and Weber, 1963). In addition to interspecific variation in their host relationships, there is some evidence that intraspecific variation may also occur in *A. brassicae* and *A. brassicicola*: Saharan and Kadian (1983) in India distinguished three races of *A. brassicae* based on a differential host series of eight test plant species or varieties.

It has been reported that there is little evidence of a range of cultivar resistance in oilseed rape to *A. brassicicola* (Dixon, 1975) and *A. brassicae* (Tewari and Skoropad, 1976). However, some sources of resistance in cauliflower and Brussels sprouts to *A. brassicicola* have been indicated (Braverman, 1971, 1977). Moreover, there are references to some resistance in rape and mustard against *A. brassicae* (Husain and Thakur, 1963; Bhander and Maini, 1965). There are also reports that the stage of plant growth may influence host response to infection:

according to Neergaard (1979), severe damage is caused when the host plant is vulnerable either at the seedling stage or at the time of flowering.

The purpose of the first of the two experiments carried out in this section was to examine further the host range and pathogenicity of different isolates of both *A. brassicae* and *A. brassicicola* using a leaf disc inoculation technique with a wide range of plants of the Cruciferae as test hosts. The use of leaf discs maintained on benzimidazole agar was considered to form an effective way of screening a large volume of host material. Jones and Hayes (1971), in a study of *Erysiphe graminis* infection of oat leaves, observed that there was a satisfactory correlation between results by this method and infection in the field. Two different incubation temperatures were used, as the two *Alternaria* species are reported to have slightly different optimal temperature requirements for infection (Deganhardt, Petrie and Morral, 1982; Humpherson-Jones and Hocast, 1983). In a second study, the progress of infection of the *Alternaria* species on different cultivars of oilseed rape was assessed over the main growth period of plants, maintained in glasshouse compartments provided with overhead misting systems. The two experiments are considered as follows:

Experiment 3.3a: Studies on the infection response of different cruciferous plants to leaf disc inoculation with different isolates of *A. brassicae* and *A. brassicicola*.

Experiment 3.3b: Studies on the development of *A. brassicae* and *A. brassicicola* infection on different cultivars of oilseed rape.

Materials and Methods

Experiment 3.3a

Studies were undertaken to investigate the infection response of 62 cultivars of cruciferous plants, included in eight species of various vegetable, forage and oilseed, cruciferous crop plants, to leaf disc inoculation with *A. brassicae* and *A. brassicicola*, using five different isolates of each species. The source of the isolates was as follows:

A. brassicae

AO1 - cabbage leaf
AO2 - cauliflower leaf
AO3 - Brussels sprout leaf
AC4 - turnip leaf
AN5 - oilseed rape leaf

A. brassicicola

BO1 - cabbage leaf
BO2 - cauliflower leaf
BO3 - Brussels sprout seed
BC4 - turnip seed
BN5 - oilseed rape seed

The plants were raised in 12.5 cm plastic pots in Levington potting compost using one plant per pot. The plants were kept in a glasshouse provided with supplementary light and heating to give more or less uniform conditions during growth. Five plants of each cultivar were grown and sampled when 7-8 weeks old. The 6th and 7th foliage leaf were collected in a polythene bag and leaf discs, 12 mm in diameter, were cut with a sterile cork borer in a laminar flow cabinet to prevent contamination. The leaf discs were transferred to petri dishes onto 0.5% water agar containing 80 ppm benzimidazole.

Spore suspensions were prepared from cultures of each isolate grown on V-8 agar for 2 weeks with NUV light periods of 12 hours alternating with 12 hours darkness. The culture plates were washed with sterile distilled water containing 0.1% Tween-21 to provide a spore suspension, and the spore load was adjusted to approximately 50,000 spores/ml. The leaf discs were each inoculated with a 0.025 ml suspension droplet at the centre of the leaf disc using a micro pipette. The plant material was prepared and tested in two series relating to particular host groups. In the first series the various cultivars of *B. oleracea* were tested, a total of 28 belonging to six different varieties (Table 3.3.1). In the second series the remaining 34 cultivars were tested: these represented nine different species or varieties (Table 3.3.2). To each petri dish, leaf discs of cultivars inoculated with one or the other of the five isolates of each of the two pathogen species were assigned along with five control leaf discs, each receiving a droplet of sterile distilled water containing 0.1% Tween-21. Four replicates were used for each host/isolate combination at each incubation temperature, the treatments being arranged in a randomised block layout with a split-split plot design, temperature representing main plots, cultivars sub-plots and pathogen species and isolates sub-sub-plots. One set was incubated at 25°C and another set of plates at 15°C. The leaf discs were scored, using 0-5 scales, after 5 days of incubation for lesion size and 10 days of incubation for spore production (Figure 3.3.1 a-b and Plate 3.3.1a-b). The length of the incubation period, spore concentration and droplet size were standardised as the most suitable in preliminary studies.

TABLE 3.3.1: Cultivars of *B. oleracea* tested for infection response to leaf disc inoculation with *A. brassicae* and *A. brassicicola* isolates.

No.	Group	Crop	Variety	Source
1	I *B. oleracea*	Cabbage	Derby Day	
2			Marner Allfruh	
3			Langedijk 4 Decema	Elsoms Seeds Ltd
4			Bartolo	
5			Clipper	
6		Cauliflower	Perfection	
7			* Snoy River	
8			Wallaby	Samuel Yates Ltd
9			No. 1468	
10			Markanta	
11		Brussels sprouts	Widgeon	Sinclair McGill Ltd
12			Top Score	
13			Peer Gynt	Unwins Seed Co.
14			* Roodnere Early Buttons	Suttons Seeds Ltd
15			Groninger Steckma Early	Sinclair McGill Ltd
16		Savoy	Ormiskirk	
17			Ormiskirk Rearguard	
18			Aquarins	Suttons Seeds Ltd
19			Alexanders	
20			Best of All	
21		Broccoli	Late Purple	
22			* Wallham	Hurst GCT Ltd
23			Late White	
24		Kale	Rape Kale	NVRS 808
25			Pentland Brig	Suttons Seeds Ltd
26			Marrowstem Kale	
27			Thousandhead	John Dunn & Co.
28			Maris Kestrel	

*Snowy River
*Roodnerf
*Waltham

TABLE 3.3.2: Cultivars of *B. campestris*, *B. napus* and other members of Cruciferae tested for infection response to leaf disc inoculation with *A. brassicae* and *A. brassicicola* isolates.

No.	Group		Crop	Variety	Source
1	II	*B. campestris*	Turnips	Stubble turnip	John Dunn & Co.
2				Yellow Tankard	John Dunn & Co.
3				The Wallace City	NVRS
4				Vobra	NVRS
5				Wallace	NVRS
6	III	*B. napus*	Swedes	Bronze Top	John Dunn & Co.
7				Green Top	John Dunn & Co.
8				Purple Top	John Dunn & Co.
9				Ruta Otofte	NVRS
10				Doon Major	NVRS
11		*B. napus*	Oilseed rape	Jet Neuf	[1] NAIB
12				Rafal	NAIB
13				Olga	NAIB
14				Line	NAIB
15				Bienvenu	NAIB
16				Quinta	NAIB
17				Lingot	NAIB
18				Korina	NAIB
19	IV	[2] *B. Juncea*	Brown mustard	Stroke - A	NAIB
20				Brown Mustard - B	W. McNair Seeds Ltd
21				Newton - C	NAIB
22	V	*B. nigra*	Black mustard	Trowse - A	NAIB
23				Black Mustard - B	W. McNair Seeds Ltd
24				Black Mustard - C	Colmans Seeds Ltd
25	VI	*S. alba*	White mustard	White Mustard - A	W. McNair Seeds Ltd
26				Bixley - B	NAIB
27				White Mustard - C	Suttons Seeds Ltd
28	VII	*B. campestris (oleifera)*	Turnip rape	Turnip rape	
29	VIII	*Raphanobrassica*	Raphanobrassica	RB 25/8/B	SCRI
30				RB 35/15/B	SCRI
31				RB 35/2/B	SCRI
32	X	*Raphanus sativus*	Fodder radish	Crail - A	SCRI
33				Slobolt - B	SCRI
34				Nerys - C	SCRI

[1] for NAIB read NIAB [2] *B. Juncea = B. juncea*

FIGURE 3.3.1: Disease assessment scales (0-5) for leaf disc inoculation studies.

(a) Leaf lesion development (Plate 3.3.1)

0 = No visible symptoms

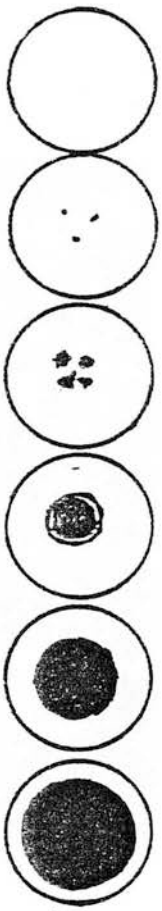
1 = Scattered small specks

2 = Distinct small specks or spots which may tend to merge

3 = Merged specks or spots affecting one-quarter of area

4 = Distinct large spot with yellow halo affecting one-half of area

5 = Large spot affecting more than three-quarters of area



(b) Sporulation

0 = No sporulation

1 = Very few scattered spores

2 = Spores produced over one-quarter of infected area

3 = Spores scattered in this layer extensively over infected area

4 = Disc fully covered with thin layer of spores

5 = Disc fully covered with thick layer of spores

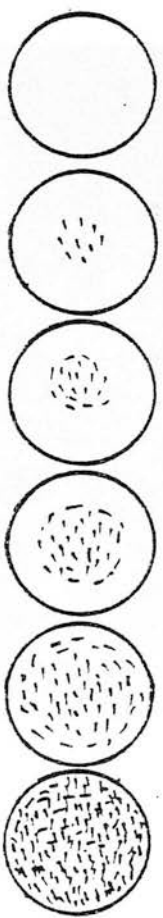
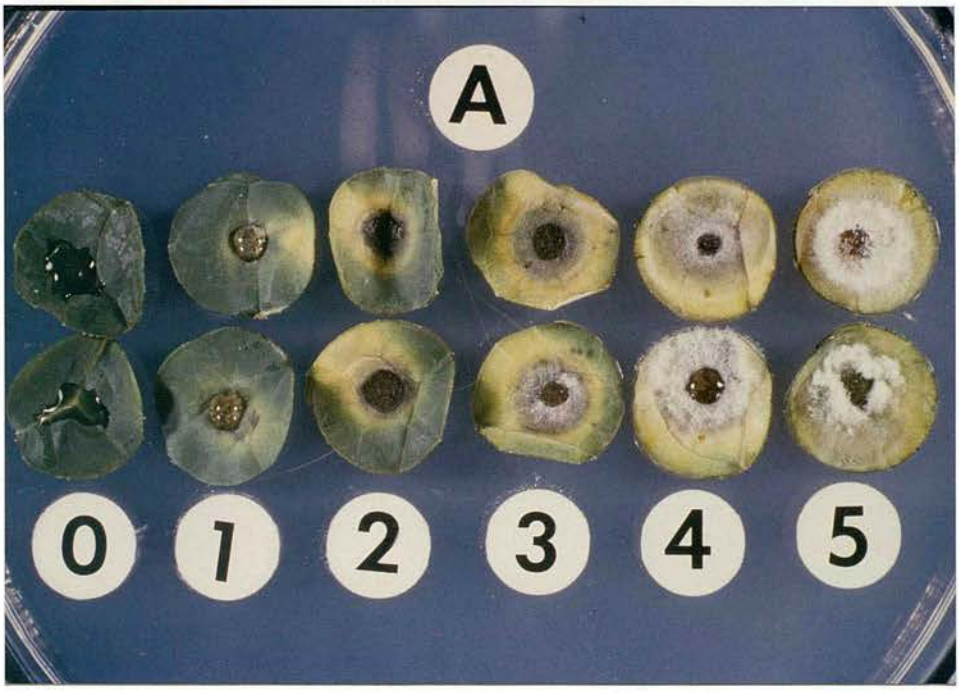
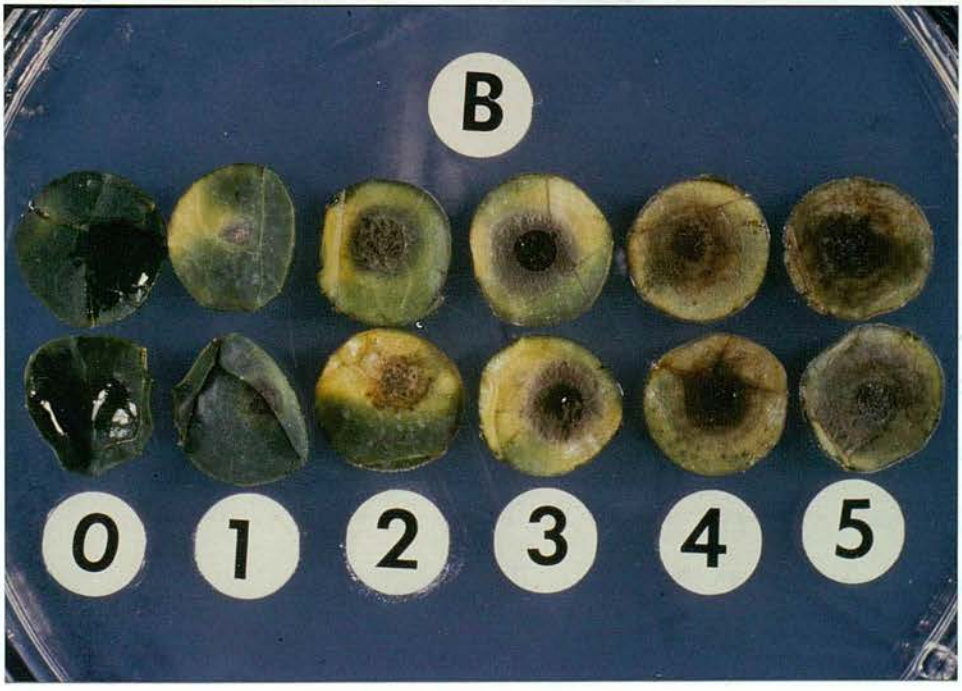


PLATE 3.3.1a: *Alternaria* leaf lesion development (0-5 scale) on oilseed rape leaf disc incubated for 10 days, on benzimidazole agar.



(a) *A. brassicae*



(b) *A. brassicicola*

Experiment 3.3b

Nineteen different cultivars of oilseed rape (Table 3.3.3) were inoculated with *Alternaria* in two separate glasshouse compartments, each fitted with an automatic misting system to maintain wet leaves and a humid atmosphere. One compartment was used for *A. brassicae* and

TABLE 3.3.3: Oilseed rape cultivars tested for the resistance to *Alternaria* inoculation at stage 3.0 until seed maturation.

No.	Variety	Source
1	Brutor	* NAIB
2	Christa	NAIB
3	Duplo	West Germany
4	Elvera	NAIB
5	Fiona	NAIB
6	Fido	NSDO
7	Gulliver	NAIB
8	Garant	NAIB
9	Jet Neuf	NAIB
10	Line	Denmark
11	Loras	NAIB
12	Lingot	NAIB
13	Mary	NAIB
14	Norli	NAIB
15	Olga	Sweden
26	Primor	NAIB
17	Quinta	NAIB
18	Rafal	NAIB
19	Willi	NAIB

the second for *A. brassicicola*. Individual plants of the 19 cultivars were grown in 18 cm pots. The plants were arranged in a randomised block layout with 10 replicates in each compartment and were inoculated at growth stage 3.0 with a spore suspension of *A. brassicae* or *A. brassicicola* isolated from oilseed rape. The spore suspensions, prepared in the same way as previously described (Experiment 3.2a), were sprayed

*should read NIAB

onto the plants to run-off. Observations were made on disease development and plants were scored 30 days after inoculation and later, at growth stage 4.2, for leaf spotting and finally before harvest, at growth stage 5.3, for pod spotting using disease scales described earlier for Experiment 3.1a. The pods were harvested from each plant, dried in the shade and the seeds were extracted manually. Seed yield was recorded from each plant. The seeds from the 10 replicates in each cultivar were bulked and samples taken for seed germination tests (ISTA, 1976) and seed health tests (Experiment 3.1).

Results

Experiment 3.3a: Studies on the infection response of different cruciferous plants to leaf disc inoculation with different isolates of A. brassicae and A. brassicicola

In both investigations, greater leaf spotting and sporulation indices were found on average at the higher incubation temperature of 25°C, compared with 15°C, and with *A. brassicicola*, compared with *A. brassicae*, inoculation treatments (Tables 3.3.4 and 3.3.5). In the first investigation, *A. brassicae* tended to show a greater response to increased temperature while, in the second, *A. brassicicola* showed the greater response. With respect to the different isolates of *A. brassicae*, in the first experiment isolate AO1 showed on average significantly smaller lesions and colonies than other isolates: AC4 tended to show, also, low indices, although this feature was more associated with low temperature incubation. In the second experiment isolate AO1 again ranked low in the order of disease development indices, but isolate AC4 was generally relatively high in order of ranking. With *A. brassicicola*, isolate BO2 was lowest in order of ranking in the first investigation and

TABLE 3.3.4: The effect of temperature, pathogen and isolate on the leaf spotting index (scale 0-5) following leaf disc inoculation with *A. brassicae* and *A. brassicicola*.

Investigation i (averaged for 28 cultivars of B. oleracea)

	Pathogen					
	A. brassicae			*A. brassicicola*		
Isolate	15°C	25°C	Mean	Isolate	15°C	25°C	Mean
AO1	0.68	1.73	1.21	BO1	1.46	2.43	1.95
AO2	0.71	2.36	1.53	BO2	1.39	2.25	1.82
AO3	0.88	2.22	1.55	BO3	1.49	2.54	2.01
AC4	0.67	2.24	1.46	BC4	1.62	2.50	2.06
AN5	1.05	2.16	1.60	BN5	1.94	2.53	2.23
Mean	0.80	2.14	1.47		1.68	2.45	2.01

SED (DF=1512)		
	Pathogen mean	± 0.03
	Isolate mean	± 0.06
	Temperature x pathogen mean	± 0.05
	Temperature x isolate mean	± 0.09

Investigation ii (averaged for 34 cultivars in the Cruciferae)

	Pathogen					
	A. brassicae			*A. brassicicola*		
Isolate	15°C	25°C	Mean	Isolate	15°C	25°C	Mean
AO1	1.63	2.39	2.01	BO1	2.07	3.35	2.71
AO2	1.83	2.68	2.25	BO2	2.02	3.24	2.63
AO3	1.83	2.68	2.26	BO3	2.11	3.32	2.71
AC4	1.88	2.71	2.29	BC4	1.86	3.29	2.58
AN5	1.79	2.60	2.19	BN5	2.10	3.33	2.71
Mean	1.79	2.61	2.20		2.03	3.31	2.67

SED (DF=1836)		
	Pathogen mean	± 0.02
	Isolate mean	± 0.04
	Temperature x pathogen mean	± 0.03
	Temperature x isolate mean	± 0.06

TABLE 3.3.5: The effect of temperature, pathogen and isolate on the sporulation index (scale 0-5) following leaf disc inoculation with *A. brassicae* and *A. brassicicola*.

Investigation i (averaged for 28 cultivars of B. oleracea)

	Pathogens					
	A. brassicae			*A. brassicicola*		
Isolate	15°C	25°C	Mean	Isolate	15°C	25°C	Mean
AO1	0.16	0.94	0.55	BO1	0.30	2.89	1.29
AO2	0.27	1.80	1.05	BO2	0.42	1.88	1.15
AO3	0.26	1.60	0.93	BO3	0.51	2.20	1.35
AC4	0.19	1.47	0.83	BC4	0.48	2.30	1.39
AN5	0.28	1.67	0.97	BN5	0.73	2.50	1.62
Mean	0.23	1.50	0.87		0.49	2.23	1.36

SED (DF=1512)	Pathogen mean	± 0.03
	Isolate mean	± 0.08
	Temperature x pathogen mean	± 0.06
	Temperature x isolate mean	± 0.11

Investigation ii (averaged for 34 cultivars in the Cruciferae)

	Pathogens					
	A. brassicae			*A. brassicicola*		
Isolate	15°C	25°C	Mean	Isolate	15°C	25°C	Mean
AO1	1.28	1.82	1.55	BO1	1.92	2.73	2.32
AO2	1.57	1.82	1.69	BO2	1.72	2.65	2.19
AO3	1.35	1.71	1.53	BO3	1.99	2.68	2.33
AC4	1.46	1.73	1.59	BC4	1.81	2.63	2.22
AN5	1.49	1.88	1.68	BN5	2.05	2.68	2.37
Mean	1.43	1.79	1.61		1.90	2.67	2.29

SED (DF=1836)	Pathogen mean	± 0.02
	Isolate mean	± 0.04
	Temperature x pathogen mean	± 0.05
	Temperature x isolate mean	± 0.07
	Except when comparing means of same level of temperature	± 0.06

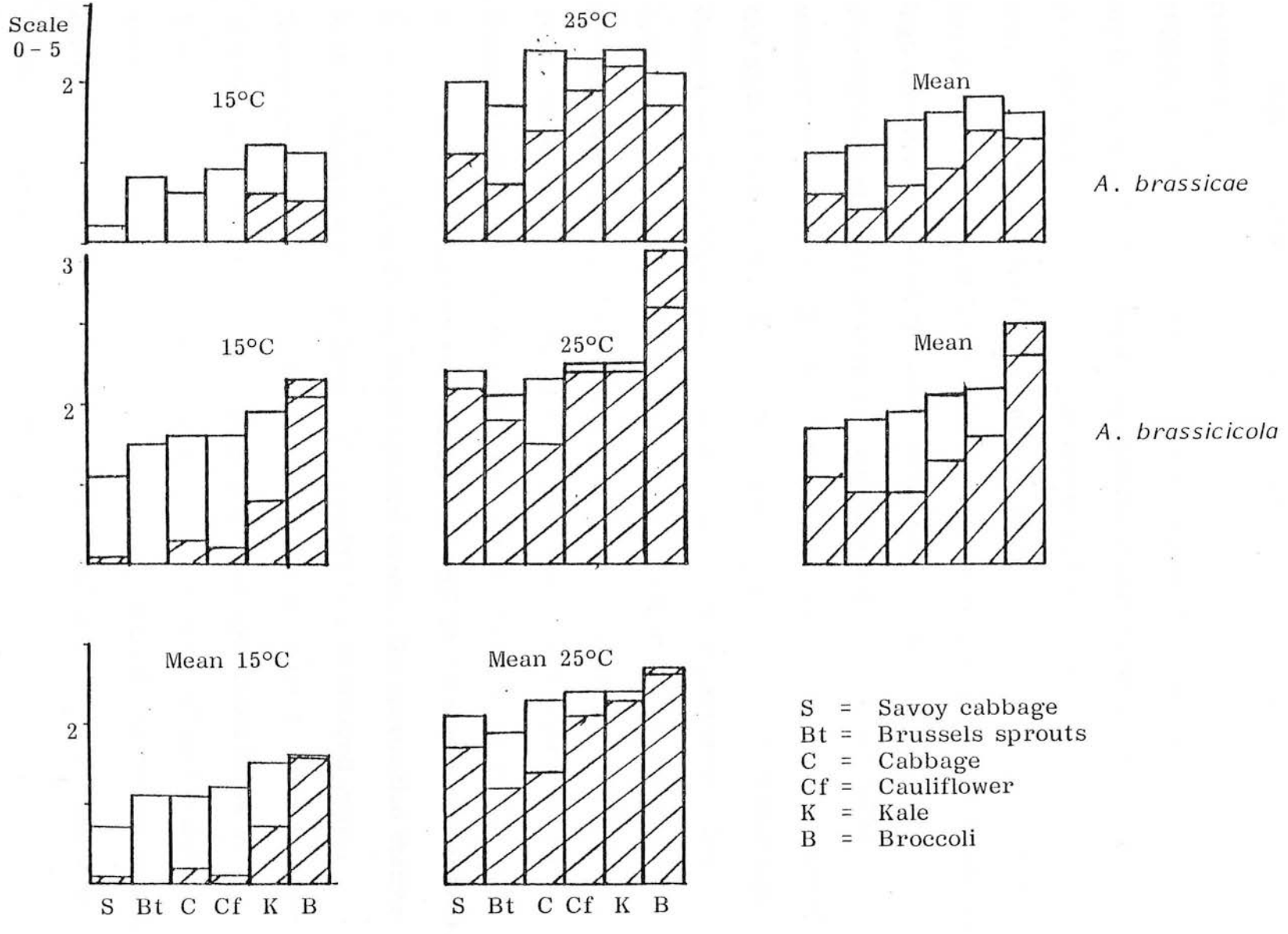
isolate BN5 the highest: in the second study, isolate BO2 again tended to be low, along with isolate BC4, but BN5 showed no significant increases over BO1 and BO3.

With the first experiment, when various groups of cultivars of *B. oleracea* were tested, the results of assessments are summarised in Table 3.3.6 averaged for the two incubation temperatures. It may be seen from Figure 3.3.2 that, for the various groups of cultivars, they behaved to some extent in a similar way in relation to pathogen and temperature: savoy cultivars tended to show the lowest levels of infection and cultivars of broccoli the highest. However, at the higher inoculation temperatures Brussels sprout cultivars tended to give the lowest levels of infection and with *A. brassicae* there was generally little difference between the groups of cultivars of cabbage, cauliflower, kale and broccoli. Broccoli tended to show relatively high sporulation indices. Cultivars notable for their low indices of leaf spotting with *A. brassicae* (<1) included Best of All, Aquarins (savoy) and Peer Gynt (Brussels sprouts). In these instances the sporulation index was 0 or only a trace (0.13 in case of Aquarins). With *A. brassicae* infection, the only cultivars showing indices for leaf spotting over 2 were Pentland Brig (kale) and Late White (broccoli). With *A. brassicicola*, infection indices were, on average, higher and the only two cultivars with leaf spotting indices of 1.5 or below were Best of All and Aquarins (savoy): the sporulation indices were 0.45 and 0.53, respectively. At least one cultivar in each group showed a leaf spot index above 2 and most or all cultivars of cauliflower, kale and broccoli were above 2: with Late White (broccoli) the index was above 3. Sporulation indices were notably high in Broccoli cultivars.

TABLE 3.3.6: Leaf spotting index and sporulation index (scale 0-5) of different cultivars of *B. oleracea* following leaf disc inoculation with *A. brassicae* and *A. brassicicola* (means of two incubation temperatures and five isolates of each pathogen).

Cultivar	*A. brassicae*		*A. brassicicola*	
	Lesion index	Sporulation index	Lesion index	Sporulation index
Savoy				
Best of All	0.68	0.00	1.20	0.45
Aquarins	0.78	0.13	1.50	0.53
Alexanders	1.10	0.63	1.85	1.83
Ormiskirk Rearguard	1.18	0.73	2.08	1.48
Ormiskirk	1.70	1.55	1.98	1.40
Brussels sprouts				
Peer Gynt	0.65	0.00	1.63	0.73
Groninger Steckma Early	1.05	0.18	1.60	0.60
Widgeon	1.48	0.73	1.60	0.80
Top Score	1.40	0.03	1.90	0.93
Roodnerf Early Buttons	1.65	0.88	2.30	1.45
Cabbage				
Bartolo	1.18	0.05	1.40	0.18
Langedijk 4 Decema	1.43	0.95	1.70	0.60
Clipper	1.65	1.23	1.80	1.00
Marner Allfruh	1.55	0.58	2.30	1.13
Derby Day	1.85	0.83	2.45	1.43
Cauliflower				
Snowy River	1.18	0.38	1.58	0.83
Perfection	1.45	1.10	2.25	1.38
No. 1468	1.60	0.80	2.13	1.23
Wallaby	1.90	1.18	2.13	1.30
Markanta	1.88	1.28	2.30	1.75
Kale				
Marrowstem kale	1.45	1.08	2.25	1.80
Maris Kestrel	1.53	1.13	2.18	1.65
Rape kale	1.93	1.65	1.93	1.63
Thousandhead	1.73	1.53	2.18	1.50
Pentland Brig	2.45	1.70	2.30	1.60
Broccoli				
Waltham	1.35	1.43	2.43	3.43
Late Purple	1.38	1.15	2.45	2.73
Late White	2.03	1.45	3.05	2.90
SED (DF=1512) ±	0.18	0.24	0.18	0.24

FIGURE 3.3.2: *Mean leaf spot and sporulation indices of different groups of B. oleracea following leaf disc inoculation with A. brassicae and A. brassicicola and incubations at 15°C or 25°C (averaged for 5 isolates of each pathogen or for cultivars within groups).*



S = Savoy cabbage
Bt = Brussels sprouts
C = Cabbage
Cf = Cauliflower
K = Kale
B = Broccoli

With the second investigation (Table 3.3.7), the groups of cultivars again tended to show similar responses to both pathogens (Figure 3.3.3), with the exception of swede which showed a lower ranking in response to *A. brassicicola* relative to other groups than it did in response to *A. brassicae*, while turnip was lower in order of ranking with *A. brassicicola* than it was with *A. brassicae* for leaf spotting index, but not for sporulation index. Moreover, turnip tended to show relatively high infection indices at the lower incubation temperature. Groups showing high infection indices as a characteristic with *A. brassicae* included oilseed rape and brown mustard, while black and brown mustard and oilseed rape showed high indices with *A. brassicicola*. Oilseed rape showed relatively high sporulation indices with both pathogens. Leaf spot indices were generally higher than in the first study and relatively few cultivars showed a leaf spot index below 2. In the case of *A. brassicae*, those with a leaf spot index below 2 were The Wallace City and Wallace (turnip), White Mustard-C and Bixley-B (white mustard), all the cultivars of fodder radish, turnip rape, RB/25/8B (*Raphanobrassica*), BLMC (black mustard) and Rafal (oilseed rape): the sporulation indices in these instances ranged from 0.55 (turnip) to 2.48 (oilseed rape). Newton (brown mustard) showed a leaf spot index over 3. With *A. brassicicola*, only two cultivars of turnip had a leaf spot index below 2, The Wallace City and Wallace. Several cultivars had leaf spot indices above 3, namely RB/35/2B (*Raphanobrassica*), BLM-B and Trowse (black mustard), Bienvenu, Line, Korina, Quinta and Olga (oilseed rape) and Stroke and Newton (brown mustard).

The response of isolates to the different groups of cultivars are illustrated in Figures 3.3.4 and 3.3.5. In the first study, with *A. brassicae*, AO1 was generally lowest with all groups of *B. oleracea*

TABLE 3.3.7: Leaf spotting index and sporulation index (scale 0-5) of different cultivars of cruciferous hosts following leaf disc inoculation with *A. brassicae* and *A. brassicicola* (mean of two incubation temperatures and five isolates of each pathogen).

Cultivar	*A. brassicae*		*A. brassicicola*	
	Lesion index	Sporulation index	Lesion index	Sporulation index
Turnip				
The Wallace City	1.73	0.78	1.90	1.20
Wallace	1.80	0.55	1.93	1.05
Yellow Tankard	2.10	1.00	2.20	1.40
Vobra	2.20	1.05	2.13	1.58
Stubble turnip	2.58	0.70	2.38	1.23
White mustard				
White mustard - C	1.80	0.88	2.30	1.88
Bixley - B	1.93	0.85	2.38	1.88
White mustard - A	2.00	0.78	2.60	2.28
Fodder radish				
Slobolt - B	1.75	1.33	2.45	2.03
Nerys - C	1.70	1.35	2.65	2.55
Crail - A	1.98	1.60	2.58	2.48
Turnip rape				
Turnip rape	1.68	1.55	2.73	2.48
Raphanobrassica				
RB25/8/B	1.50	1.55	2.20	2.18
RB35/15/B	2.00	1.83	2.53	2.53
RB35/2/B	2.00	1.93	3.15	3.10
Swedes				
Purple Top	2.05	1.03	2.25	1.48
Bronze Top	2.30	1.50	2.20	1.35
Ruta Otofte	2.35	1.38	2.23	1.78
Green Top	2.30	1.93	2.55	1.88
Doon Major	2.70	1.98	2.20	1.78
Black mustard				
Black mustard - C	1.68	0.78	2.90	2.00
Black mustard - B	2.38	1.03	3.18	2.60
Trowse - A	2.40	1.38	3.25	2.35
Oilseed rape				
Rafal	1.98	2.48	2.35	3.03
Jet Neuf	2.48	2.95	2.80	3.35
Bienvenu	2.28	2.63	3.03	3.18
Lingot	2.43	2.70	2.88	2.83
Line	2.55	3.05	3.20	3.48
Korina	2.48	2.73	3.45	3.38
Quinta	2.53	2.83	3.53	3.50
Olga	2.83	2.85	3.30	3.48
Brown mustard				
Brown mustard - B	2.45	1.35	2.75	2.25
Stroke - A	2.78	1.50	3.20	2.68
Newton - C	3.20	1.00	3.45	2.18
SED (DF=1836)	0.15	0.16	0.15	0.16

FIGURE 3.3.3: *Mean leaf spot and sporulation indices of different groups of cruciferous species following leaf disc inoculation with A. brassicae and A. brassicicola and incubated at 15°C or 25°C (average for 5 isolates of each pathogen and for* cultivars within groups).

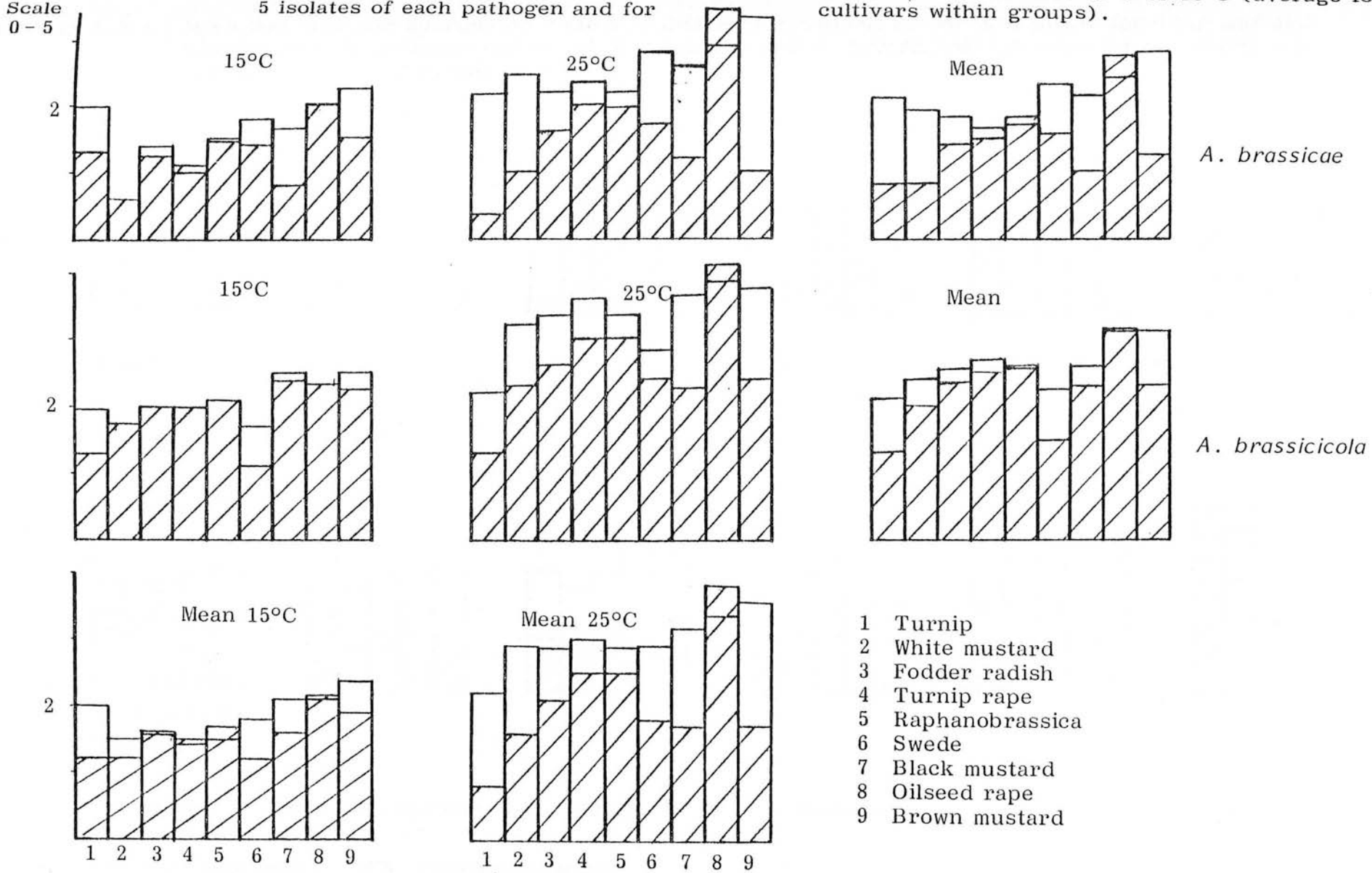


FIGURE 3.3.4: Mean leaf spot and sporulation indices of different groups of *B. oleracea* plants following leaf disc inoculation with different isolates of *A. brassicae* and *A. brassicicola* (average for incubation temperature and cultivars within groups).

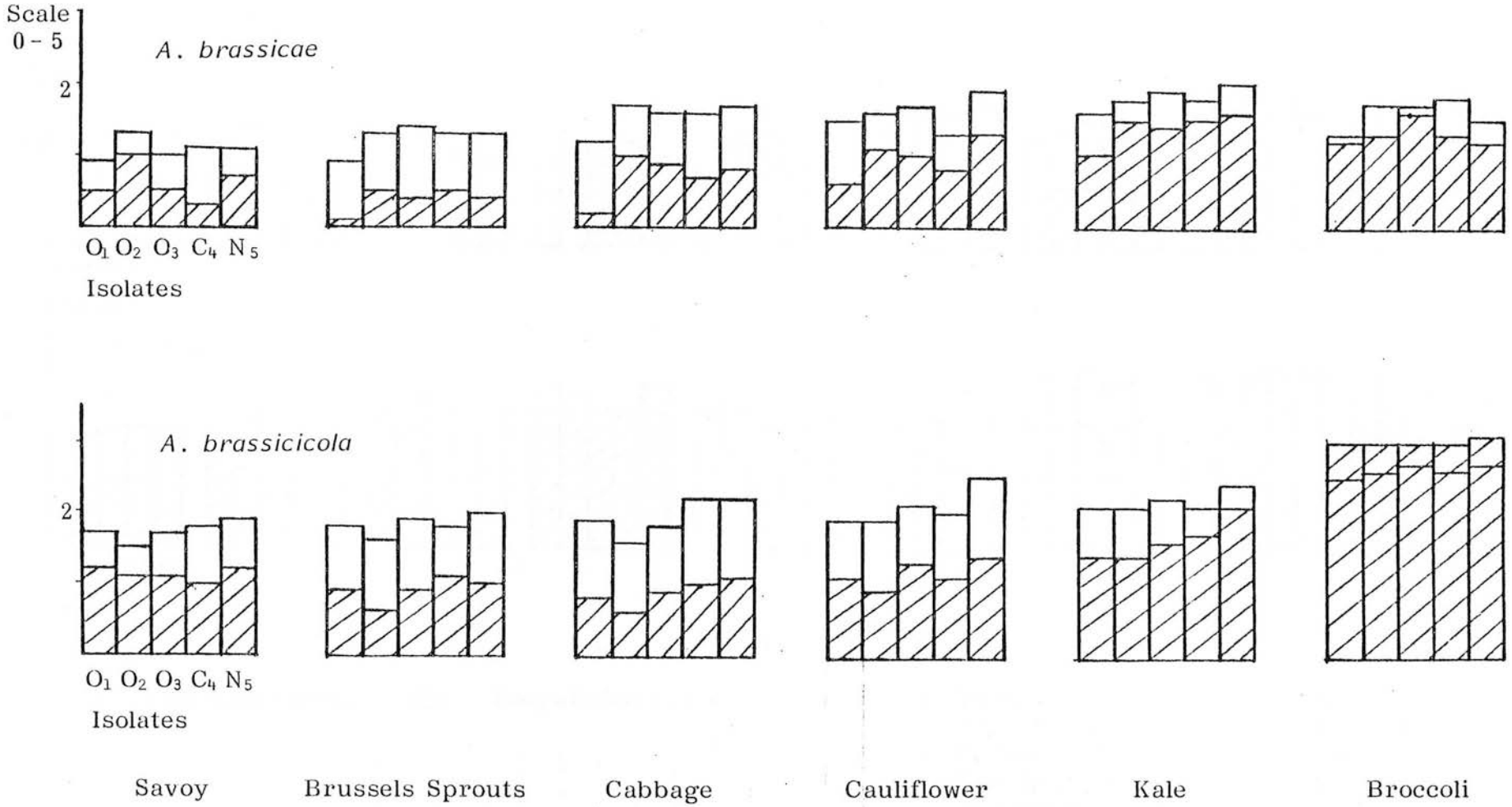


FIGURE 3.3.5: Mean leaf spot and sporulation indices of different groups of cruciferous plants following leaf disc inoculation with different isolates of *A. brassicae* and *A. brassicicola* (averaged for incubation temperatures and cultivars within groups).

and in the second study showed relatively low indices with radish, turnip, rape, Raphanobrassica, swede and white, black and brown mustard, but not with turnip and oilseed rape. With *A. brassicicola*, isolate BO2 tended to be low with most of the groups of *B. oleracea*, but in the second investigation the behaviour of isolates was less consistent: BC4 gave low disease indices with fodder radish and turnip rape, while BO1 and BN5 gave higher indices with turnip rape. However, in all cases with isolates, the range of variation was small.

The behaviour of some of the groups are illustrated in Plates 3.3.2 and 3.3.3 using material from the preliminary studies, allowing some general observations to be made on infection 10 days after inoculation. It may be seen that leaf discs tended to yellow more rapidly at the higher temperature, but that in some groups yellowing is also associated in particular with *Alternaria* infection. However, leaf discs of some groups, notably turnips, appeared to yellow independent of infection. The mustards and turnip rape seemed to be characterised by green island effects.

Experiment 3.3b: Studies on the development of A. brassicae and A. brassicicola on different cultivars of oilseed rape

The infection response of the 19 oilseed rape cultivars to inoculation with *A. brassicae* and *A. brassicicola*, subsequent disease development and transfer at progressive growth stages, along with seed yields and seed viability, are summarised in Tables 3.3.8 and 3.3.9. It may be seen that, although the data for the two pathogens are not directly comparable, the general levels of leaf spotting at growth stages 3.0 and 4.2 seemed higher with *A. brassicae* than with *A. brassicicola*, levels of pod spotting were generally similar for both pathogens, while the

PLATE 3.3.2: *Alternaria* infection on leaf discs of cruciferous crop plants incubated at 15°C or 25°C.

(a) Cabbage, cauliflower, Brussels sprouts.

(b) Broccoli, raphanobrassica, fodder radish.

A = *A. brassicae* (five isolates arranged in duplicate rows)
B = *A. brassicicola* " " " " "
C = uninoculated

PLATE 3.3.3

(a) Swede, turnip, oilseed rape.

(b) White / black / brown mustard, turnip rape.

TABLE 3.3.8: The effect of *A. brassicae* inoculation of 19 oilseed rape cultivars on leaf spot, pod spot, seed yield, seed infection and seed germination.

	Leaf spot (i) scale 0-5[1]	Leaf spot (ii) scale 0-5[1]	Pod spot scale 0-5[1]	Seed yield g	Seed infection %[1]	Seed germination %[1]
Brutor	1.6	2.1	1.9	2.0	42.7	56.7
Christa	1.6	2.0	2.0	0.7	35.3	62.1
Duplo	1.5	1.9	3.1	0.6	31.2	61.8
Elevra	1.5	1.9	1.5	2.2	30.7	67.5
Fiona	1.5	1.8	1.6	3.8	35.3	66.5
Fido	1.5	2.0	1.7	0.8	46.4	52.0
Gulliver	1.6	2.2	1.8	0.5	42.4	57.3
Garant	1.7	2.0	1.8	5.5	13.3	69.0
Jet Neuf	1.6	2.0	1.8	3.5	34.6	60.9
Line	1.5	2.0	2.1	0.6	33.8	48.2
Loras	1.7	2.1	1.9	0.7	41.0	50.3
Lingot	1.6	1.9	1.7	5.6	40.7	61.4
Mary	1.5	2.0	1.8	0.5	35.8	57.3
Norli	1.6	2.0	1.4	3.9	15.0	71.6
Olga	1.6	2.0	2.2	0.8	38.3	57.7
Primor	1.5	2.0	1.5	0.9	28.9	67.9
Quinta	1.5	1.9	1.6	7.3	33.5	63.8
Rafal	1.4	1.8	1.6	2.3	32.2	66.1
Willi	1.6	2.0	1.9	0.8	39.8	59.8
SED±	0.09	0.13	0.22	1.05	3.16	1.84
		(DF = 162)			(DF = 54)	

[1] transformed values.

amount of seed infection was greater with *A. brassicicola* compared with *A. brassicae*.

The symptoms of *A. brassicae* on oilseed rape plants appeared on lower and middle leaves one week after inoculation as small, circular, sunken, dark brown to black points less than 0.5 mm in diameter. There was some change in colour as the spots enlarged to form light grey centres

TABLE 3.3.9: The effect of *A. brassicicola* inoculation of 19 oilseed rape cultivars on leaf spot, pod spot, seed yield, seed infection and seed germination.

Cultivar	Leaf spot (i) scale 0-5[1]	Leaf spot (ii) scale 0-5[1]	Pod spot scale 0-5[1]	Seed yield g	Seed infection %[1]	Seed germination %[1]
Brutor	0.9	1.3	1.8	3.6	53.5	52.4
Christa	1.1	1.3	2.0	1.2	45.6	63.4
Duplo	1.0	1.2	1.9	1.2	65.9	59.3
Elevra	1.1	1.2	1.3	2.0	47.3	62.9
Fiona	1.0	1.3	1.5	5.2	87.1	58.1
Fido	1.1	1.3	2.0	0.8	87.1	55.7
Gulliver	1.1	1.2	2.0	1.1	88.0	48.9
Garant	1.1	1.2	1.5	2.4	48.5	52.9
Jet Neuf	1.1	1.2	1.8	2.8	76.5	59.7
Line	1.0	1.4	1.9	0.3	88.0	54.5
Loras	1.0	1.3	2.0	1.0	80.7	52.0
Lingot	1.0	1.3	1.7	5.3	77.4	53.5
Mary	1.1	1.4	2.0	0.3	83.9	44.4
Norli	1.1	1.3	1.4	3.6	41.5	62.2
Olga	1.0	1.4	1.9	2.3	85.9	53.7
Primor	1.0	1.2	1.1	1.6	85.1	63.1
Quinta	1.1	1.3	1.7	6.4	72.2	65.5
Rafal	1.1	1.4	1.9	2.2	51.7	62.2
Willi	1.0	1.4	2.0	1.8	90.0	47.0
SED±	0.05	0.06	0.17	1.29	2.93	2.41
		(DF = 162)			(DF = 54)	

[1] transformed data.

with dark margins (Plate 3.3.4a); some spots were entirely black and some surrounded by chlorotic haloes with concentric zonations giving a target-like appearance. The individual, large spots measured up to 2.5 cm in diameter with thin, papery, light brown centres which could break to give a shot-hole appearance. The spots were also observed on midribs in the form of oblong, sunken, linear lesions. On young foliage, as it developed, the spots were small, less than 1 mm, restricted

and dark brown to blackish in colour. On stems, the spots were more elongated, brownish grey, often with a dark margin, measuring from 1-3 mm in size; the spots often united forming large irregular dark areas on stems. As the plants started flowering, the fungus produced numerous spots on leaves and stems, and small tiny spots on flower buds; the infected buds became dry and dropped off, reducing the number of seed-bearing pods on the raceme. The spots were seen as small, sunken, dark points on young, green pods, initially less than 0.5 mm in diameter, enlarging up to 2-4 mm and becoming round to oblong and dark brown to black in colour. The infected pods were small in size with very few seed in them. Severely infected, under-developed pods started yellowing and pods infected at the seed setting (green pod) stage had half-filled and chaffy seeds.

The symptoms of *A. brassicicola* infection appeared quite late, 4 weeks after inoculation, on the outermost, old and weakened leaves as small, brown, necrotic, scattered specks less than 0.5 mm in size (Plate 3.3.4b). Numerous little streaks, measuring 1-3 mm in length, appeared on the midribs of the leaves and were dark brownish in colour. The upper young leaves were free from infection. The spots on lower leaves spread rapidly to form circular lesions measuring 1-2 mm in diameter. The fungus was found to establish itself rapidly on leaves which were damaged or injured by insects and especially slugs. Oblong lesions were also seen on stems in the form of dark brown stains which gradually changed into large necrotic areas, changing the colour of stems from green to dark, almost black. Infection was very severe on flower buds and flowers (Plate 3.3.5). On pods, the symptoms were seen as minute lesions which were dark brown to black in colour, measuring less than 0.5 mm in size: the lesions eventually coalesced producing

PLATE 3.3.4: Symptoms of *Alternaria* infection on leaves at growth stage 3.0 in a humid glasshouse, 4 weeks after inoculation.

(a) *A. brassicae*

(b) *A. brassicicola*

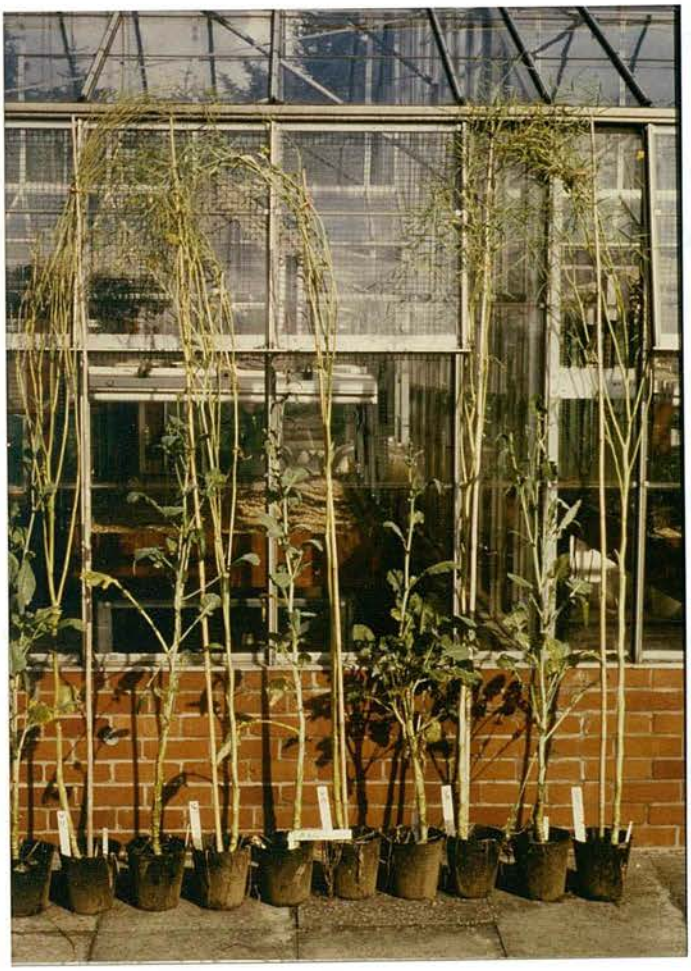
PLATE 3.3.5: Symptoms of *A. brassicicola* on oilseed rape flowers and young developing pods.

large affected areas which covered the full pods and carried dark conidia giving black sooty patches covering, in severe cases, the infected pod.

Plants where the flower buds were infected by either pathogen before elongation did not produce any shoots bearing either flowers or pods and the plants remained green and dwarf: a few plants produced side branches which grew very tall, bearing green pods (Plate 3.3.6), and contributed to seed yield. A few plants which escaped infection by one or the other of the two pathogens recorded high seed yields. Although overall yield was similar in the two glasshouse compartments, representing the two *Alternaria* pathogens, the seed viability was poorer from plants inoculated with *A. brassicicola*.

Within the compartments where plants were inoculated with *A. brassicae* there were no marked differences in levels of leaf spotting among different cultivars, but the incidence of pod spotting for different cultivars varied significantly (Table 3.3.8). Cultivars showing more pod spotting included Brutor, Christa, Duplo, Line, Loras, Olga and Willi: those showing less infection included Elevra, Norli and Primor. With regard to seed infection, significant differences were also found among cultivars, Garant and Norli showing a remarkably low incidence, and Fido showing the highest infection level, followed by Brutor and Gulliver. Seed yield varied greatly among cultivars, but this was not necessarily linked with infection levels: Christa, Duplo, Fido, Gullivar, Line, Loras, Mary, Olga, Primor and Willi showed very low yields, and Garant, Lingot and especially Quinta, showing relatively high yields. Seed germination rates among cultivars varied significantly, those showing high rates of germination, including Elevra, Fiona, Garant, Norli, Primor and Rafal, while Line had a relatively low germination rate.

PLATE 3.3.6: Oilseed rape plants with early severe damage to flower buds due to *A. brassicae*, showing stunted growth without pods, along with plants which have escaped flower infection, showing good growth with green pods.



With *A. brassicicola* infection, there was little evidence of marked variation in the extent of leaf spotting, but at the second observation Duplo, Elvera, Gulliver, Garant, Jet Neuf and Primor tended to be less affected than the average, while Line, Olga and Willi had disease scores above average (Table 3.3.9). Any differences, however, were very slight. As with *A. brassicae*, cultivar differences were not marked until pod spotting assessments were made, when lower rates of infection were associated with Elvera, Fiona, Garant, Norli and Primor and higher rates associated with Christa, Fido, Gulliver, Loras, Mary and Willi. Cultivars also showed differences in the extent of seed infection, those showing low rates of infection, including Brutor, Christa, Elevra, Garant, Norli and Rafal. High levels of seed infection were shown by Fiona, Fido, Gulliver, Line, Olga and Primor, while all seeds of Willi were infected. Seed yields varied significantly with cultivars, low yields being evidenced by Christa, Duplo, Fido, Gulliver, Line, Loras and Mary, and high yields being associated with Fiona, Lingot and Quinta. Seed germination rates were relatively low with Gulliver, Mary and Willi, and relatively high with Christa, Elvera, Norli, Primor, Quinta and Rafal.

Discussion

From the leaf disc inoculation studies (Experiment 3.3a), significant effects of temperature and pathogen on lesion and colony development were evident, with the higher temperature and *A. brassicicola*, compared with *A. brassicae*, giving higher indices. Between the pathogens, there was no consistent variation in response to temperature. It was observed, however, that the influence of temperature was not only on the pathogen but also on host tissues and leaf discs were seen to

yellow more rapidly at higher incubation temperatures. Within each pathogenic species some variation in the general effects of different isolates was observed, but the differences were not marked and there was no obvious host specificity linked with individual isolates. Neergaard (1945), in a pathogenicity test of various isolates of *A. brassicicola* on various cruciferous host plants, reported that different isolates of *A. brassicae* were alike in their pathogenicity. Similarly, Humpherson-Jones and Hocart (1983), while studying the cross-infectivity of isolates of *A. brassicae* and *A. brassicicola* from a range of cultivated and wild hosts of Cruciferae, indicated that neither fungus was host specific. However, Saharan and Kadian (1983) identified some physiologic specialisation in *A. brassicae* and described three distinct races.

In comparing different host groups in the first study, relating to varieties of *B. oleracea*, some cultivars of savoy and Brussels sprouts, in particular, were notable in showing some resistance to both pathogens, while there was a general trend for different host groups or cultivars to respond in the same way to the different pathogens. Kale and broccoli cultivars appear to be most susceptible to *Alternaria* infection among *B. oleracea* groups. In a seedling inoculation study on *B. oleracea* groups (cauliflower, Brussels sprouts and broccoli), Braverman (1971, 1977) indicated promising resistance to *A. brassicicola* in cauliflower and Brussels sprouts cultivars, whereas none of the broccoli accessions were resistant. Changsri and Weber (1963) observed that *A. brassicicola* infection was greater on cabbage, collards and kale compared with *A. brassicae*.

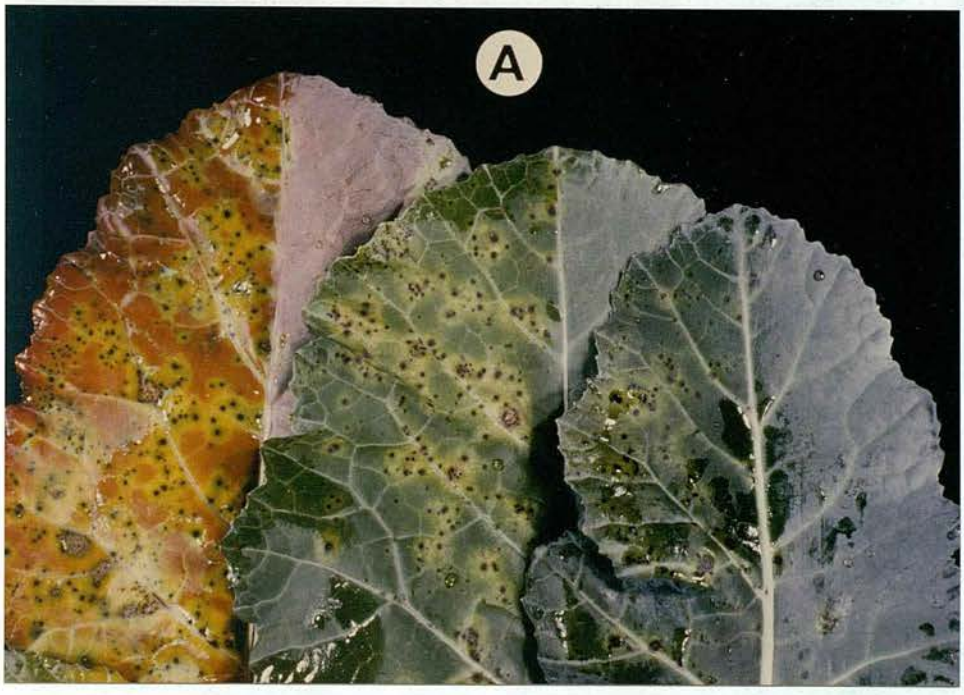
In the second experiment, infection levels, although not strictly comparable with the first, tended to be higher and, of the range of cruciferous hosts tested, those showing less infection included turnip

and white mustard. It is interesting to note that Husain and Thakur (1963) reported that white mustard was most resistant to *A. brassicae*, while Changsri and Weber (1963) indicated that *A. brassicae* leaf infection was higher on mustard, turnip and radish compared with *B. oleracea* cultivars. Oilseed rape and brown mustard were the most susceptible groups in the present study with respect to both pathogens, while black mustard also showed relatively high susceptibility to *A. brassicae*. Bhander and Maini (1965) described brown, yellow and black mustard as moderately susceptible to *A. brassicae*.

The sporulation indices were relatively low at the lower incubation temperature and cultivars of Brussels sprouts and cauliflower did not produce spores of *A. brassicae* at 15°C while *A. brassicicola* failed to sporulate on Brussels sprouts at this temperature in the first study. However, all cultivars showed high sporulation indices at 25°C, especially those of broccoli. In the second study, radish, turnip rape, Raphanobrassica and oilseed rape showed relatively high sporulation indices even at 15°C.

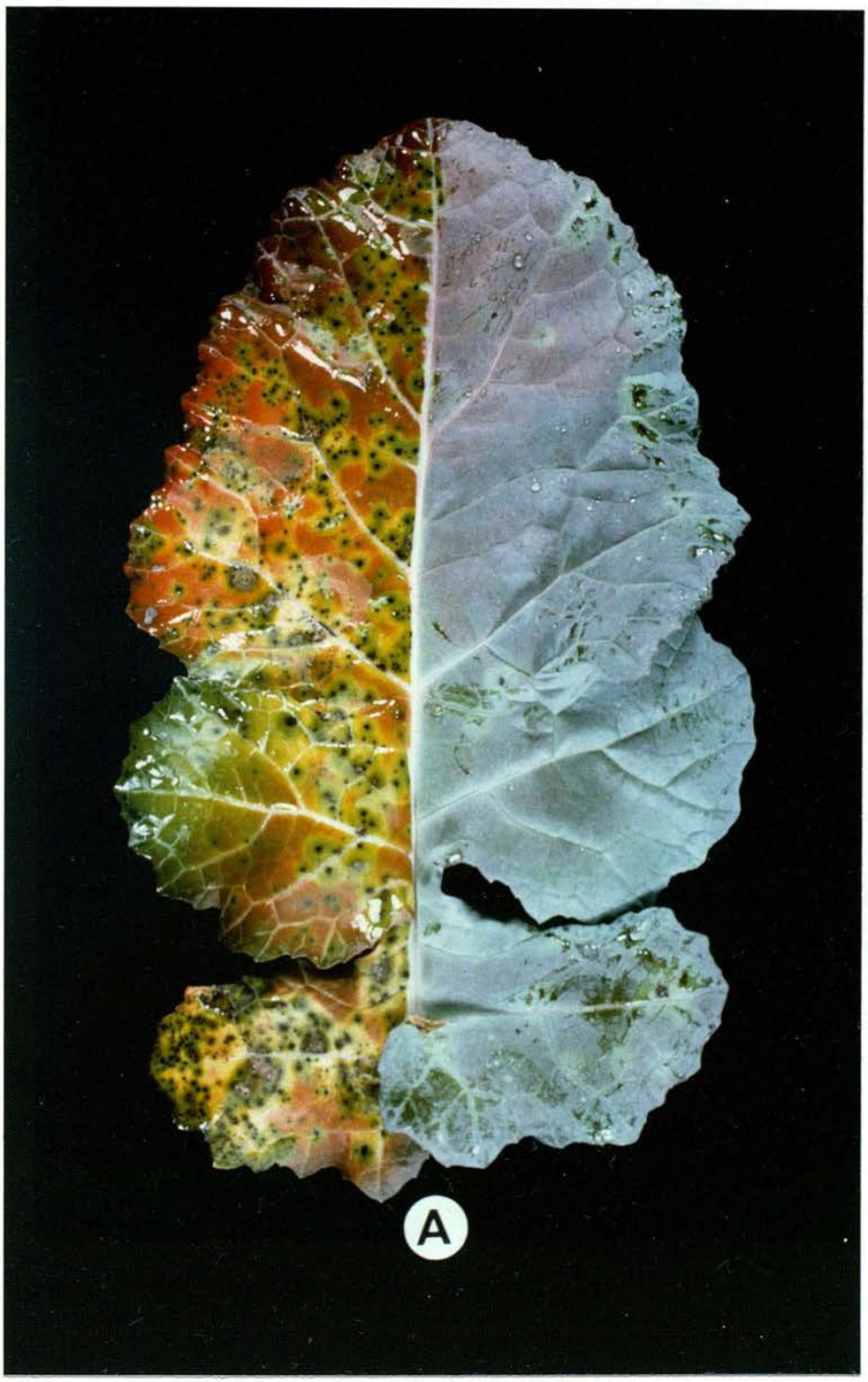
As in the previous experiment, *A. brassicicola* gave greater leaf lesion development than *A. brassicae*. This did not reflect the behaviour of the two pathogens on growing plants in the field, but in this present work the conditions were controlled to give maximum infection and the more prolific growth of *A. brassicicola* compared with *A. brassicae*, evidenced in axenic culture, may have accounted for the greater development of this species where circumstances were substantially weighted in favour of pathogen development. A feature noted in black, brown, white mustard and also turnip rape was the green-island effect on leaf discs beneath the *Alternaria* spore drop, when the surrounding tissues started yellowing. This effect has been linked with an increased output

PLATE 3.3.7: Symptom development of *A. brassicae* infection on leaves of oilseed rape in relation to removal of wax layers by rubbing.



(a) Older leaves / younger leaves

PLATE 3.3.7:



(b) Rubbed / not rubbed

of cytokinins in studies on *A. brassicicola* infection of mustard (Suri, Mandahar and Gill, 1983).

With respect to the leaf disc inoculation studies in general, it was observed that host groups differed in their infection responses but that this variation was quantitative rather than qualitative in nature. In considering factors that might account for lower levels of infection, it seemed that the apparently more resistant groups, e.g. savoy, cabbage and Brussels sprouts, exhibited greater waxiness of the leaf surface and this might be viewed as providing a physical barrier which impeded infection. In a small test where the outer wax layers were rubbed from half the surface area of oilseed rape leaves prior to spraying with a spore suspension of *A. brassicae*, infection developed most extensively only on the side of the leaf which had been rubbed (Plate 3.3.7a). Moreover, the infection was greater on older leaves (Plate 3.3.7b) which have less wax and wax is reported to decline with the processes of ageing (Skoropad and Tewari, 1977).

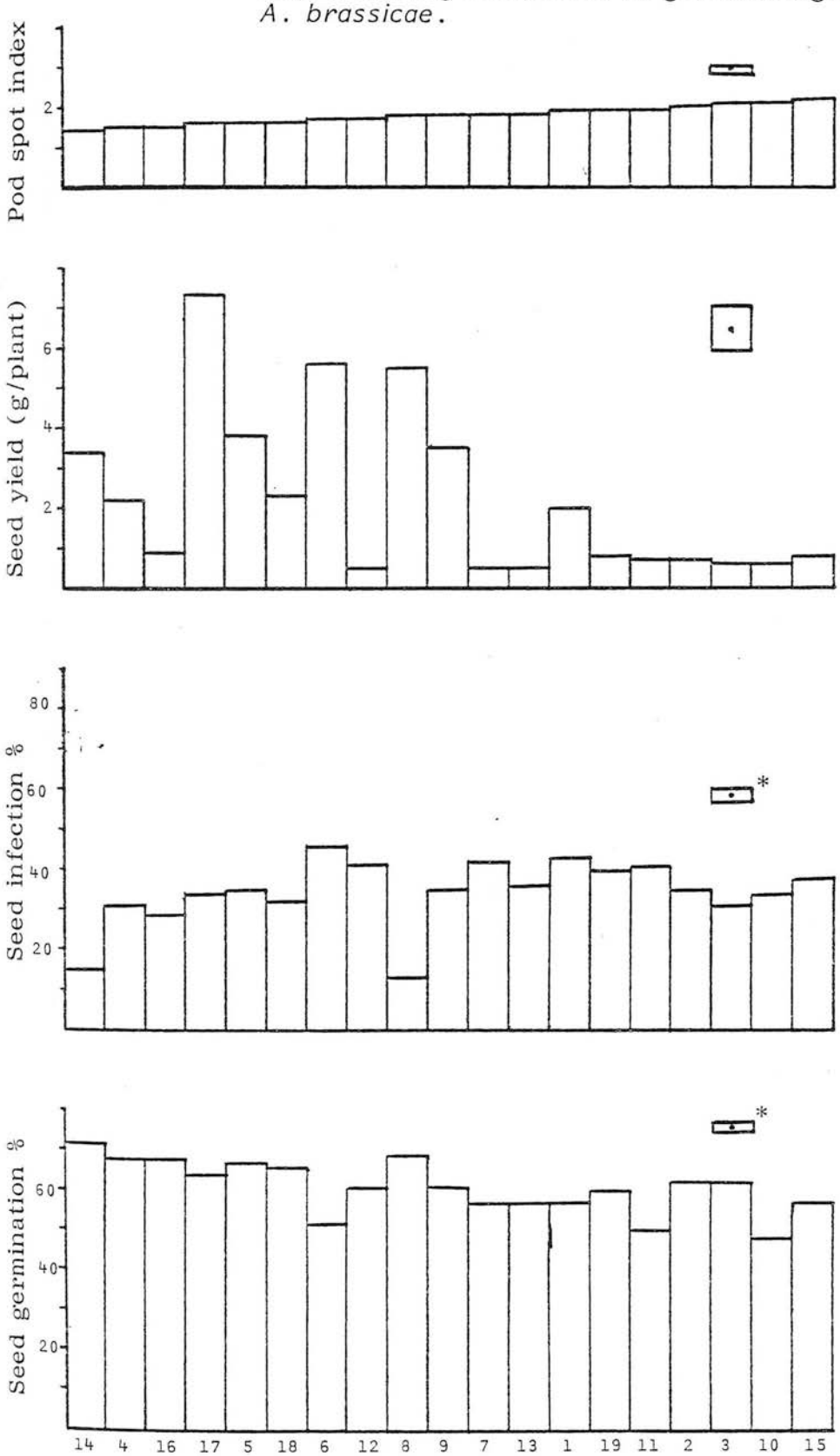
In Experiment 3.3b, among the 19 cultivars of oilseed rape tested for their response to inoculation of growing plants with *A. brassicae* or *A. brassicicola*, none were found to be highly resistant to either *Alternaria* species. The two pathogens showed differences in their effects in the early growth stages, but the disease response to both at later stages of flowering and seed formation was generally similar. *A. brassicae* produced distinct spots 1 week after inoculation on all cultivars tested, whereas *A. brassicicola* failed to produce clear symptoms even 4 weeks after inoculation. Earlier workers described *A. brassicicola* as the less aggressive pathogen of the foliage (Knox-Davies, 1979), but causing severe damage at the seedling stage in seed beds or when the host becomes weaker at the seed maturation stage (Neergaard, 1979).

Further, it was observed that *A. brassicae* spots were noticed on all leaves of the plants, both young and old, but *A. brassicicola* lesions were only confined to the lowermost leaves in vigorously growing plants. *A. brassicae* differed from *A. brassicicola* in the early stages of disease development in the form of spots produced: *A. brassicae* produced spots with a definite zonation, whereas *A. brassicicola* produced countless, small, blackish-brown specks on leaf blades. Weimer (1924) reported that attacks of *A. brassicicola* in the form of leaf spots on plants in the field is rarely of any particular significance and, as a rule, it is chiefly the old and weakened leaves that are damaged. Further, he reported in 1926 that *A. brassicae* leaf spotting on the lower leaves of cabbage and cauliflower reduced the size of the crop significantly. As the host reached the flowering stage, *A. brassicicola* symptoms were seen on old foliage and more frequently on flowers and young pods. Green (1947) reported the frequent incidence of *A. brassicicola* on seed-bearing stocks of various *Brassicas*. Although pod spotting assessments due to *A. brassicae* and *A. brassicicola* were similar, *A. brassicae* produced more distinctly pronounced, larger spots on pods, stems, as well as leaves, resulting in a reduced photosynthetic area and earlier senescence. Seed infection was more frequent with *A. brassicicola* than *A. brassicae*: this may be due to the larger amount of inoculum of *A. brassicicola* associated with its prolific spore production: moreover, its smaller spores were often carried in the hilum cavities as indicated by the escape of much of the fungus from sodium hypochlorite treatment, and internal infection of the testa is reported to occur at the hilum (Knox-Davies, 1979). Thus, there was usually a greater reduction in seed germination in seeds infected with *A. brassicicola*, compared with *A. brassicae*. These results, based on one experiment in one season

and set of conditions, may be inadequate to derive any precise conclusions, but there is some evidence to suggest that *A. brassicae* is the more aggressive foliage pathogen, whereas *A. brassicicola* proved to be serious at later stages of flower development and seed maturation.

Although there were no highly resistant varieties among the 19 oilseed rape cultivars tested, there was a significant variation in their responses at different growth stages to inoculation from both *Alternaria* species (Figures 3.3.6 and 3.3.7). Although the figures show that infection, seed yield and seed viability were not necessarily related, the cultivars Quinta, Lingot and Garant with *A. brassicae* showed the highest yields coupled with below average infection and above average seed germination rates. With *A. brassicicola*, the cultivars Fiona, Lingot and Quinta showed relatively high yields along with the low pod spotting indices, but these cultivars tended to give high or moderately high seed infection rates; however, the seed germination rate in cultivar Quinta was relatively high. In general, based on arbitrary classification, a high incidence of leaf spot and seed infection with one or both pathogens and a low seed yield and low germination rate was seen in the cultivar Line, whereas a low disease incidence and low seed infection and higher seed yield with a high germination rate was associated with Norli.

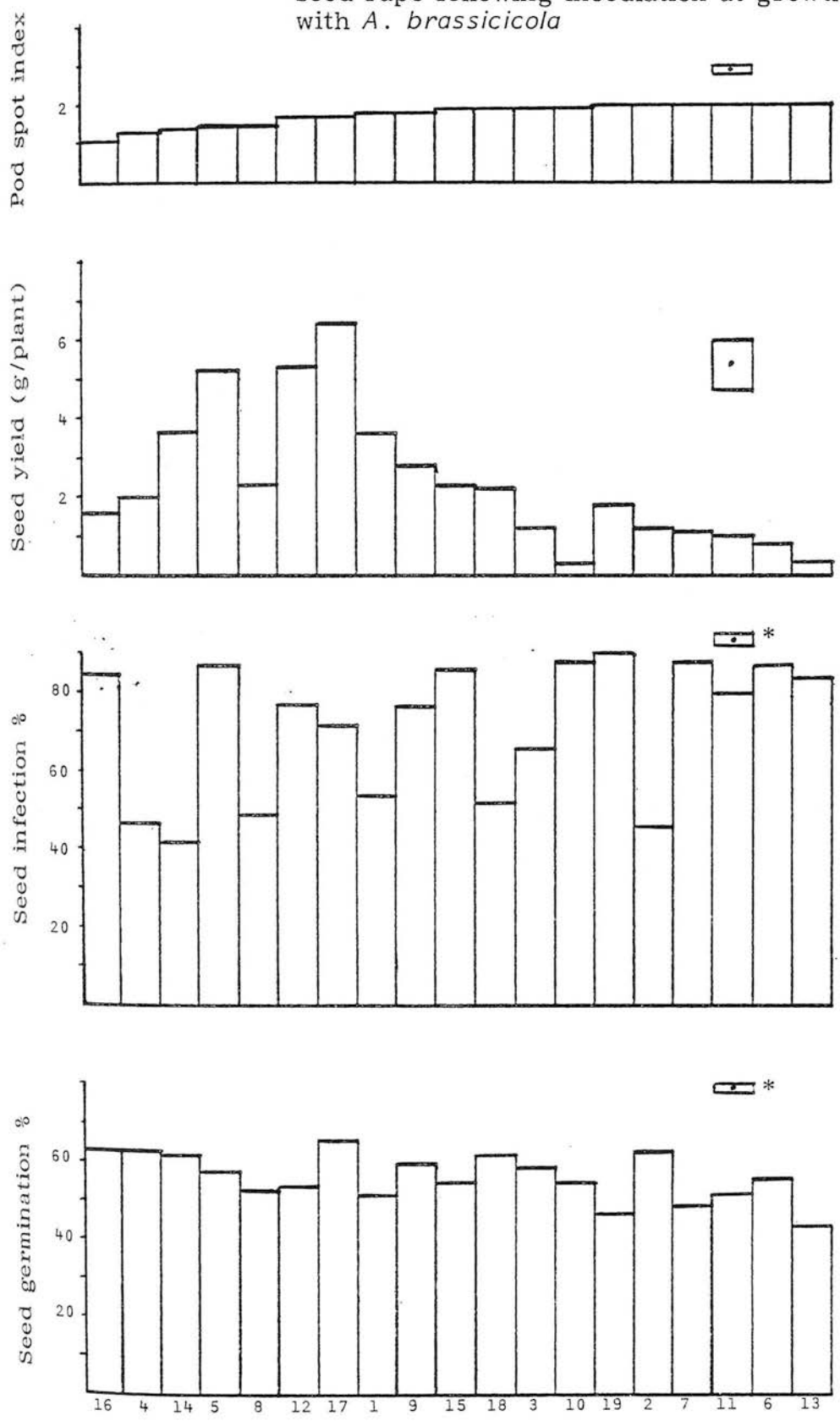
FIGURE 3.3.6: Pod spot index (transformed value), seed yield (g/plant), seed infection and seed germination per centage (transformed values) in 19 cultivars of oilseed rape following inoculation at growth stage 3.0 with *A. brassicae*.



(cultivars as numbered in Table 3.3.3)

SED (DF=162); *SED (DF=54)

FIGURE 3.3.7: Pod spot index (transformed value) seed yield (g/plant), seed infection and seed germination percentage (transformed values) in 19 cultivars of oilseed rape following inoculation at growth stage 3.0 with *A. brassicicola*



(cultivars as numbered in Table 3.3.3)

SED (DF=162); * SED (DF=54)

3.4: THE INFLUENCE OF HUMIDITY AND TEMPERATURE ON THE DEVELOPMENT OF LEAF SPOT SYMPTOMS ON OILSEED RAPE CAUSED BY *A. BRASSICAE* AND *A. BRASSICICOLA*

Introduction

Humidity and temperature are well recognized as important environmental factors determining the severity of *Alternaria* disease. *A. brassicicola* is reported to develop rapidly in warm and rainy weather conditions (Eddins and Burrell, 1949) and infection has been found to be rapid in glasshouses with high humidity conditions (Milbraith, 1922; Raabe, 1939). The outbreak of a serious epidemic of *A. brassicicola* presupposes a humidity level of 95-100% RH for at least 18 hours (Domsch, 1957). Jorgensen (1967) observed that *A. brassicae* causes severe damage under humid conditions and maximum *A. brassicae* infection has been associated with wet years (Louvet, 1958; McDonald, 1959): a wetting period of 18 hours is required for *Alternaria* infection according to Rangel (1945).

It has been demonstrated that the two *Alternaria* species have different optimum temperatures for infection. Degenhardt, Petrie and Morrall (1982) gave optimum temperatures of 19-23°C and 23-25°C for *A. brassicae* and *A. brassicicola*, respectively. Humpherson-Jones and Hocart (1983) indicated an optimum temperature of 15°C for *A. brassicae* and 25°C for *A. brassicicola*. In earlier reports, Van Schreven (1953) had noted that 20-24°C gave maximum disease development for *A. brassicae* and Domsch (1957), working with *A. brassicicola*, considered 21-27°C as the optimum temperature range for infection. However, *A. brassicicola* infection may be evident even at 7°C in 5 days (Weimer, 1924).

Two studies were made in the present work, one relating to humidity and the other to temperature as follows:

Experiment 3.4a: The effects of atmospheric humidity on leaf spot development, following inoculation of leaf discs with *A. brassicae* or *A. brassicicola*.

Experiment 3.4b: The effect of temperature on leaf spot development following inoculation of leaf discs with *A. brassicae* and *A. brassicicola*.

Materials and Methods

Experiment 3.4a

In this study, five *Alternaria* isolates each of *A. brassicae* and of *A. brassicicola* were inoculated on to the leaf discs of two oilseed rape cultivars, Rafal and Jet Neuf. The plants were raised in a glasshouse and leaf discs prepared as explained in the previous experiment. Leaf discs were maintained on benzimidazole agar following inoculation with a 0.025 ml droplet of 50,000 spores/ml of an isolate per leaf disc (Experiment 3.3). In a second duplicate set of plates, following inoculation of spore suspension as before, the droplets were carefully dried to study the infection rates of dry spores compared with wet spores. The leaf discs were incubated at five different humidity levels obtained in desiccators with different concentrations of sulphuric acid, according to the method described by Johnston and Booth (1983) and as listed in Table 3.4.1. The inoculated leaf discs in the desiccators were incubated at 20 ± 2°C for 5 days in a 12 hour light period alternating with 12 hours darkness in each 24 hours cycle. Within each dish, the leaf discs of the two cultivars inoculated with the different isolates of the two pathogen species were arranged at random. The experiment

was arranged in a split-plot design with humidity treatments representing main plots, dry and wet spores sub-plots, and sub-sub-plots consisting of each cultivar/pathogen/isolate combination. The experiment was repeated four times to give a randomised block layout with four replicates.

TABLE 3.4.1: Relative humidities brought about by different sulphuric acid concentrations.

Relative humidity (%)	Sulphuric acid concentration (w/w) (%)
56.8	40
66.8	35
75.6	30
88.5	20
98.5	5

At the end of the incubation period, the leaf discs were scored for disease development using the same 0-5 disease index scale as in Experiment 3.3.

Experiment 3.4b

The effect of various temperature levels on disease development was studied by incubating the leaf discs of Rafal and Jet Neuf cultivars inoculated with spore suspension droplets of isolates of *A. brassicae* and *A. brassicicola*, as explained in Experiment 3.4a. In this instance, however, the inoculated leaf discs were incubated in darkness at 5°C, 10°C, 15°C, 20°C, 25°C and 30°C for 5 days in closed plastic containers to maintain a high humidity. At the end of the incubation period, the disease development at different incubation temperatures was then assessed using the 0-5 disease scale. The experiment was in the form

of a split-plot design, with temperature treatments representing main plots and the various cultivar/pathogen species/isolate combinations representing sub-plots. A randomised block layout was used which was replicated four times.

Results

EXPERIMENT 3.4a: The effect of atmospheric humidity on leaf spot development following inoculation of leaf discs with A. brassicae and A. brassicicola

From the analysis of variance of the data, significant effects were associated with humidity, the form of inoculum and pathogen species, and there were significant interactions between these factors (Table 3.4.2). Cultivars and isolates had no significant effects on the results. Dry spore inoculation produced, on average, larger lesions than wet inoculation, a disease index of 2.56 compared with 1.66. There was a general trend for lesion size to increase with increasing humidity but this was evident only where 'dry spore' inoculation as opposed to 'wet spore' inoculation was used (Plate 3.4.1). In comparing the overall means for the different pathogens, *A. brassicae* gave a very slightly larger disease index than *A. brassicicola*, 2.21 compared with 2.01, but their respective responses differed in relation to humidity and form of inoculum (Plate 3.4.1). Both pathogens showed increased disease indices at higher humidities with dry spore inoculum, *A. brassicicola* tending to show the greatest response. However, with wet spores, *A. brassicae* showed only a slight increase with increasing humidities and *A. brassicicola* showed a slight decrease.

TABLE 3.4.2: The effect of atmospheric humidity and form of inoculum on lesion development on leaf discs inoculated with *A. brassicae* and *A. brassicicola* (mean of two cultivars and five isolates of each pathogen).

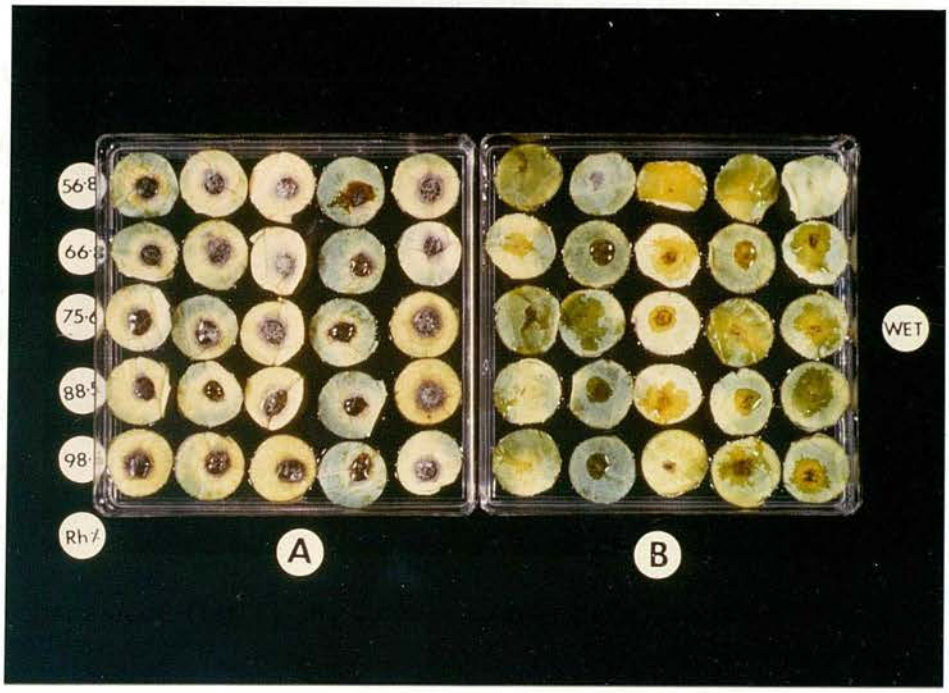
Humidity (%) RH	Disease Index (0-5 scale) Dry drop *A. brassicae*	*A. brassicicola*	Mean	Wet drop *A. brassicae*	*A. brassicicola*	Mean	*Grand mean*
56	1.73	1.73	1.73	1.73	1.68	1.70	*1.71*
68	1.75	1.78	1.76	2.00	1.70	1.85	*1.80*
75	2.00	2.53	2.26	2.00	1.00	1.50	*1.88*
88	3.00	3.30	3.15	2.13	1.00	1.56	*2.35*
98	3.40	4.40	3.90	2.33	1.00	1.66	*2.78*
Mean	2.38	2.75		2.04	1.28		

SED
Humidity (H) mean ± 0.05 (DF=12)
Inoculum (I) mean ± 0.03 (DF=15)
Pathogen (P) mean ± 0.03 (DF=94)

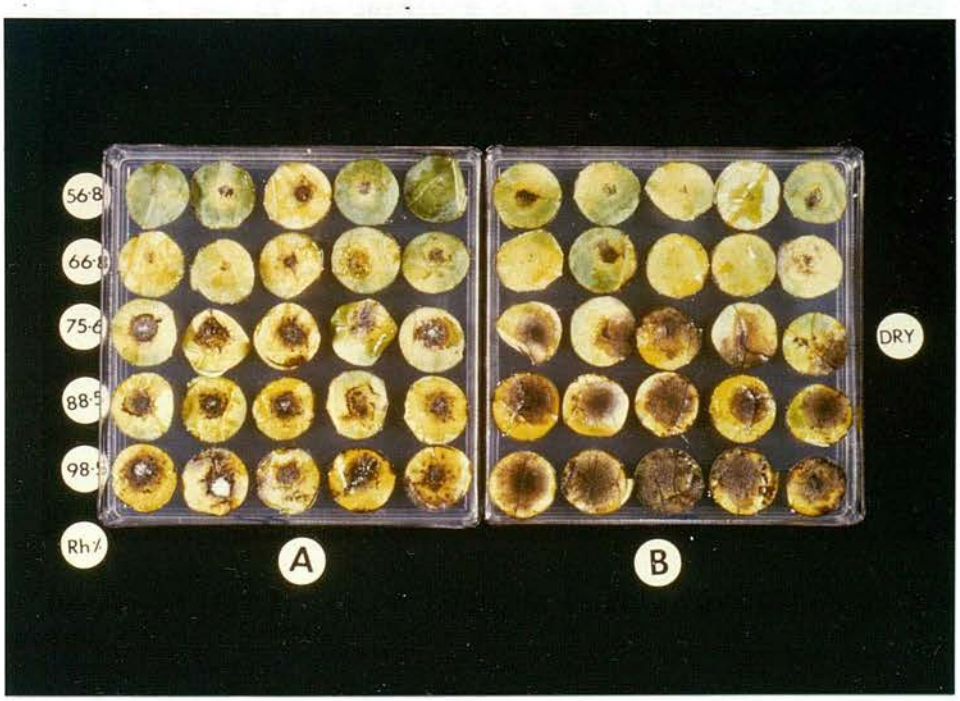
H x I mean ± 0.08 (DF=15)
H x P mean[1] ± 0.07 (DF=94)
[1]except when comparing at same level of H ± 0.06 (DF=94)

I x P mean ± 0.04 (DF=94)
H x I x P mean[2] ± 0.10 (DF=94)
[2]except when comparing at same level of H x I ± 0.08 (DF=94)

PLATE 3.4.1: Effect of different humidity levels on *Alternaria* disease development on oilseed rape leaf discs.



(a) *A. brassicae* (left), *A. brassicicola* (right), inoculated as wet droplet.



(b) *A. brassicae* (left), *A. brassicicola* (right), inoculated as dry spores.

EXPERIMENT 3.4b: The effect of temperature on leaf spot development following inoculation of leaf discs with A. brassicae and A. brassicicola

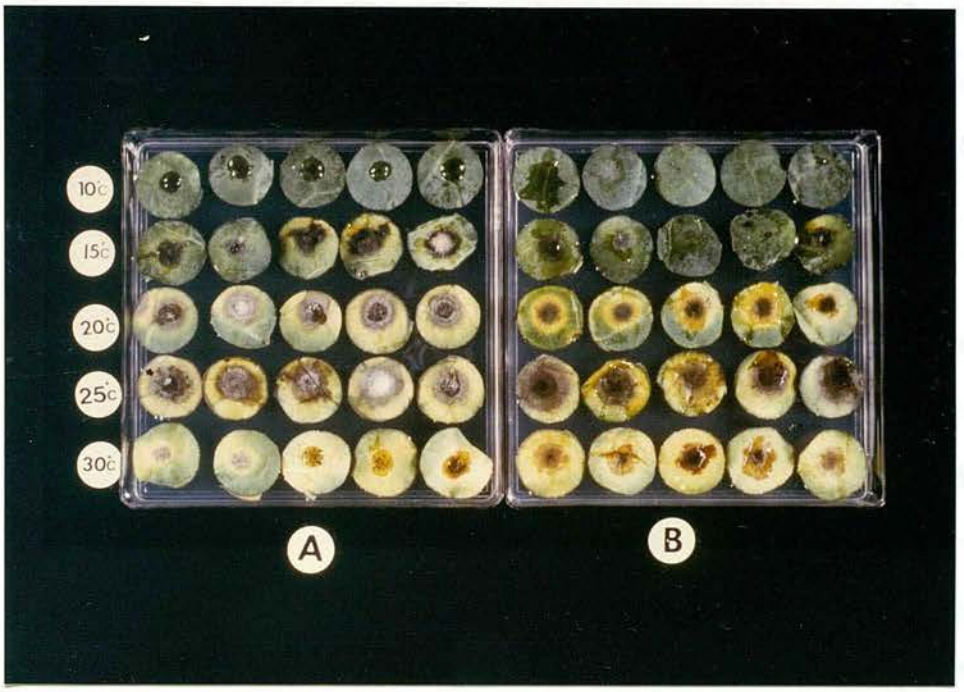
It was observed that at high temperature leaf discs appeared to yellow more rapidly independently of infection. At the lowest temperature of 5°C, infection symptoms occurred in the form of minute light brown specks visible only under microscope. However, at 10°C the spots were clearly visible to the naked eye, *A. brassicae* generally showing clear symptoms. Temperature and species of *Alternaria* were found to have significant effects, with a significant interaction between the factors (Table 3.4.3). With both pathogens, greatest lesion development occurred at 25°C and *A. brassicae* tended to give larger lesions than *A. brassicicola* overall (Plate 3.4.2). At lower temperatures than 25°C, *A. brassicae* showed larger lesions than *A. brassicicola*. Neither host cultivar or pathogen isolate affected lesion development significantly.

TABLE 3.4.3: Effect of temperature on lesion development on leaf discs of oilseed rape inoculated with *A. brassicae* and *A. brassicicola* (mean of two cultivars and five isolates of each pathogen).

Temperature (°C)	Disease Index (0-5 scale)		
	A. brassicae	*A. brassicicola*	Mean
5	0.61	0.23	0.42
10	1.20	0.74	0.97
15	2.68	1.73	2.20
20	3.00	2.70	2.85
25	4.00	4.18	4.09
30	2.00	2.50	2.25
Mean	2.25	2.01	

SED Temperature mean = ± 0.03 (DF=15)
Pathogen mean = ± 0.06 (DF=54)
Temperature x Pathogen mean = ± 0.08 (DF=54)

PLATE 3.4.2: Effect of different temperatures on *Alternaria* disease development on oilseed rape leaf discs.



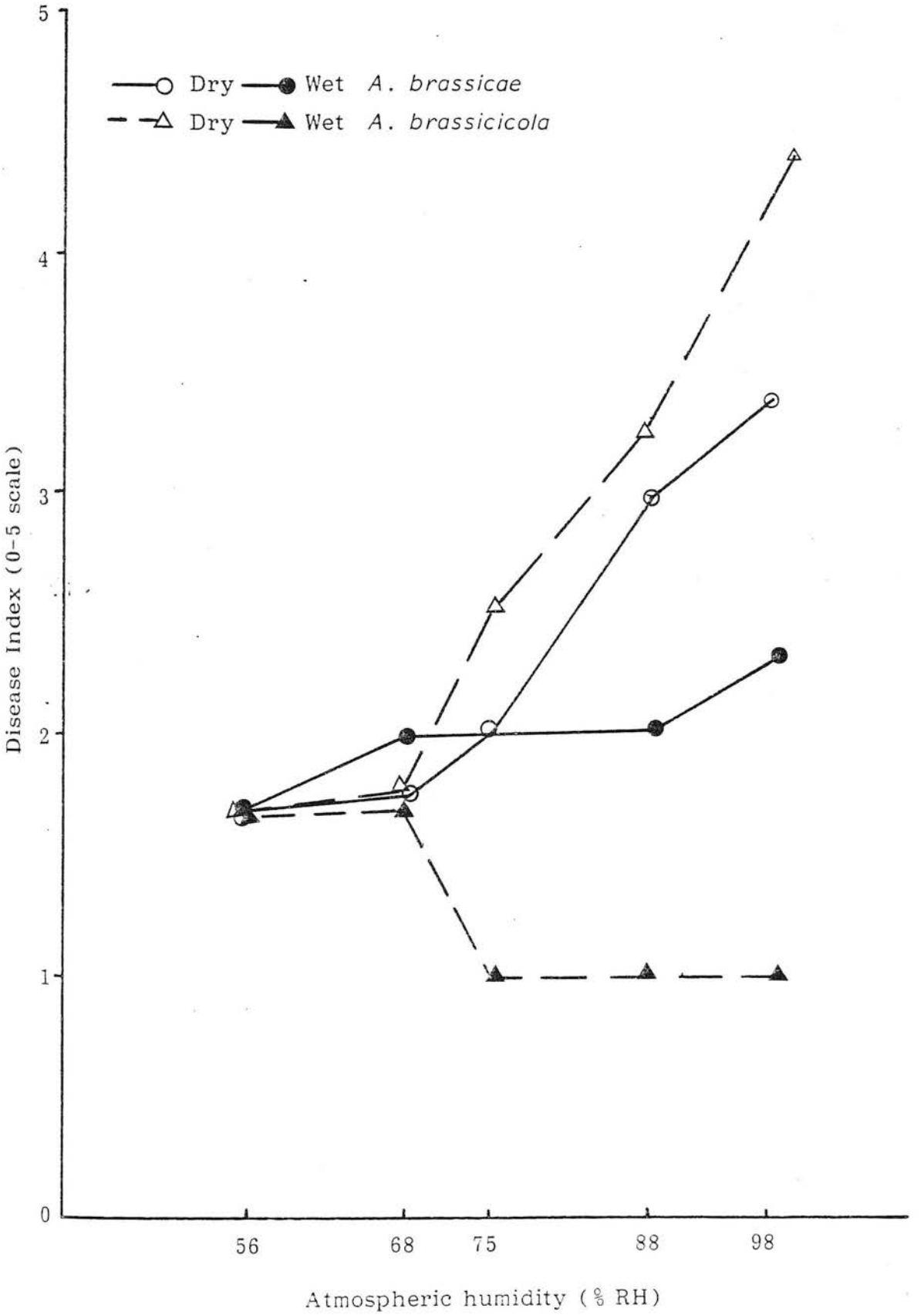
A. brassicae (left), *A. brassicicola* (right).

Discussion

Although many workers have reported that *Alternaria* pathogens require free water (Humpherson-Jones and Hocart, 1983) or a humidity more than 95% (Domsch, 1957) for disease development, in the present studies it was observed that *Alternaria* leaf spotting was recorded over the full range of humidities tested down to 56% RH. However, maximum leaf disease development occurred at the highest humidity with dried spore droplets (Figure 3.4.1). Karwasra and Saharan (1983) reported a progressive development of *A. brassicae* leaf spotting on Chinese fodder cabbage, *B. pekinensis*, over a 3 month period where the relative humidity fluctuated within the range of 55-75%.

With both *Alternaria* species, disease development was greater when they were inoculated as a dried drop as opposed to wet droplet inoculation. When the spore drop was dried, it tended to spread over the leaf disc and disease development was seen as number of large spots scattered all over the leaf disc compared with a confined area of a few small spots with wet droplet inoculation (Plate 3.4.1). The more restricted spotting with wet drops may be associated with self-inhibitory factors which accumulate when spores are clustered together within a small droplet. Mukadam (1982) reported that in *A. brassicicola* self-inhibition of spore germination was associated with spore load, and the inhibitory effect decreased with increased dilution. It is possible that spores of *A. brassicicola* show greater self-inhibition than those of *A. brassicae* from the results of this present study. A further factor may have related to the physical properties of the droplets which impeded effective contact of spores with the leaf surface. Royle (1976) has indicated that the capacity of the inoculum itself to be wetted and the effect of spores on the surface tension of water drops containing them

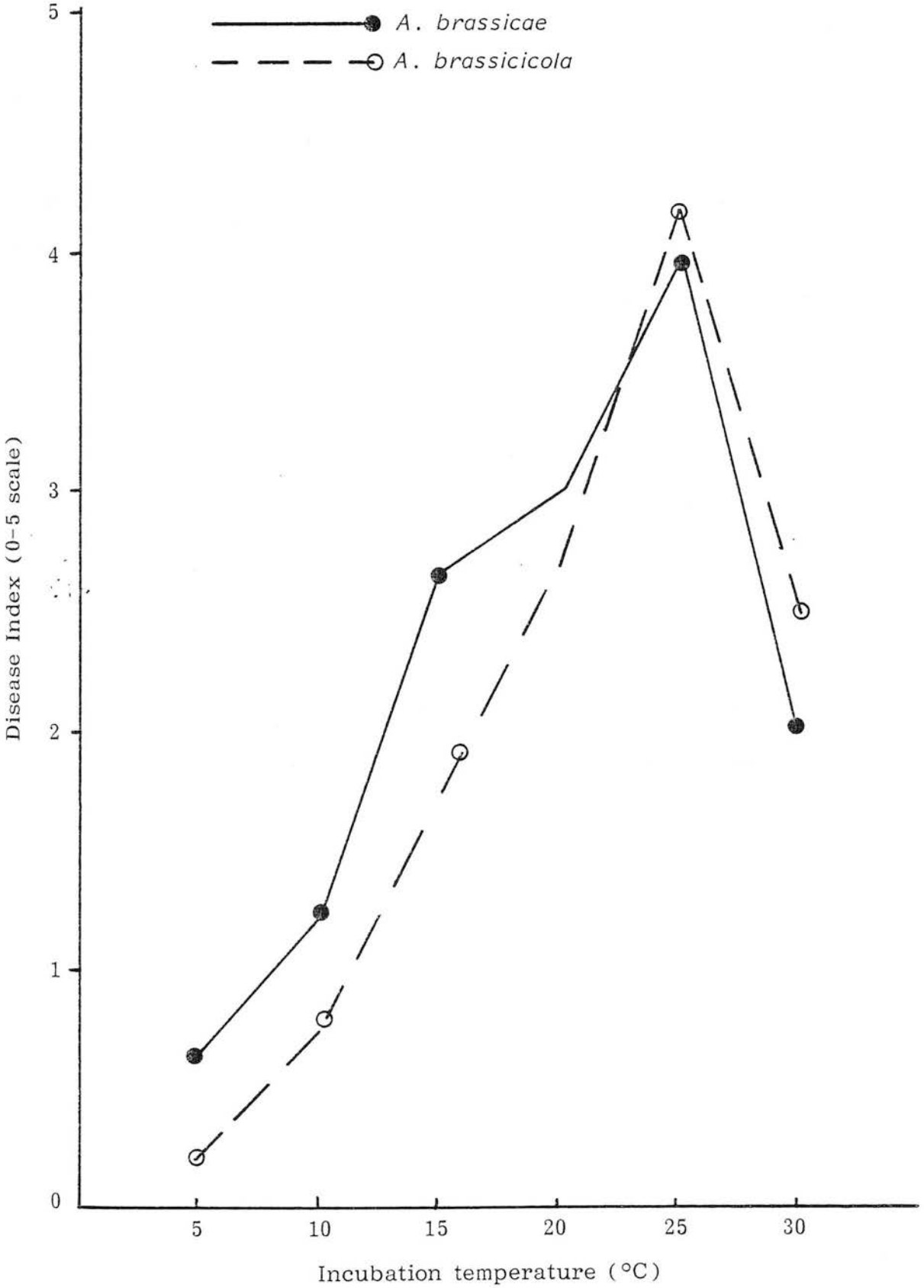
FIGURE 3.4.1: Lesion development on oilseed rape leaf discs following inoculation with *A. brassicae* and *A. brassicicola* in relation to atmospheric humidity and form of inoculum (dry or wet).



can influence the degree to which plant surface wettability contributes to disease resistance. The differential response of *A. brassicae* and *A. brassicicola* to increasing humidity with drops (Figure 3.4.1) may be possibly associated with higher humidities prolonging the period of intact droplets, intact droplets being assumed to interfere with infection, and the two species possessing spores with different abilities to lower the surface tension of water drops.

With regard to temperature requirements of *Alternaria* species, Degenhardt *et al.* (1982) and Humpherson-Jones and Horcot (1983) suggest that *A. brassicae* infection is favoured at lower temperatures of 19-23°C or 15°C and *A. brassicicola* at higher temperatures of 23-25°C or 25°C. In the present study both *Alternaria* species, when inoculated on to oilseed rape leaf discs, gave maximum infection indices at 25°C (Figure 3.4.2). Infection occurred to declining levels at lower temperatures down to 5°C when the indices were very low (Plate 3.4.2). *A. brassicicola* gave slightly lower indices than *A. brassicae* at temperatures below 25°C, but at 30°C *A. brassicicola* gave the higher index. Weimer (1924) reported that the optimum temperature for *A. brassicicola* infection on detached cauliflower leaves in moist chambers was between 28 and 31°C, although clear disease symptoms were seen at 7°C in 5 days. Some of the differences in the findings of different workers may be due to different hosts and it was observed in the present study that temperature affected rate of leaf disc yellowing independently of inoculation, leaves yellowing more rapidly at higher temperatures. However, the results are in keeping with the general impression that at temperatures below 25°C conditions are more favourable for *A. brassicae* than for *A. brassicicola*, whereas at 25°C or above conditions favour *A. brassicicola*.

FIGURE 3.4.2: Lesion development on oilseed rape leaf discs following inoculation with *A. brassicae* and *A. brassicicola* in relation to incubation temperature.



3.5: STUDIES ON OILSEED RAPE SEED AND SEEDLING INFECTION BY *A. BRASSICAE* AND *A. BRASSICICOLA*

Introduction

Both *A. brassicae* and *A. brassicicola* are primarily seed-borne fungi and cause a range of effects on germination from seed rot to wire stem of seedlings (Rangel, 1945; Schimmer, 1953; Holtzhausen and Knox-Davies, 1974) and damping-off (Groves and Skolko, 1944; Changsri and Weber, 1963; Taber, Vanderpool and Taber, 1968). There is, however, little information on the comparative behaviour and effects of the two fungi in association with seed.

Many workers have reported that *Alternaria* infection is confined to only the seed coat and that the fungi cannot penetrate the hard pericarp: according to Boek (1952), *A. brassicicola* was never encountered in the embryo below the infected seed coat. Domsch (1957) established that *A. brassicicola* penetrates the epidermis of the seed coat of maturing seed of *B. oleracea*: the large epidermal cells may be densely colonised by heavily sporulating mycelium but the fungus seemed incapable of penetrating into the deeper layers of mature seed, apparently because of thick walled palisade cells immediately beneath the epidermis which formed a mechanical barrier. However, Maude and Humpherson-Jones (1980) showed that *A. brassicicola* is capable of infecting embryos in heavily diseased samples of cabbage and kale.

Neergaard (1979) noted that *A. brassicicola* inoculum which is present in the seed, outside the embryo, is carried during germination either on the cotyledons or in the seed coat, and the spores are transmitted to young plants by air currents. It may be expected that the effect of fungal infection on germination and seedling growth will be

influenced by environmental factors, and there is some evidence that temperature may determine differentially the behaviour of the two *Alternaria* species (Humpherson-Jones and Hocort, 1982).

The experiments in this section were carried out to study further the forms of association of *A. brassicae* and *A. brassicicola* with seed, their effects on seed germination and seedling development, and the influence of moisture and temperature on seedling infection. The experiments are considered under the following headings:

Experiment 3.5a: The distribution of *A. brassicae* and *A. brassicicola* in oilseed rape seed tissues.

Experiment 3.5b: The effect of oilseed rape seed inoculation with *A. brassicae* and *A. brassicicola* isolates on seed germination.

Experiment 3.5c: The effects of oilseed rape seed inoculation or infection with *A. brassicae* and *A. brassicicola* on seedling disease.

Experiment 3.5d: Seedling infection by *A. brassicae* and *A. brassicicola* from inoculated seed of oilseed rape in relation to moisture conditions.

Experiment 3.5e: The effect of temperature on the development of *A. brassicae* and *A. brassicicola* from infected oilseed rape seed.

Materials and Methods

Experiment 3.5a

The distribution of *A. brassicae* and *A. brassicicola* in infected oilseed rape seed of the cultivar Rafal was studied using two seed lots each of *A. brassicae* infected samples and of *A. brassicicola* infected samples. Seeds were collected from plants inoculated before flowering in humid chambers, selecting those from severely or moderately spotted pods to make sure that infection was likely in the seed. The seeds were washed in running water to remove contaminating spores then surface sterilized with 1% sodium hypochlorite solution for 5 minutes. Each seed was then soaked separately in multi-compartment discs for 8 hours and 100 seeds in each seed lot were separated into their seed coat and embryo components aseptically using a stereo-binocular microscope. The separated seed parts were dipped in 1% sodium hypochlorite solution and incubated on V-8 agar supplemented with 40 ppm rose bengal and 100 ppm streptomycin to prevent bacterial contamination. The plates were incubated for 7 days under 12 hours NUV light alternating with 12 hours darkness. Observations were recorded on the number of colonies of *A. brassicae* and *A. brassicicola* recovered from the seed coat and embryo parts.

Experiment 3.5b

(i) The effects of *A. brassicae* and *A. brassicicola* on seed germination of oilseed rape were studied by inoculating healthy seed with isolates of each species. Two oilseed rape samples, one of Jet Neuf and another of Rafal, which were free from seed-borne fungi and showed good germination (above 95%), were soaked overnight with isolates of *A. brassicae* and *A. brassicicola* using in each case a spore

suspension of 50,000 spores/ml. Five isolates of each species, one each from cabbage, cauliflower, Brussels sprouts, turnip and rape, were tested. The seeds were dried on dry blotters for 10 minutes before sowing. Samples of 400 seeds of each cultivar/pathogen species/isolate were tested in groups of 100 seeds using the standard rolled towel test (ISTA, 1976). The paper towels were kept upright in a wire basket in a randomised block arrangement, covered with a polythene bag to prevent drying and incubated at 20 ± 2°C. The incubated samples were scored for healthy and infected seedlings after 7 days of incubation.

(ii) In a second investigation the same seed lots were inoculated with one isolate of *A. brassicae* and one of *A. brassicicola* in the same way as previously explained and 100 seeds in each seed lot were incubated on moist blotter paper discs (9 cm) in four replicates of 25 seeds each. The moist paper discs were placed in plastic humid chambers and incubated at 20 ± °C with a randomised block arrangement. The experiment was continued up to 2 weeks to give sufficient time for infection to become established on seedlings and to allow damping-off symptoms to develop. The seedlings were scored for disease development, the number of infected and dead (with damping-off) seedlings being recorded.

Experiment 3.5c

(i) In this experiment oilseed rape seed lots were inoculated with an isolate of *A. brassicae* and one of *A. brassicicola*, as in Experiment 3.5b, and then sown in malachite in 8 cm plastic pots. One hundred seeds of each seed lot/pathogen species were sown at the rate of five seeds per pot. Sufficient water was applied to support germination and

seedling growth, the pots being kept in a plastic tray within a transparent plastic chamber in a glasshouse. After 3 weeks, the seedlings were removed from the pots and the malachite was washed away by dipping the seedlings in water in a glass beaker. The seedlings were scored for disease symptoms and the number of infected and healthy seedlings recorded. The experiment was arranged as a randomised block design with four replicates, each treatment plot being represented by 5 pots grouped together.

(ii) The transfer of disease from naturally infected seeds of Rafal collected from plants heavily infected with *A. brassicae* or *A. brassicicola* was studied using perspex 'Fiji' boxes lined with blotters and filled with sterile Vermiculite. The seeds were sown between the vertical walls of the box and the blotter sheet. Lots of 50 seeds each infected with *A. brassicae* and *A. brassicicola* were used, arranging five boxes of 10 seeds for each pathogenic species. The plastic boxes were contained in a polythene bag to prevent drying. The seeds were incubated for 3 weeks and observations were made on disease development from infected seed and its progress from seed to seedling. The number of dead seeds, dead seedlings, seedlings showing wire stem symptoms and healthy seedlings were noted.

Experiment 3.5d

The transfer of *Alternaria* from seed to seedling was studied by sowing inoculated oilseed rape seed lots in malachite in 5 cm plastic pots. The seed lots of Rafal and Jet Neuf were inoculated as described in Experiment 3.5b. One lot of pots was kept in a propagating mist chamber fitted with an automatic mist spraying system and the other

set of pots was kept in a second propagating chamber without an automatic misting arrangement so that the atmospheric humidity and moisture in pots were low. The temperature was adjusted to 22 ± 2°C and the humidity was maintained around 85-90% in the first propagating chamber. The seedlings were allowed to grow for 4 weeks until clear damping-off symptoms were seen in the chambers where a high humidity was maintained. The seedlings were examined individually after removing them from each plastic pot. The number of germinated and ungerminated seeds were counted and the frequencies of pre-emergence and of post-emergence damping-off were recorded.

Experiment 3.5e

The effect of incubation temperature on the development of *A. brassicae* and *A. brassicicola* colonies on agar medium from infected oilseed rape was studied by incubating two infected seed lots on V-8 agar at 5, 10, 15, 20, 25 and 30°C for 7 days. Samples of 200 seeds in each seed lot infected with *A. brassicae* or *A. brassicicola* were tested in four replicates of 50 each. The seeds were pretreated with 1% sodium hypochlorite for 1 minute before placing on the agar medium. At the end of the incubation period the number of colonies developed at each incubation temperature was noted.

Results

EXPERIMENT 3.5a: The distribution of A. brassicae and A. brassicicola in oilseed rape seed tissues

The extent of recovery of *A. brassicae* and *A. brassicicola* from infected oilseed rape seed lots is indicated in Table 3.5.1.

TABLE 3.5.1: The location of *A. brassicae* and *A. brassicicola* in different seed parts of oilseed rape.

Seed tissue	*A. brassicae*		*A. brassicicola*	
	Lot 1	Lot 2	Lot 1	Lot 2
	*%	%	%	%
Seed coat	72	55	100	53
Embryo	45	24	80	48

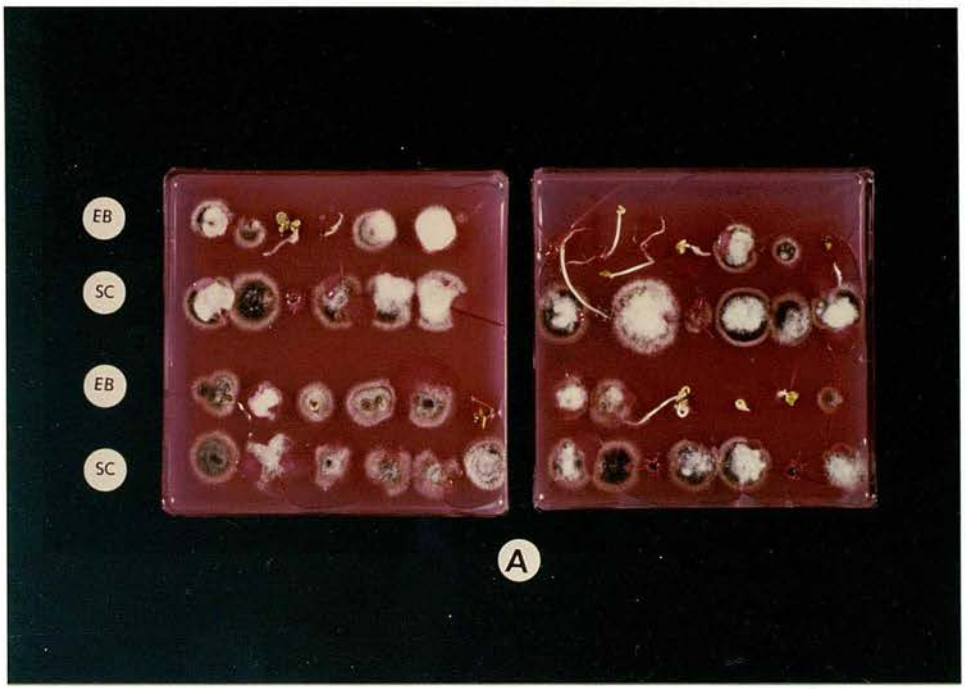
*% of seed showing infection

Both species were frequently associated with seed coats, the incidence ranging from just over 50% to 100% with one seed lot infected with *A. brassicicola*. The rates of embryo infection tended to be greater for *A. brassicicola* compared with *A. brassicae*; moreover, the ratio of embryo to seed coat infection was greater with *A. brassicicola* than with *A. brassicae* (Plate 3.5.1). With heavily diseased seeds, where the infection was deep seated, the separation of the seed coat from the embryo was noted to be difficult, especially in small size seeds.

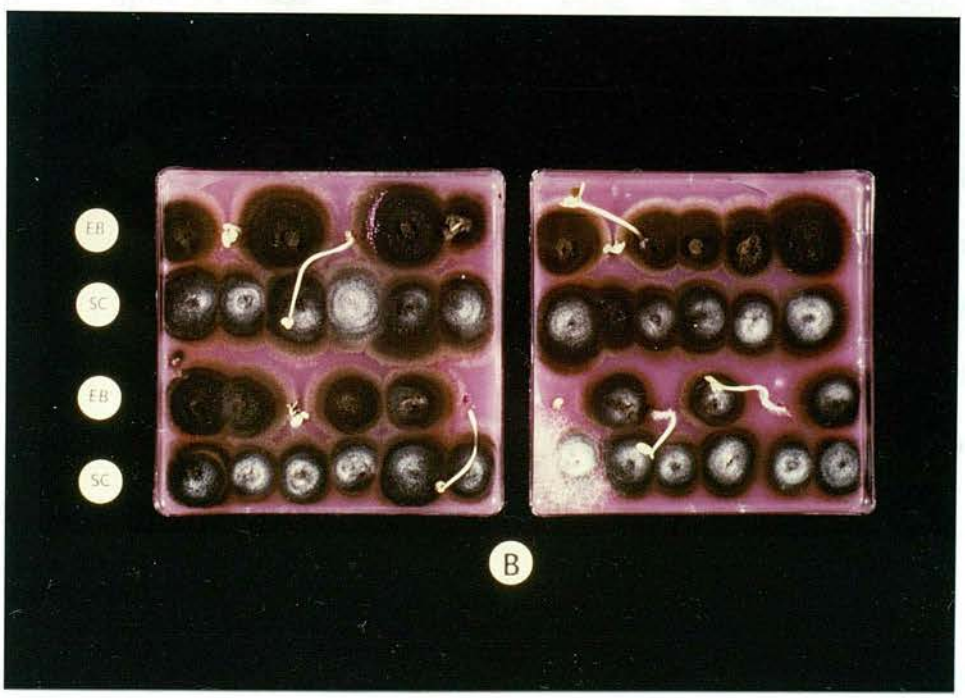
EXPERIMENT 3.5b: The effects of oilseed rape seed inoculation with A. brassicae and A. brassicicola isolates on seed and seedling infection

(i) The comparative effects of inoculating oilseed rape seed with different *Alternaria* isolates on seed and seedling infection in paper towels are indicated in Table 3.5.2. No differences were found among the isolates of *A. brassicae* and *A. brassicicola* in their effects on seed germination failure or on the production of abnormal seedlings which in some instances appeared to occur independently of *Alternaria* infection. The symptoms on seedlings were expressed in the form of a brown or grey lesions on the hypocotyl, associated with seedling decay or rot. Differences in the incidence of seedling infection due

PLATE 3.5.1: The distribution of *Alternaria* species in infected oilseed rape seed tissues (SC = seed coat; EB = embryo).



A. brassicae



A. brassicicola

TABLE 3.5.2: The percentage of seed/seedling death and seedling infection (transformed values) in oilseed rape seed inoculated with different isolates of *A. brassicae* or *A. brassicicola* and germinated in rolled paper towels.

Alternaria isolates	Percentage of seed/seedlings dead	Percentage of infected seedlings
	A. brassicae	
1	15.0	18.0
2	17.1	20.8
3	17.1	20.0
4	17.6	18.7
5	16.3	20.0
Mean	16.6	19.5
	A. brassicicola	
1	17.5	19.2
2	16.3	18.3
3	16.6	19.6
4	16.5	17.7
5	16.6	17.6
Mean	16.7	18.5
SED species mean	±0.7	±0.7
(DF=67) isolate mean	±1.5	±1.5

to the various isolates of *A. brassicae* and *A. brassicicola* were slight and non-significant. Infection symptoms were generally found where emerged seedlings carried up the seed coat, with which they remained in close contact. On the other hand, those seedlings where the seed coat carrying inoculum was pushed away from the seedling during the germination process, escaped infection.

(ii) From the results of assessments of the effects of *A. brassicae* and *A. brassicicola* on seed germination on blotters discs following incubation for 2 weeks in humid chambers (Table 3.5.3), *A. brassicicola*

TABLE 3.5.3: Effect of the inoculation of seed with *A. brassicae* and *A. brassicicola* on the incidence of seed/seedling death and percentage seedling infection (transformed values) on blotter discs in humid chamber after 2 weeks of incubation.

Infection response	*A. brassicae* Jet Neuf	*A. brassicae* Rafal	*A. brassicicola* Jet Neuf	*A. brassicicola* Rafal	SED± (DF=9)
Seedling infection	64.4	65.1	78.9	74.7	5.9
	64.7		76.8		4.2
Seed/seedling death	5.8	5.8	11.1	15.3	6.0
	5.8		13.3		4.2

was found to cause a higher rate of seed germination failure and of seedling infection than *A. brassicae* with both cultivars. The symptoms of *A. brassicae* infection of seedlings, including symptoms on the cotyledon leaves, are illustrated in Plate 3.5.2, along with symptoms of severe infection by *A. brassicicola*. *A. brassicae* produced dark brown lesions and spots on cotyledons and also necrotic streaks on the hypcotyl, whereas *A. brassicicola* produced profuse sporulation covering the entire emerging seedling. The seed coats which carried the inoculum produced light brown to dark lesions in association with the root or the shoot of the seedlings. The seedlings remained healthy when the seed coats were not attached to the seedlings. Both fungi covered the slowly emerging seeds with a profuse light pink grey mycelium, in the case of *A. brassicae*, and a dark grey spore mass, in the case of *A. brassicicola*, resulting in seed/seedling death: as already indicated, *A. brassicicola* tended to cause more seed/seedling death than *A. brassicae*.

PLATE 3.5.2: *Alternaria* symptoms on oilseed rape seedlings.

A. brassicae infection of germinating seedling showing characteristic lesions on the hypocotyl and cotyledons.

A. brassicicola symptoms on a germinating seedling showing profuse sporulation.

EXPERIMENT 3.5c: The effects of oilseed rape seed inoculation or infection with A. brassicae and A. brassicicola on seedling infection

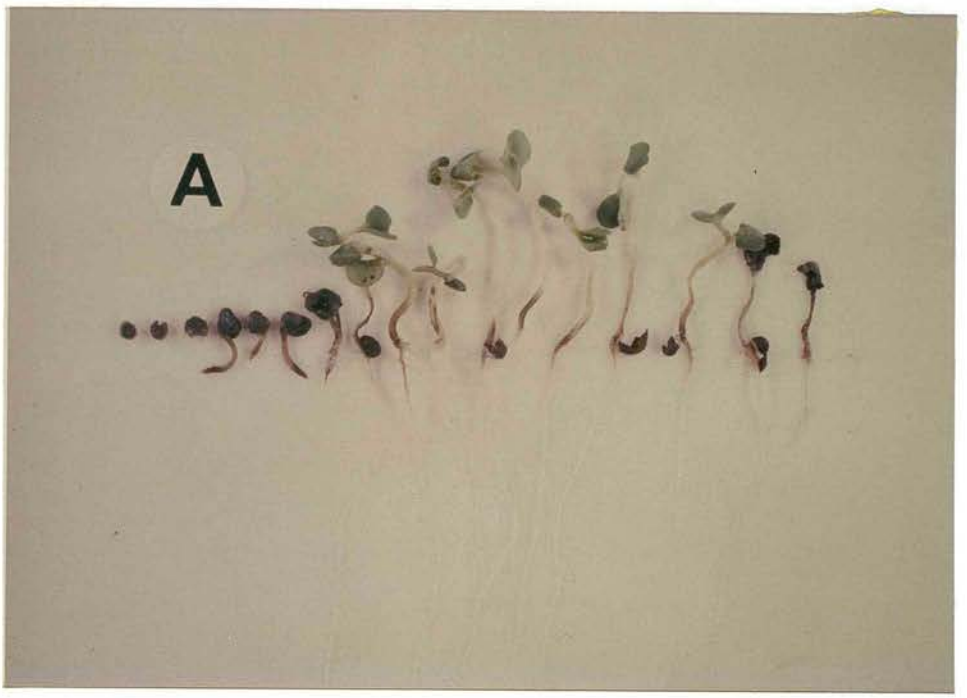
(i) Inoculation of seed with *Alternaria* did not show any adverse effect on seed germination and seedling emergence but infection symptoms were seen as light grey to dark superficial specks at the crown of the root where it was associated with the seed coat. There were no distinct symptoms on either the cotyledons or first leaves. There were no significant differences in the level of seedling infection associated with pathogen species or cultivar, although *A. brassicicola* gave slightly more infection (Table 3.5.4).

TABLE 3.5.4: The effect of seed inoculation with *A. brassicae* and *A. brassicicola* on percentage seedling infection (transformed values) of oilseed rape.

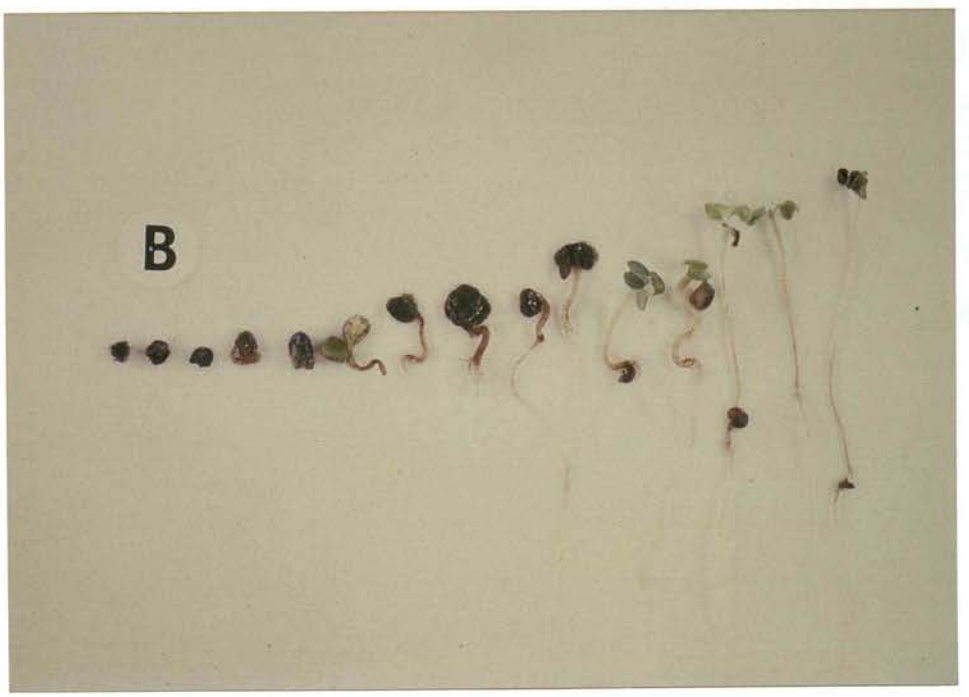
	A. brassicae		*A. brassicicola*	
	Jet Neuf	Rafal	Jet Neuf	Rafal
Seedling infection	18.1	23.5	28.7	28.5
SED± (DF=9)		4.7		

(ii) The results of observations on the development of infection symptoms in seedlings from naturally infected seeds, relating to seed rot, seedling decay and wirestem are summarised in Table 3.5.5. The number of dead seeds was slightly higher with *A. brassicae* infected seeds, whereas the incidence of pre-emergence seedling mortality and of wire stem were greater in seeds infected with *A. brassicicola*: the percentage of seedlings free from symptoms was lower with *A. brassicicola* compared with *A. brassicae*. The symptoms associated with infected seed of the two species are illustrated in Plate 3.5.3: severely infected

PLATE 3.5.3: Symptoms of seed rot, seedling decay, wire stem and damping-off on oilseed rape seedlings caused by *Alternaria* species.



A. brassicae



A. brassicicola

seeds failed to germinate or to give rise to normal seedlings, with deep-seated infection producing seed rot or seedling decay.

TABLE 3.5.5: The effect of *A. brassicae* and *A. brassicicola* infection of oilseed rape seed on the incidence of seed rot, seedling pre-emergence and post-emergence mortality and wire stem.

Infection category	*A. brassicae*	*A. brassicicola*
Dead seed	14	6
Pre-emergence death	20	40
Post-emergence death	28	24
Wire stem	8	18
Seedling without symptoms	30	12

EXPERIMENT 3.5d: Seedling infection by A. brassicae and A. brassicicola from inoculated seeds of oilseed rape in relation to moisture conditions

The assessment of the effects of *Alternaria* infection on seedlings from artificially inoculated seeds, summarised in Table 3.5.6, showed a significant effect of moisture treatment on seedling response (Plate 3.5.4). High moisture conditions gave a high incidence of pre-emergence seedling mortality, particularly in the case of *A. brassicicola*: where automatic misting was applied, all the seedlings that emerged were subsequently killed with both fungal species. In the dry chamber, where the incidence of seedling death was generally lower, there was no marked difference in the incidence of mortality relating to the two *Alternaria* species. Although there was no significant overall effect of cultivar on mortality, there was a significant interaction between cultivar, humidity treatment and pathogen. In the case of *A. brassicicola* the response of the two cultivars was similar. However, with *A. brassicae* the two

TABLE 3.5.6: The percentage incidence of pre-emergence and post-emergence seedling mortality (transformed values) in relation to seed inoculation with *Alternaria* species and humidity (mean of two cultivars).

Seedling response	Pathogen	Humidity condition: Wet	Dry	Mean
Pre-emergence mortality	*A. brassicae*	71.7	31.8	51.7
	A. brassicicola	83.5	38.0	60.8
	Mean	77.6	34.9	
Post-emergence mortality	*A. brassicae*	18.3	25.9	22.1
	A. brassicicola	6.5	28.2	17.3
	Mean	12.4	27.1	
Total mortality	*A. brassicae*	90.0	57.8	73.9
	A. brassicicola	90.0	66.2	78.1
	Mean	90.0	62.0	

			Pre-emergence mortality	Post-emergence mortality	Total mortality
SED (DF=21)	Pathogen mean	±	2.4	2.4	1.8
	Humidity mean	±	2.4	2.4	1.8
	Pathogen x humidity mean	±	3.4	3.4	2.5

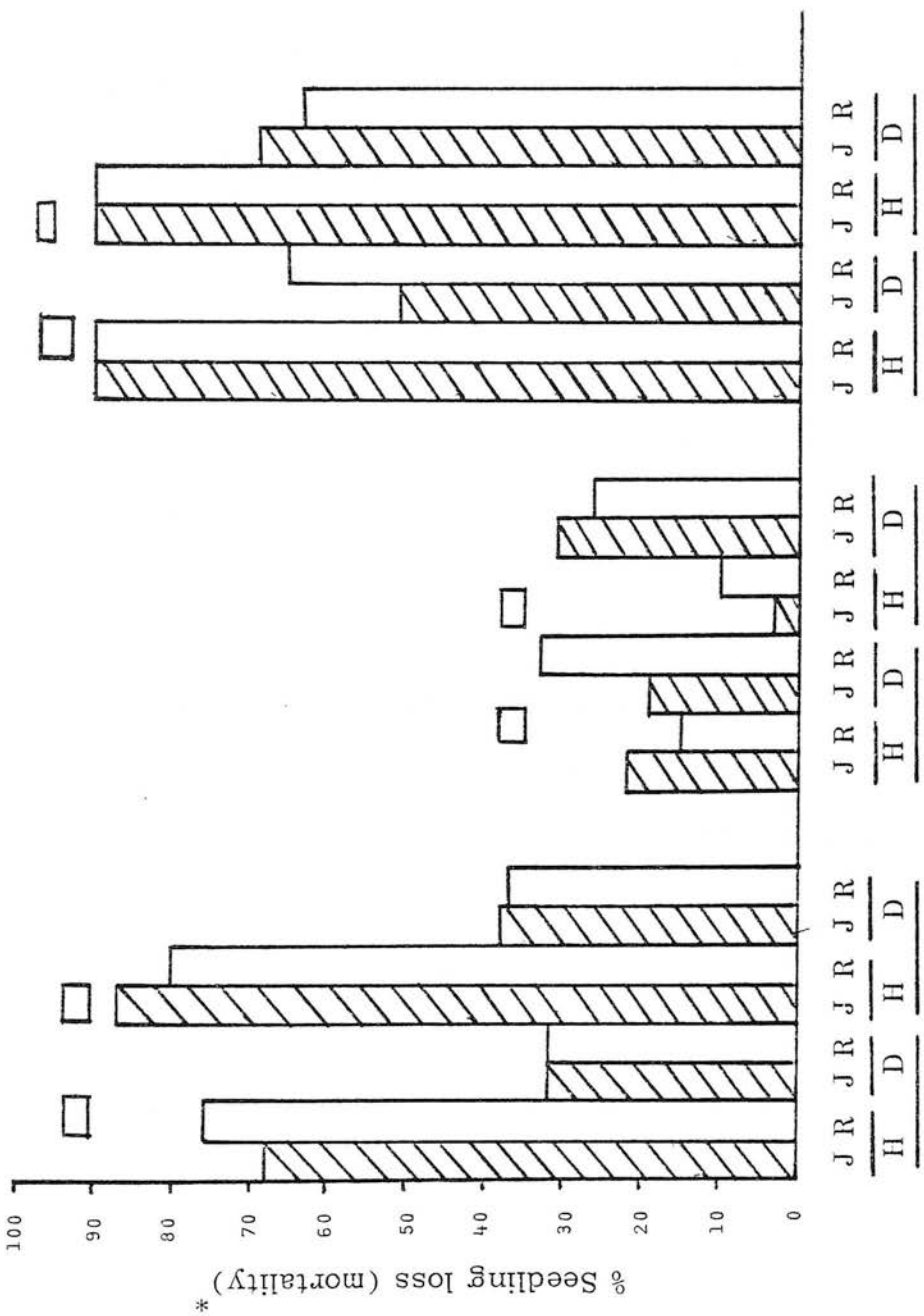
cultivars showed similar levels of post-emergence mortality where there was automatic misting, but in the dry chamber Jet Neuf gave significantly less post-emergence death than Rafal (Figure 3.5.1): the difference, however, was only slight.

PLATE 3.5.4: Seedling infection from *Alternaria* inoculum applied to seed of oilseed rape (cultivar Rafal).

A. brassicae: (1) Humid, and (2) Dry chambers.

A. brassicicola: (1) Humid, and (2) Dry chambers.

FIGURE 3.5.1: The percentage pre- and post-emergence loss of Jet Neuf (J) and Rafal (R) seedlings inoculated with *A. brassicae* (A) and *A. brassicicola* (B), and incubated in humid (H) and dry (D) conditions.



(*Transformed values)

EXPERIMENT 3.5e: The effect of incubation temperature on the development of A. brassicae and A. brassicicola from infected oilseed rape seed

The growth responses to various incubation temperatures of *A. brassicae* and *A. brassicicola* from infected oilseed rape seed samples are given in Table 3.5.7.

TABLE 3.5.7: Effect of incubation temperature on seed infection counts for *A. brassicae* and *A. brassicicola* in agar plate tests.

Incubation temperature (°C)	Percentage infection of *A. brassicae*		*A. brassicicola*	
	Lot A	Lot B	Lot C	Lot D
5	5	4	13	6
10	28	12	100	20
15	28	13	100	19
20	27	14	100	20
25	31	12	100	21
30	9	5	99	18

The seed samples infected with *A. brassicae* showed a more or less similar number of colonies over the range of 10-25°C, while, at 5° and 30°C, fungal growth was markedly reduced. With *A. brassicicola*, the maximum colony production rate was again evident over the range 10-25°C, but there was virtually no decline at 30°C: at 5°C growth was again markedly reduced.

Discussions

In the seed lots investigated it was found that, with both *A. brassicae* and *A. brassicicola*, a proportion of infected seed showed embryo infection. This deep-seated internal infection may explain the reasons for lower seed viability in naturally infected *Brassica* seed

samples. The ratio of seed coat to embryo infection was higher in the case of *A. brassicicola*. Maude and Humpherson-Jones (1980) reported that *A. brassicicola* is capable of infecting embryos in heavily infected samples of cabbage and kale. Although earlier workers (Domsch, 1957; Neergaard, 1979) reported that *Alternaria* is confined to the seed coat in *Brassica* seeds, it is also possible that, when the infection occurs at an early seed maturation stage, the mycelium invades the soft tissues and becomes established in the embryo under prolonged humid conditions.

When seed was inoculated with *A. brassicae* and *A. brassicicola* isolates and germinated in paper towels for 7 days, responses to the different inoculation treatments were slight and similar. The seedlings exhibited rapid extension growth and appeared to escape much of the infection. However, where paper discs were used and the incubation period was longer, more infection was found with both pathogen species, possibly due to the slower extension growth of seedlings: *A. brassicicola* gave rise to more infection than *A. brassicae*, which may be attributed in part to its greater sporulation capacity.

In comparing inoculated (contaminated) with infected seed, much greater damage occurred in the latter case for both *Alternaria* species, expressed in the form of seed decay and seedling damping-off. This would suggest that most seedling emergence damage due to *Alternaria* relates to early pod infection in the field rather than to late, superficial contamination of seed.

When inoculated seed was incubated in malachite chippings in a glasshouse in dry conditions or wet conditions, there was little difference in the effects of the two *Alternaria* fungi on emergence and seedling infection apart from the slightly greater pre-emergence mortality with *A. brassicicola*. In comparing the effects of moisture, wet conditions

aggravated infection and resulted in 100% seedling mortality with both *Alternaria* species. In dry conditions seedling mortality, more especially pre-emergence mortality, was considerably reduced.

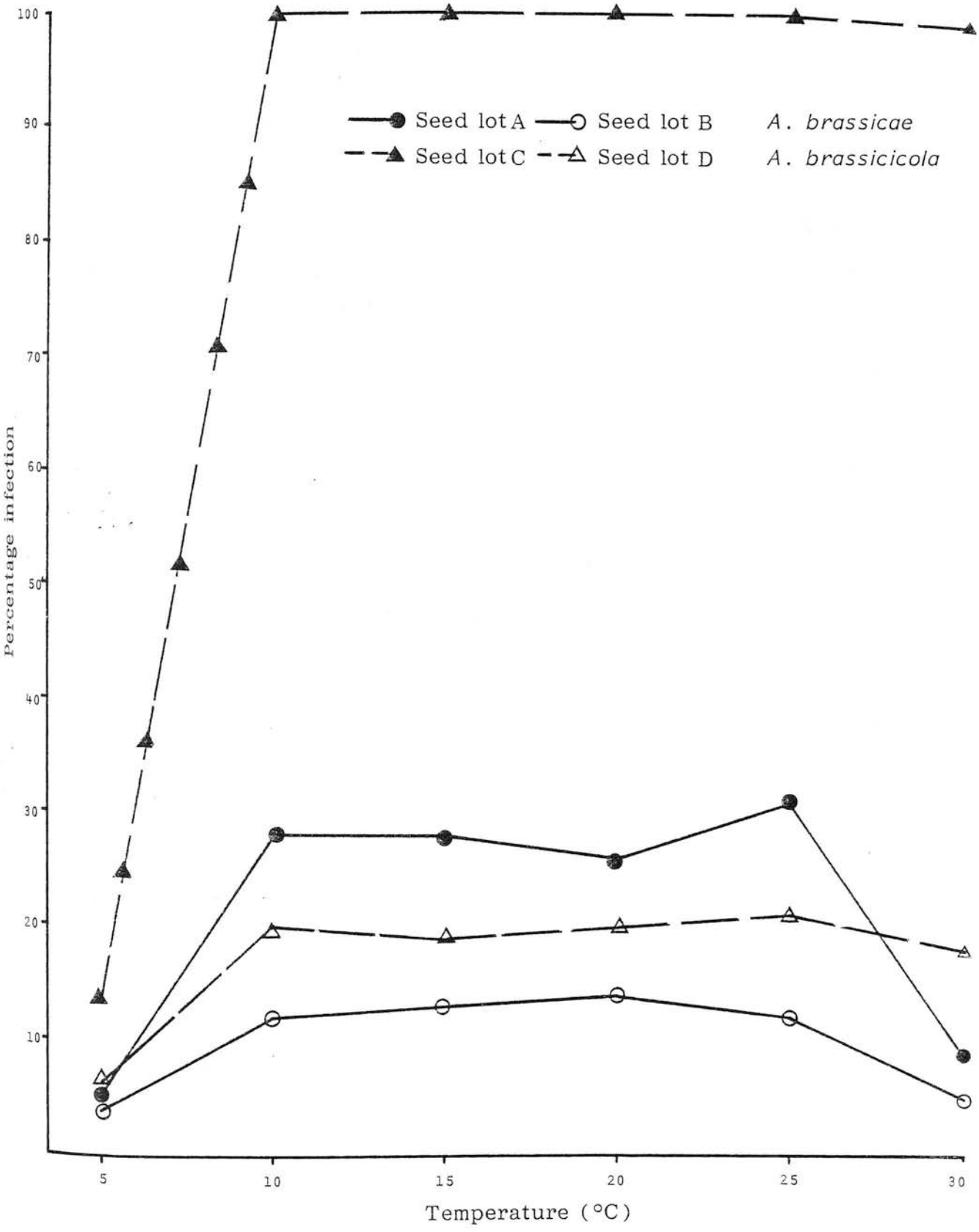
There was no evidence of variation in the effects of the different isolates of both *Alternaria* species on seed germination.

Richardson (1970) reported that *A. brassicae* and *A. brassicicola* associated with *Brassica* seeds had no effect on the number of seedlings produced in seed samples. Petrie (1974a) reported that seed germination, seedling emergence, and seedling survival in naturally infected seeds of *B. napus* or *B. campestris* sown in sand or soil appeared to be completely unrelated to the level of *Alternaria* in the sample: when seeds of rape and turnip rape were heavily inoculated with *A. brassicae* and sown in soil the reduction in stand was no more than 8%. Similar observations were also made by Vannacci (1981) on cauliflower samples naturally infected with *A. brassicicola*: no influence of the fungus on seed germination was found in germination tests using Jacobson's apparatus (ISTA, 1976). However, Bassey and Gabrielson (1983a) recorded a reduction in cabbage seedling emergence in seed naturally infected with *A. brassicicola*. In the present investigations it was observed that both *Alternaria* species could cause 100% seedling mortality when inoculated seeds are incubated for extended periods of 3-4 weeks at high moisture levels in a glasshouse. It is clear that either external contamination or superficial infection could cause seedling infection, resulting in wirestem or damping-off under prolonged wet and warm conditions.

The results of the assessments of the effects of incubation temperature on *A. brassicae* and *A. brassicicola* colony development from seed on V-8 agar suggest that both *Alternaria* species are capable of developing actively at a wide range of temperatures from 10-25°C, while

A. brassicicola also showed active growth at 30°C (Figure 3.5.2). Both species showed a marked reduction in infection counts at 5°C. Bassey and Gabrielson (1983b) recorded maximum *A. brassicicola* infection at 20 and 25°C using the 2-4D method in Percival growth chambers with white fluorescent light. The slight variation in results associated with different studies may be attributed to different incubation conditions, but the observations are in keeping with the general findings indicating a higher temperature tolerance for *A. brassicicola* than for *A. brassicae*.

FIGURE 3.5.2: Effects of incubation temperature on the development of *A. brassicae* and *A. brassicicola* from infected seed lots.



3.6 STUDIES ON THE EFFECTIVENESS OF DIFFERENT SEED TREATMENTS FOR THE CONTROL OF *A. BRASSICAE* AND *A. BRASSICICOLA* OF *BRASSICA* CROP PLANTS

Introduction

In the general review of literature (Section 2), the importance of seed-borne transmission as a primary source of *Alternaria* infection was indicated and the use of various seed treatments considered. In this experimental section, interest is focussed on the relative effectiveness of different treatments as practical methods for controlling seed-borne inoculum. According to Dixon (1981), hot water treatment at 50°C for 25 minutes is the traditional method of decontaminating seed: Schimmer (1953) observed that seed infection may be largely controlled by steeping in hot water at 50°C for 18 minutes. Neergaard (1969) reduced substantially the infection of *A. brassicicola* in cabbage with 0.2% Germisan (mercury chlorophenol) at 50°C for 5 minutes (65% to less than 1%). Muade, Vizor and Shuring (1969) reported that thiram (tetramethylthiuram disulphide) soaking at low temperatures (0.2% thiram for 24 hours at 30°C) gave good control of disease, besides keeping a high germinability of seed. More recently, iprodione has been used, not only as a spray, but also as a seed dressing (Maude, Humpherson-Jones, Bambridge and Spencer, 1980).

In the present studies the effects of hot water treatments and of fungicide soak treatments have been assessed, along with hot air treatments of seed and use of fungicide dressings. Four studies were carried out as follows:

Experiment 3.6a: The effects of hot water treatments of seed on the control of *Alternaria* infection.

Experiment 3.6b: The effects of fungicide soak treatments of seed on the control of *Alternaria* infection.

(i) High temperature soak
(ii) Low temperature soak

Experiment 3.6c: The effects of hot air treatments of seed on the control of *Alternaria* infection.

Experiment 3.6d: The effects of fungicide treatments of seed on the control of *Alternaria* infection.

Materials and Methods

Experiment 3.6a

Two oilseed rape seed lots (1 and 2) infected with *A. brassicae* and a further two lots (1 and 2) infected with *A. brassicicola* were treated with hot water, using a standard laboratory water bath in which the seed samples were held loosely in a muslin cloth suspended in hot water. Three temperature levels (±0.2°C) were applied, 50°C, 55°C and 60°C, and seed samples were exposed for periods of 5, 10, 15, 20, 25 and 30 minutes. At the end of each treatment the samples were removed from the water bath and dried in a laminar flow cabinet on filter paper. From each seed lot and temperature/time treatment, 200 seeds were incubated in four replicates of 50 seeds each on V-8 agar for seed infection counts, and 400 seeds were tested in four replicates of 100 seeds each for germination effects (ISTA, 1976).

Experiment 3.6b

(i) Samples from the same seed lots as in the previous experiment were held at 50°C and 55°C in a fungicide suspension (0.2%) of thiram and iprodione liquid or iprodione dust (Rovral) in water using similar

procedures and times as described in Experiment 3.6a. The treated seeds were tested as previously for infection and germination.

(ii) Further samples from the seed lots were held at 30°C for 24 hours, in water alone or in thiram, iprodione liquid and iprodione dust liquid suspensions at a 0.2% concentration. The seeds were then tested for infection and germination.

Experiment 3.6c

The four oilseed rape samples were exposed to hot air using a "T.S. crop tester" (Tower Silos Ltd, Bath). Seeds were placed on a metal grid through which heated air was passed. The crop tester contains a small heating unit comprising a 1 kW heater and a 40 W fan, and was fitted with a 5 amp rheostat to allow the temperature of the air to be adjusted. Temperature was measured throughout each treatment with a Chromel-Alumel type K thermocouple (Model 1751, Digitron Instrumentation Ltd) at an accuracy of ±1°C. The seeds were treated at 50°C, 55°C and 60°C for 10, 20 and 30 minutes. Two hundred seeds were tested for *Alternaria* infection and 400 seeds were tested for germination as described in Experiment 3.6a.

Experiment 3.6d

Samples of oilseed rape infected with *A. brassicae* and *A. brassicicola* were treated at 2 g/kg as dry dressing with six seed dressing chemicals, copper oxychloride (Blitox), benomyl (Benlate), captan (Orthocide), iprodione (Rovral), thiram (Tripomol 80) and thiobendazole (Storite). Seeds were treated by mixing the seed and chemical in a flask and shaking for 10 minutes. Two hundred seeds in each treatment

in four replicates of 50 seeds per treatment were incubated 1 day after treatment on V-8 agar for seed infection assessments, and 400 seeds were tested in replicates of 100 each for seed germination using rolled paper towels (ISTA, 1976).

Results

EXPERIMENT 3.6a: The effects of hot water treatment of seed on the control of Alternaria infection

From the analysis of variance of the transformed percentage data, levels of seed infection were found to vary significantly with seed lot, temperature and exposure time and there were significant interactions between the factors for both pairs of seed lots. The results of assessments of seed infected with *A. brassicae* and with *A. brassicicola* in relation to seed lot and treatment are summarised in Table 3.6.1 (overleaf). Seed lot 1 gave more infection than seed lot 2, but in both cases treatment at 60°C for 5 minutes or more completely controlled *A. brassicae*: with lot 2, treatments at 50°C or 55°C for 15 minutes or, with lot 1, treatment at the same temperatures for 20 minutes eliminated infection. At 50°C and 55°C, shorter periods of exposure of 5, and particularly 10, minutes still gave significant reductions in infection levels. *A. brassicicola* infection was very high in one seed lot and at a moderate level in the other. However, complete control of *A. brassicicola* seed infection was obtained in both seed lots with a hot water temperature of 60°C for 15 minutes or more; none of the other combinations of temperature and time completely controlled *A. brassicicola* but 50°C or 55°C for 30 minutes or 60°C for 10 minutes produced very low levels of infection.

The effects of hot water treatments on seed germination are summarised in Table 3.6.2. From the analyses of variance of the data,

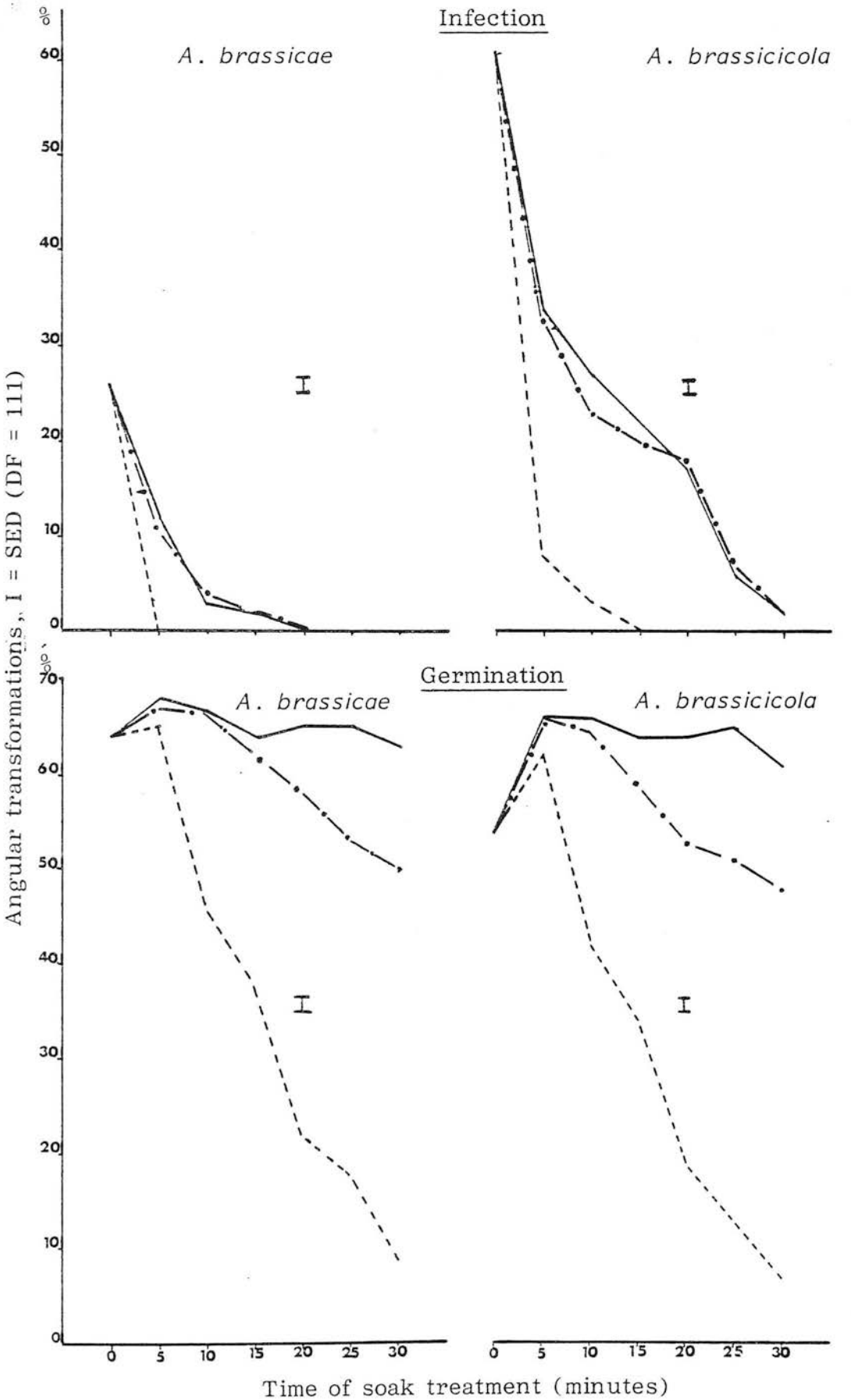
TABLE 3.6.1: The effects of hot water treatments of oilseed rape on the percentage (transformed values) of *Alternaria* infection in different seed lots.

Temperature (°C)	Time (minutes)	*A. brassicae* infection		*A. brassicicola* infection	
		Seed lot 1	Seed lot 2	Seed lot 1	Seed lot 2
50	5	15.8	7.8	48.2	20.1
	10	4.9	2.0	40.9	13.1
	15	4.7	0.0	38.5	6.1
	20	0.0	0.0	29.4	4.9
	25	0.0	0.0	8.5	4.1
	30	0.0	0.0	2.9	2.0
55	5	13.4	6.9	45.9	19.7
	10	6.1	2.0	33.3	12.4
	15	4.9	0.0	30.0	9.8
	20	0.0	0.0	30.1	7.0
	25	0.0	0.0	9.7	4.1
	30	0.0	0.0	2.9	2.0
60	5	0.0	0.0	11.9	4.9
	10	0.0	0.0	4.1	2.0
	15	0.0	0.0	0.0	0.0
	20	0.0	0.0	0.0	0.0
	25	0.0	0.0	0.0	0.0
	30	0.0	0.0	0.0	0.0
Control (untreated)	-	33.0	19.9	88.0	34.0
SED ± (DF = 111)	1.9	1.9	1.9	3.4	3.4

TABLE 3.6.2: The effects of hot water treatments of oilseed rape seed on the percentage germination (transformed values) in different seed lots.

Temperature (°C)	Time (minutes)	*A. brassicae* infection		*A. brassicicola* infection	
		Seed lot 1	Seed lot 2	Seed lot 1	Seed lot 2
50	5	66.3	70.3	64.0	68.3
	10	64.8	68.6	64.2	67.7
	15	64.4	64.5	64.4	64.4
	20	64.0	65.2	64.1	64.4
	25	63.7	64.4	64.8	64.8
	30	63.3	63.5	60.6	61.8
55	5	64.9	69.1	64.0	68.7
	10	66.0	67.1	64.6	64.0
	15	61.5	63.7	58.6	59.5
	20	55.9	60.1	52.0	54.0
	25	52.7	53.9	49.4	52.4
	30	47.2	52.3	46.9	49.2
60	5	64.0	66.2	60.9	63.1
	10	47.2	44.9	41.1	43.8
	15	36.6	39.2	32.4	36.4
	20	22.8	22.5	17.0	20.2
	25	18.0	18.5	11.3	15.4
	30	8.8	9.2	4.9	8.4
Control	-	63.5	64.5	53.5	53.8
SED ± (DF = 111)		2.4	2.4	2.3	2.3

FIGURE 3.6.1: Seed infection percentage and germination percentage in seed lots of oilseed rape infected with *A. brassicae* and *A. brassicicola* in relation to hot water treatment at ——— 50°C, —·— 55°C and --- 60°C.



significant overall differences were associated with seed lot, temperature and time treatments, but with significant interactions between time and temperature. The differences between seed lots in both infection groups were very slight: seed lots with less infection tended to give higher rates of germination. With *A. brassicae* infected lots, germination rates declined relative to those of untreated seed with increased periods of exposure above 15 minutes at 55°C or above 5 minutes at 60°C. This decline was very marked at 60°C. Treatment at 50°C had little effect on germination. In the case of *A. brassicicola* infected seed lots, water at 50°C over the range of times, at 55°C for up to 15 minutes and at 60°C for up to 5 minutes improved germination slightly but longer periods of treatment at 55°C and especially at 60°C reduced germination. The responses, averaged for the seed lots within each infection group, are illustrated in Figure 3.6.1.

EXPERIMENT 3.6b: The effect of fungicide soak treatments of seed on the control of Alternaria infection

(i) High temperature

The percentage of *Alternaria* infection and seed germination in oilseed rape samples soaked in different fungicides at 50°C or 55°C are given in Table 3.6.3. In general, levels of *Alternaria* were low in all treated samples: complete control of *A. brassicae* was obtained with all the three fungicides using soaks of 20 minutes at 50°C or of 15 minutes at 55°C. With *A. brassicicola* infection, longer soaks of 25 minutes at 55°C were required for complete elimination of the fungus: at 50°C, there was still slight infection in the more heavily infected seed lot after 30 minutes soaking. In comparing various fungicides, iprodione tended to be more effective than thiram in reducing *A. brassicae*

TABLE 3.6.3: The effects of different fungicide soak treatments of oilseed rape on the percentage (transformed values) of *Alternaria* infection in different seed lots (average of three fungicides).

Temperature of soak (°C)	Time of soak (minutes)	*A. brassicae* infection		*A. brassicicola* infection	
		Seed lot 1	Seed lot 2	Seed lot 1	Seed lot 2
50	5	8.3	4.7	32.1	13.5
	10	4.7	1.4	24.0	10.9
	15	2.7	0.0	17.1	6.7
	20	0.0	0.0	11.4	4.1
	25	0.0	0.0	5.5	2.0
	30	0.0	0.0	0.7	0.0
55	5	6.7	3.4	7.9	4.7
	10	2.0	0.7	2.0	0.7
	15	0.0	0.0	0.7	0.0
	20	0.0	0.0	0.7	0.0
	25	0.0	0.0	0.0	0.0
	30	0.0	0.0	0.0	0.0
Control	-	33.0	19.9	88.0	34.0
SED ± (DF = 223)		0.9	0.9	1.5	1.5

but the response of *A. brassicicola* was similar to all three fungicide treatments (Figure 3.6.2).

The germination rates of seed lots soaked in fungicide at different temperatures for different times is presented in Table 3.6.4. There was no significant reduction in germination rates with 50°C soaks and levels were improved compared with untreated seed lots infected with *A. brassicicola*. However, at 55°C germination rates were adversely affected at longer periods of exposure above 15 minutes.

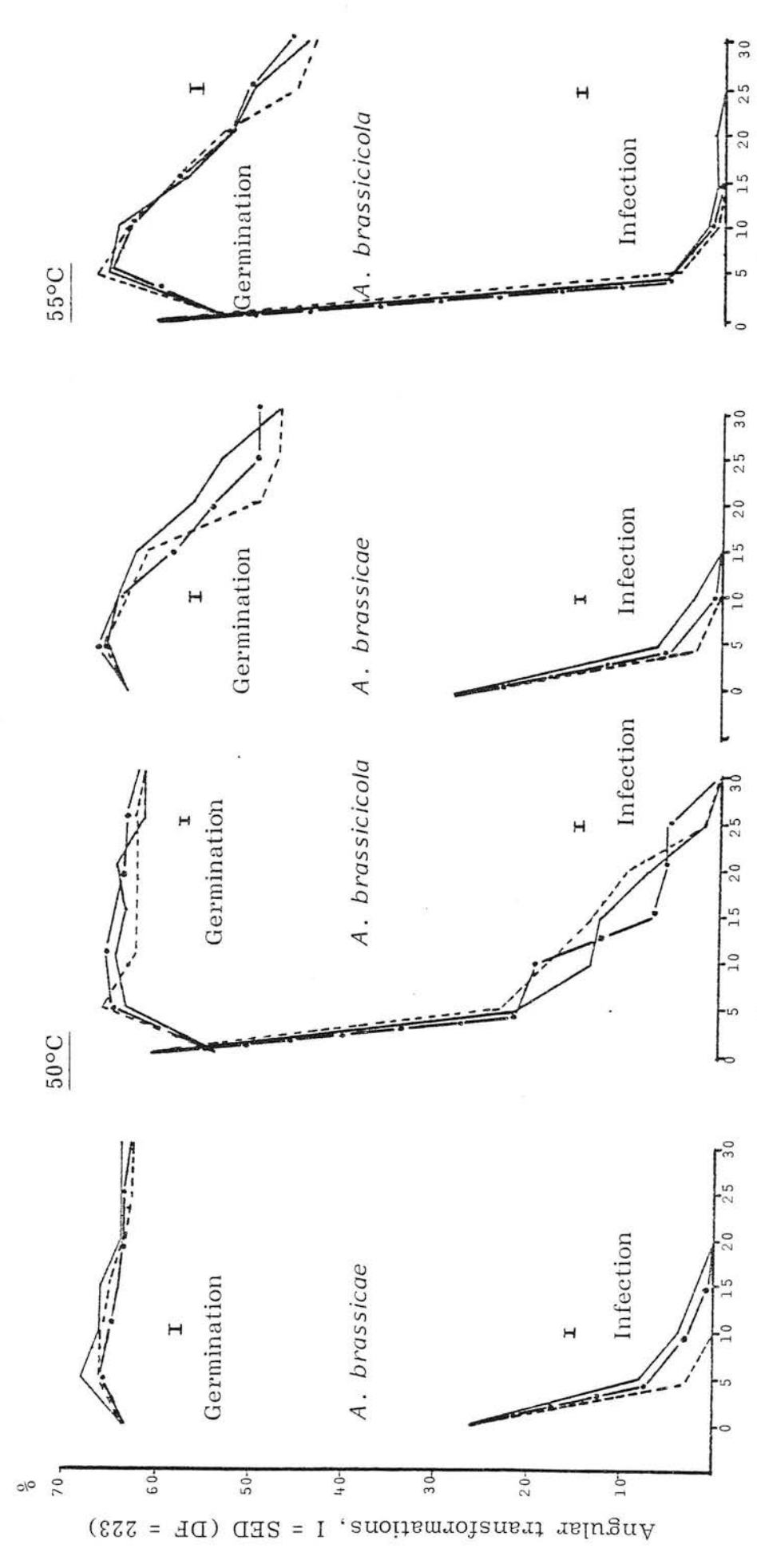


FIGURE 3.6.2: Germination percentage and infection percentage in oilseed rape seed lots infected with either *A. brassicae* or *A. brassicicola* in relation to different fungicide soak treatments for different times at 50°C or 55°C. —— Thiram, ·-·-· Iprodione liquid and ----- Iprodione dust.

TABLE 3.6.4: The effects of different fungicide soak treatments of oilseed rape in the percentage (transformed values) germination in different seed lots (average of three fungicides).

Temperature of soak (°C)	Time of soak (minutes)	*A. brassicae* infection		*A. brassicicola* infection	
		Seed lot 1	Seed lot 2	Seed lot 1	Seed lot 2
50	5	65.2	68.4	63.7	66.7
	10	64.4	67.1	63.9	65.6
	15	64.0	65.7	63.8	64.5
	20	63.8	64.5	63.6	64.2
	25	63.6	64.0	62.6	63.2
	30	63.4	63.4	61.4	63.0
55	5	64.9	68.6	65.0	68.0
	10	64.3	65.5	64.5	64.5
	15	60.2	62.2	57.5	60.0
	20	52.6	56.1	49.2	53.0
	25	48.8	52.6	47.7	51.2
	30	46.8	49.8	42.8	47.9
Control		63.5	64.5	53.5	53.8
SED ± (DF = 223)		1.2	1.2	1.1	1.1

(ii) Low temperature

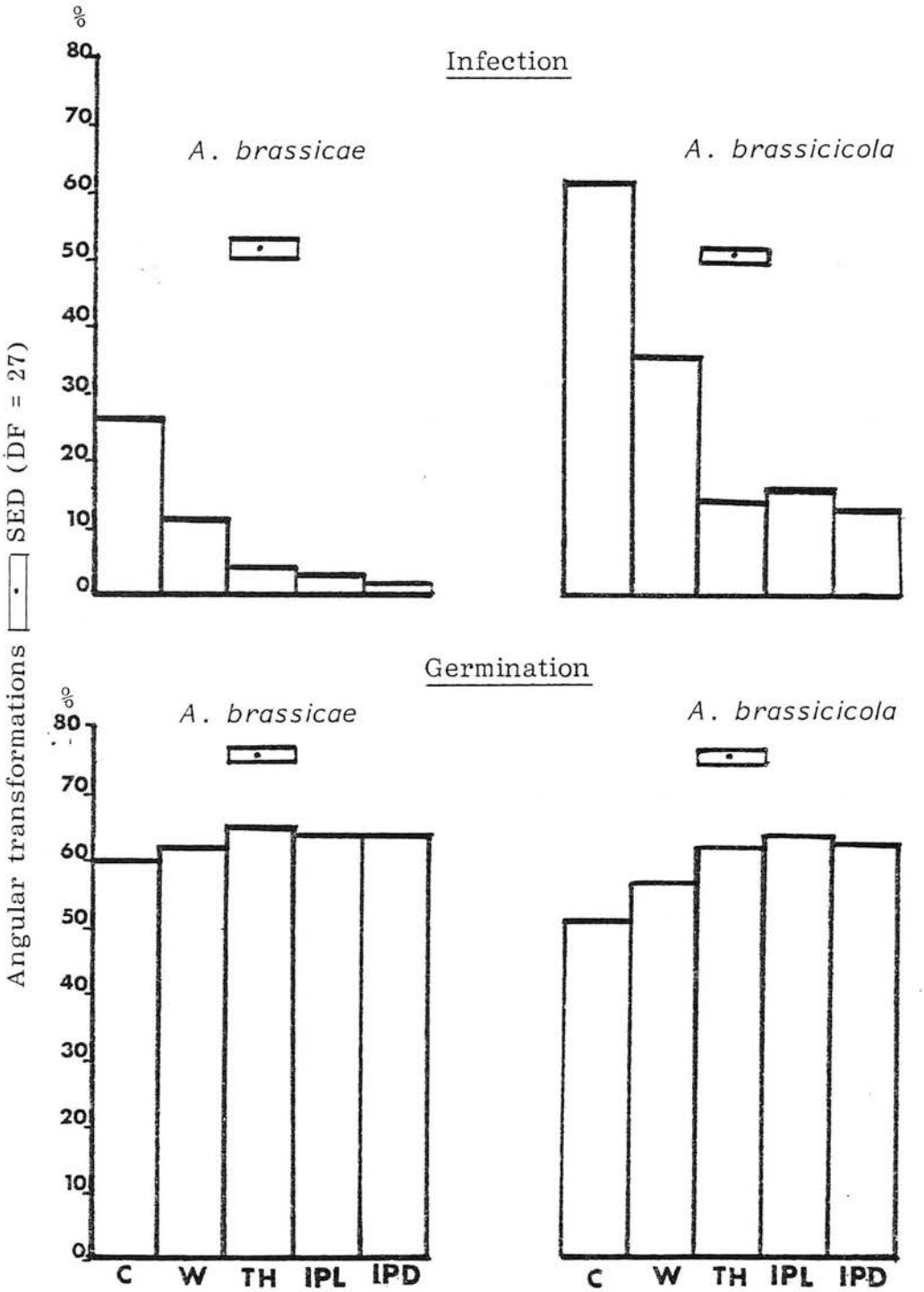
The results of soak treatments at 30°C for 24 hours are given in Table 3.6.5 and illustrated in Figure 3.6.3.

TABLE 3.6.5: The effects of fungicide soak treatments at low temperature (30°C) for 24 hours on the percentage of *Alternaria* infection and germination (transformed values) in different seed lots of oilseed rape.

Seed treatment	Seed lot 1	Seed lot 2	Seed lot 1	Seed lot 2
	A. brassicae infection		*A. brassicicola* infection	
Untreated	31.1	20.9	88.0	35.1
Water 30°C, 24 hours	15.2	7.0	48.2	23.8
Fungicide:				
thiram	6.1	2.0	23.0	5.0
iprodione liquid	4.1	2.0	23.8	9.0
iprodione dust	2.0	0.0	23.1	2.0
SED ± (DF = 27)	3.0	3.0	2.5	2.5
	Seed germination			
Untreated	60.0	60.4	47.6	55.2
Water 30°C, 24 hours	60.9	62.4	56.8	56.8
Fungicide:				
thiram	64.6	66.1	60.9	63.7
iprodione liquid	64.0	64.3	63.1	64.0
iprodione dust	65.0	63.2	62.6	63.3
SED ± (DF = 27)	2.3	2.3	1.7	1.7

Soaking in water gave some reduction in infection, while all three fungicides reduced infection substantially but did not eliminate it. In the case of *A. brassicicola* infection, there was a significant interaction between seed lot and treatment. With the seed lot severely infected with *A. brassicicola*, there was still an appreciable level of infection after treatment and the relative extent of reduction was less

FIGURE 3.6.3: Percentage infection and percentage germination of oilseed rape lots infected with *A. brassicae* or *A. brassicicola* in relation to soak treatment at 30°C for 24 hours with different fungicides.



Key:

C = Untreated control
W = Water
TH = Thiram
IPL = Iprodione liquid
IPD = Iprodione dust

than with seed lot 4. There was no significant difference between the different fungicides. Germination rates were slightly improved by water soak alone and slightly improved a little more by adding fungicide.

EXPERIMENT 3.6c: The effect of hot air treatment of seed on the control of Alternaria infection

The effect of hot air treatment on the level of *Alternaria* infection in different seed lots is given in Table 3.6.6.

TABLE 3.6.6: The effects of hot air treatments of oilseed rape on percentage (transformed values) of *Alternaria* infection in different seed lots.

Temperature (°C)	Time (minutes)	*A. brassicae* infection		*A. brassicicola* infection	
		Seed lot 1	Seed lot 2	Seed lot 1	Seed lot 2
50	10	31.7	19.4	90.0	29.2
	20	24.1	20.3	88.0	25.7
	30	13.9	11.1	87.1	19.2
55	10	20.1	9.0	85.1	21.2
	20	16.7	6.4	84.2	23.0
	30	12.6	7.0	84.2	17.3
60	10	18.2	9.0	90.0	20.2
	20	14.6	8.5	85.1	20.4
	30	12.6	7.0	88.0	21.4
Control	-	31.4	23.9	90.0	32.8
SED ± (DF = 57)		3.3	3.3	3.3	3.3

In the case of *A. brassicae*, increasing temperature and increasing times of exposure to hot air gave some reduction in infection levels; with *A. brassicicola*, there was no significant difference between different temperature/time treatments and hot air treatments on average gave only a small reduction in level of infection. Germination rates

tended to be reduced with hot air treatments, the reduction being particularly marked from exposure to 60°C for 20 minutes or more (Table 3.6.7). The overall effects are summarised in Figure 3.6.4.

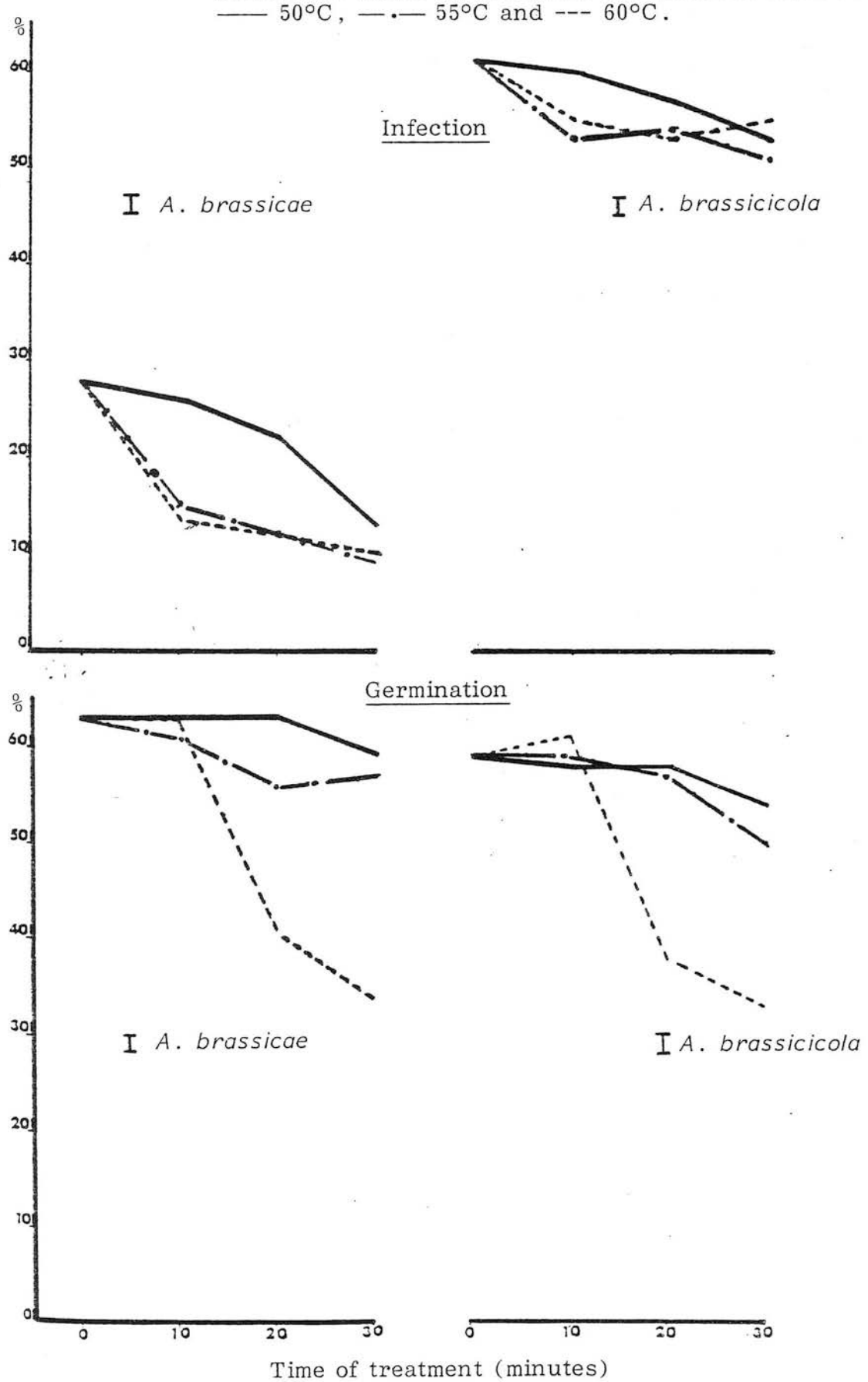
TABLE 3.6.7: The effects of hot air treatments of oilseed rape on the percentage (transformed values) germination in different seed lots.

Temperature (°C)	Time (minutes)	*A. brassicae* infection		*A. brassicicola* infection	
		Seed lot 1	Seed lot 2	Seed lot 1	Seed lot 2
50	10	61.6	64.9	55.7	61.0
	20	62.6	63.4	54.8	61.2
	30	56.5	61.2	55.0	57.6
55	10	60.4	61.9	56.5	61.2
	20	53.9	57.6	56.8	56.5
	30	56.7	56.5	48.5	51.0
60	10	63.0	63.6	59.9	61.4
	20	40.0	42.0	33.9	41.4
	30	33.8	35.2	30.9	35.3
Control	-	62.1	63.3	56.0	62.9
SED ± (DF = 57)		2.1	2.1	2.8	2.8

EXPERIMENT 3.6d: The effects of fungicide seed treatment on the control of Alternaria infection

From the results of seed treatment with different fungicides on seed infection (Table 3.6.8) it may be seen that, in the case of *A. brassicae* seed infection, iprodione dust followed by thiram and captan gave relatively good control in both seed lots while thiobendazole, benomyl and copper oxychloride gave only moderate levels. With *A. brassicicola*, there was a significant interaction between fungicide treatment and seed lot: with more severely infected samples, thiram,

FIGURE 3.6.4: Percentage infection and percentage germination of oilseed rape seed lots infected with *A. brassicae* and *A. brassicicola* in relation to hot air treatment for different times at —— 50°C, —·— 55°C and --- 60°C.



captan and iprodione only gave some control, the degree of control being best with thiram; with less severely infected samples, only thiobendazole failed to give a significant reduction while iprodione, captan and thiram gave similar levels of control which were superior to those of benomyl and copper oxychloride.

TABLE 3.6.8: The effects of different fungicide seed treatments of oilseed rape on the percentage (transformed values) of *Alternaria* infection in different seed lots.

Treatment (Fungicides)	*A. brassicae* infection		*A. brassicicola* infection	
	Seed lot 1	Seed lot 2	Seed lot 1	Seed lot 2
Copper oxychloride	14.1	7.0	90.0	22.3
Benomyl	14.1	9.8	86.0	20.0
Captan	9.8	6.1	34.1	7.0
Iprodione	4.1	4.1	42.0	6.1
Thiram	7.8	6.1	28.0	9.0
Thiobendazole	12.9	9.0	88.0	24.0
Control	30.8	18.7	90.0	29.1
SED ± (DF = 39)	2.6	2.6	3.3	3.3

Germination rates were slightly improved by treatment with captan, iprodione and thiram with both infection groups of seed, particularly with *A. brassicicola* (Table 3.6.9). In the case of *A. brassicicola* infection there was a significant interaction between seed lot and treatment: the less heavily infected lot showed no response to fungicide treatment and improved germination with thiram, iprodione, captan and, also, benomyl was seen only in the severely infected group. The effects of different fungicides on seed infection and seed germination are illustrated in Figure 3.6.5.

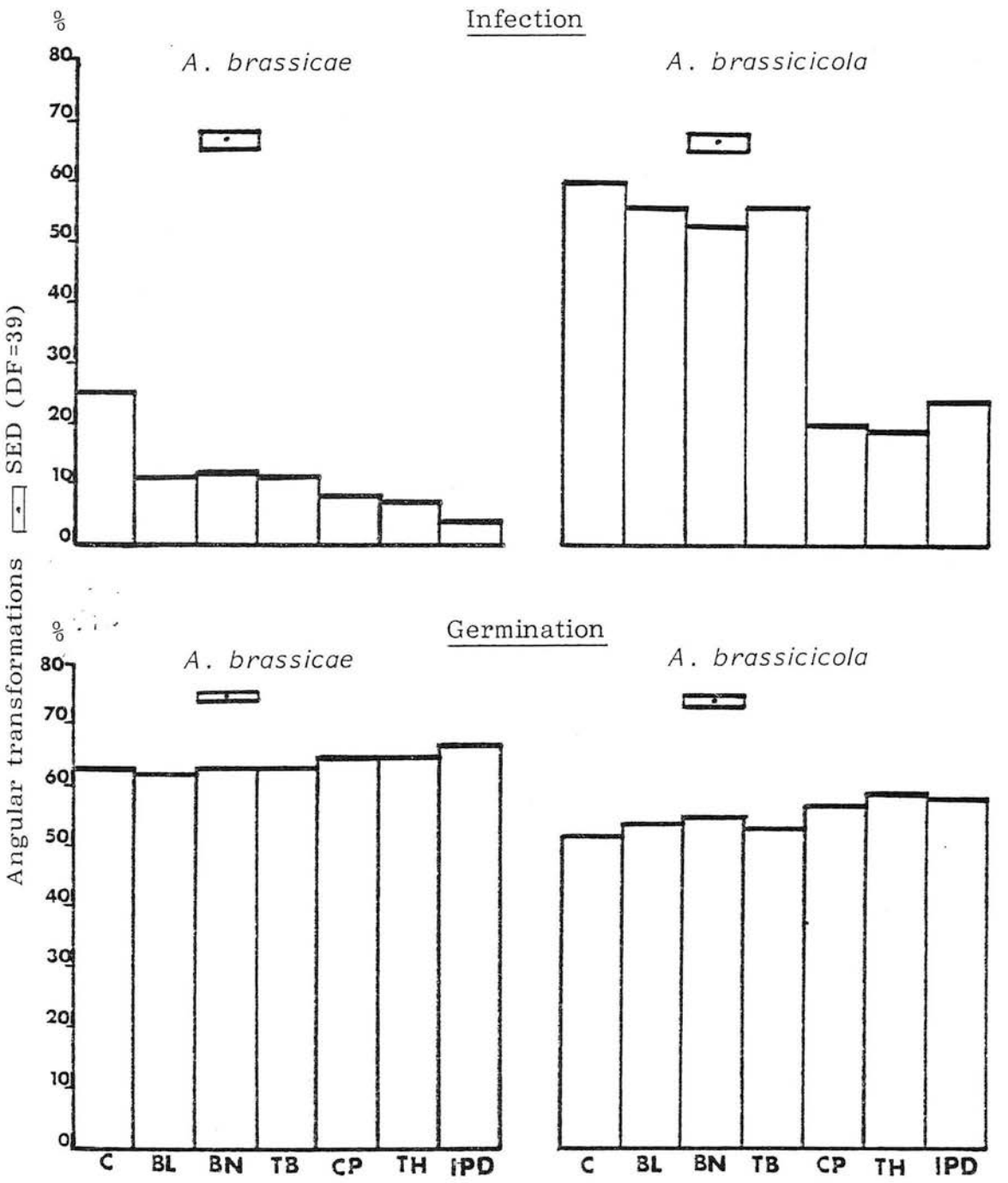
TABLE 3.6.9: The effects of different fungicide seed treatments of oilseed rape on the percentage (transformed values) seed germination in different seed lots.

Treatment (Fungicides)	*A. brassicae* infection		*A. brassicicola* infection	
	Seed lot 1	Seed lot 2	Seed lot 1	Seed lot 2
Copper oxychloride	59.7	64.0	50.4	58.1
Benomyl	61.9	63.7	53.9	55.3
Captan	64.7	66.3	56.7	56.5
Iprodione	66.4	67.1	57.8	58.4
Thiram	64.2	66.5	59.5	57.6
Thiobendazole	62.2	64.2	50.4	54.8
Control	63.3	62.8	47.7	56.8
SED ± (DF = 39)	1.3	1.3	2.0	2.0

Discussion

Although hot water treatment at 60°C for 15 minutes or more gave complete control of both *Alternaria* pathogens it substantially reduced germination and would thus be an inappropriate treatment in practice. Hot water treatment at 50°C or 55°C for as long as 30 minutes still did not give complete control of *A. brassicicola* in infected oilseed rape samples and, at longer exposure times, there was no benefit of a reduced infection from the higher temperature: thus there was no advantage in a temperature of 55°C compared with 50°C in controlling *Alternaria*, but 50°C showed superior germination. Hot water treatment at 50°C for 30 minutes gave complete control of *A. brassicae* and substantially reduced *A. brassicicola* without impairing germination and proved the most satisfactory treatment. Similar

FIGURE 3.6.5: Percentage infection and percentage germination of oilseed rape lots infected with *A. brassicae* or *A. brassicicola* in relation to different fungicide dust seed treatments.



Key:

C = Untreated control
BL = Copperoxylchloride
BN = Benomyl
TB = Thiobendazole
CP = Captan
TH = Thiram
IPD = Iprodione dust

observations were reported by Maude (1967): when cabbage seeds infected with *A. brassicicola* were treated for 25 minutes at 50°C in water, infection was reduced from 95.5% to 4.5%. Rangel (1945) isolated *A. brassicicola* from kale seeds treated at 52°C for 20 minutes but Schimmer (1953) observed that *A. brassicicola* infection in cauliflower seed was reduced from 96% to 1, 0 and 0% in three seed lots when treated at 50°C for 18 minutes. Complete control of *A. brassicicola* was obtained in cabbage seed when treated for 10 minutes at 56°C (Chupp, 1923) and in cabbage and cauliflower when treated for 20 and 30 minutes at 50°C and 45°C respectively (Nielson, 1936). Holtzhausen (1978) obtained good control of *A. brassicicola* in radish seeds by treating at 50°C for 25 minutes, and McLean (1947) obtained a similar level of control in radish. Complete control of *A. brassicae* with hot water treatment at 50°C for 30 minutes has been obtained in cabbage (Walker, 1922; Porter and Rice, 1944), and in turnip and swede by treating at 50°C for 10-25 minutes (Chupp, 1935; Ogilvie, 1954). Myers (1942) reported good control of *A. brassicae* in cabbage seed by hot water treatment at 50°C for 25 minutes followed by 20 minutes steeping in 1 in 1000 mercuric chloride solution.

In the present tests with dry heat, hot air treatment gave only a slight level of control with no improvement with increased time of exposure. The results suggest that hot air controlled superficial infection but dry heat failed to reduce further the main residue of infection: whether this related to more resistant fungal structures such as spores as opposed to mycelium surviving or to more deep-seated infection or both is not clear. Neergaard (1979) reported that dry heat has been little used, although different combinations of temperature have given promising results against certain pathogens (*Colletotrichum* spp.

Puccinia spp.), while attempts against other pathogens gave negative results.

The results of tests on fungicide soak treatments at higher temperatures demonstrate that all the three fungicides investigated are capable of reducing seed infection in infected seed samples. Fungicide soak at 50°C gave good control of both *Alternaria* species without impairing germination: *A. brassicae* was controlled with a short exposure time of 10 minutes with iprodione dust in suspensions, or 20 minutes with thiram or iprodione liquid, whereas with more severe *A. brassicicola* infection 30 minutes exposure was required. A similar trend was also seen at 55°C but germination was reduced at 55°C with exposure times above 15 minutes. Crosier and Patrick (1940) obtained good control of both *Alternaria* species by soaking at 50°C for 25 minutes in 0.2% New Ceresan (ethylmercuric phosphate). Neergaard (1969) reduced *A. brassicicola* infection below 1% by treating cabbage seed with 0.2% Germisan (mercury chlorophenol).

The results of low temperature fungicide soak confirm the work of a number of experimentors (Maude, 1967; Harman and Nash, 1978; Chirco and Harman, 1979) who found that thiram soak (0.2%) reduced seed infection but could not eliminate *A. brassicicola* in cabbage completely. However, Holtzhausen (1978) reported the complete control of *A. brassicae* with 0.5% thiram soak for 24 hours at 30°C in radish. In the present study, *A. brassicae* was reduced to very low levels but, with severe *A. brassicicola* infection, levels were still substantial after treatment. The improvement in germination was greater in the case of *A. brassicicola* infected seed compared with *A. brassicae* infected seed: this may be due to the more severe *A. brassicicola* infection including some deep-seated infection which was associated with reduced germination counts and was partially controlled by the treatment.

Studies with chemical seed treatment illustrate essential differences between the two *Alternaria* species and different chemicals. Thiram, captan and iprodione were more effective than other fungicides tested: iprodione tended to be more effective than the others against *A. brassicae*, whereas captan and thiram gave slightly better control of *A. brassicicola*. Captan, thiram and iprodione treatments improved the germination compared to copper oxychloride, benomyl and thiobendazole which gave only moderate control of infection. In a similar study, Maude *et al.* (1980) obtained good control of *A. brassicicola* by treating infected cabbage seed with iprodione and thiram whereas benomyl and thiobendazole were ineffective. Darpoux *et al.* (1957) reported that organic fungicides such as thiram, captan, quinone and quinoline derivatives gave effective disinfection of *A. brassicae* in infected rape seed. Richardson (1970) found that captan treatment was effective in reducing *A. brassicicola* infection in *Brassica* seeds, but less so in controlling *A. brassicae*. Kanwar and Khanna (1979), however, obtained complete control of *A. brassicae* infection in mustard seed treated with thiram and Difolatan (captafol). Maude (1978) reported that systemic fungicides such as benomyl, have a more narrow range of action than most contact fungicides and are not effective against dark spore fungi like *Alternaria*.

An attempt to summarise the relative performances of the different treatments is presented in Table 3.6.10. Substantial control relates to a reduction in infection percentage to about 1%.

TABLE 3.6.10: Relative effectiveness of the various seed treatments in controlling *Alternaria* infection in oilseed rape.

Pathogen	Treatment		Period of treatment	Complete control	Substantial control	Germination*
A. brassicae	Water	30°C	24 h	NO	NO	+
		50°C	20 min	YES	-	+
		55°C	20 min	YES	-	-
		60°C	5 min	YES	-	+
	Fungicide	30°C	24 h	NO	YES	+
		50°C	20 min	YES	-	o
		55°C	15 min	YES	-	-
	Hot air	50°C	30 min	NO	NO	-
		55°C	30 min	NO	NO	-
		60°C	30 min	NO	NO	-
	Fungicide (dust)		24 h	NO	NO	o
A. brassicicola	Water	30°C	24 h	NO	NO	+
		50°C	30 min	NO	YES	+
		55°C	30 min	NO	YES	+
		60°C	15 min	YES	-	-
	Fungicide	30°C	24 h	NO	NO	+
		50°C	30 min	NO	YES	+
		55°C	25 min	YES	-	-
	Hot air	50°C	30 min	NO	NO	o
		55°C	30 min	NO	NO	-
		60°C	30 min	NO	NO	-
	Fungicide (dust)		24 h	NO	NO	+

*Key

o unchanged

+ improved

- lowered

4. GENERAL SUMMARY

In the present studies, various aspects of *Alternaria* disease of cruciferous plants have been considered: these aspects include the features of the two main pathogenic species involved, *A. brassicae* and *A. brassicicola*; factors associated with the incidence of infection; the effects of infection on plant growth and development; the nature of host-pathogen relationships with respect to levels of responses to infection (susceptible or resistant) of a wide range of cruciferous plants and the degree of host specificity exhibited by different isolates of each pathogen species; the effects of temperature and humidity on infection; and a consideration of seed borne infection or transmission and its control.

The main interest centred on oilseed rape and the work coincided with a remarkable extension in the area of production of this crop in Scotland from less than 200 hectares in 1981 to several thousand in 1984. Moreover, *Alternaria* infection became recognized as a major problem in the crop. From a survey of *Alternaria* infection of oilseed rape in east Scotland, over the 3-year period 1982 to 1984, *A. brassicae* was identified as the predominant species. *A. brassicicola* produced symptoms only occasionally and its distribution was associated mainly with market garden areas, where vegetable *Brassica* crops apparently provided the source of inoculum. With *A. brassicae*, the greater incidence of infection was associated with higher rainfall and a longer history of oilseed rape growing and a greater intensity of production, while dense crops with luxuriant foliage and lodging predisposed crops to severe infection.

The results of a survey of seed samples from various *Brassica* crops indicated that, while *A. brassicae* is the principal pathogen of oilseed rape, *A. brassicicola* is the major pathogen in vegetable *Brassica* crops. The findings are in keeping with the observations of other workers, although the reasons are not altogether clear. The literature

on the economic effects of the two pathogens suggests that it is in seed crops that the fungi are linked with the most serious damage: from leaf disc inoculation studies there was little evidence of a differential response of different cruciferous plants to the two pathogens but it is possible that developmental characteristics of different hosts coupled with epidemiological factors may account for the difference in importance of the two pathogens in different crops. The reason why their importance is linked with seed crops rather than vegetable or forage crops may lie in the time of their economic production: seed crops developing at warmer times of the year are, hence, more liable to attack than crops developing in late autumn or winter. There does seem to be evidence that *A. brassicicola* is favoured at somewhat higher temperatures. The significance of oilseed rape production, in relation to the disease problems, is that it is being developed in cooler areas and this may allow *A. brassicae* to assume greater importance. It may be noted that on overwintering oilseed rape plants, only *A. brassicae* was observed. From artificial inoculation studies on growing plants of various cultivars of oilseed rape, differences in the characteristics of the two pathogens emerged. *A. brassicae* appeared as the most aggressive leaf pathogen producing more obvious and severe symptoms than *A. brassicicola*, but the latter fungus evidenced a greater spore production capacity and ability to colonise drying or immature flower parts: thus its incidence in seed in these particular experiments was higher than that of *A. brassicae*. The adverse effects of infection relate to reduced seed yield and seed quality and the use of iprodione spray treatment gave significant improvements in both factors. However, field observations suggest that one spray application did not necessarily afford adequate control in practice.

In comparing different cultivars of oilseed rape, differences in susceptibility or resistance were quantitative rather than qualitative in character and did not appear to be associated with major gene effects. Likewise, there was little evidence of any marked interaction between host and different isolates of the pathogen. A leaf disc assessment method was developed to examine the response of a range of cruciferous plants and the pattern of reaction to the two pathogens was similar. One factor associated with resistance was the waxiness of the cuticle and removing the wax layer increased the infection level. The presence of wax may provide a physical barrier to infection, but wax may also play a role in shedding water droplets away from leaves or maintaining droplet integrity due to its effect on surface tension characteristics. It was observed that where spores were contained in water droplets which persisted, particularly in the case of *A. brassicicola*, infection was reduced. Both *Alternaria* spores produce dry as opposed to slimy spores, the latter being more readily wetted. With dry spore inoculation, increasing humidity favoured infection. The optimum temperature for leaf infection with both pathogens was 25°C, with a sharp decline in lesion development above or below this: however, *A. brassicicola* was slightly more tolerant of temperatures above optimum, and *A. brassicae* was more tolerant of temperatures below optimum. With respect to incubation of infected seed, colonies of both species emerged at a maximum level over a wide range of temperature from about 10 to 25°C, suggesting that the optimum temperature for infecting leaf tissue is more critical than that for colonisation of inert substrate.

Seed infection studies indicated that both *Alternaria* pathogens may give rise to deep-seated infection, as well as being carried in the seed coat, deep-seated infection being the form of infection mainly

associated with emergence failure which is aggravated by wet soil conditions. With respect to transmission of the pathogen from seed to seedlings, the position of the seed coat in relation to the cotyledons, i.e. whether it is shed early or carried up with the cotyledons determines the success of seed to plant transfer. The results of assessing various seed treatments for the control of seed-borne *Alternaria* confirm that infection is often internal: thus only those treatments which had a penetrative effect, i.e. hot water treatment, rather than hot dry air or fungicide soak treatment, rather than fungicide dust, gave substantial control

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APPENDICES

APPENDIX I: Glossary of common plant-names of family Cruciferae (ISTA, 1982).

Latin name	Common name in the UK
Brassica chinensis L.	Chinese cabbage
Brassica juncea (L.) Czern. et Coss. in Czern.	Brown mustard
Brassica napus L.	Rape
Brassica napus L. var. *napobrassica* (L.) Rchb.	Swede
Brassica nigra (L.) W.D.J. Koch	Black mustard
Brassica oleracea L.	Brassicas, cabbage
B.o. convar. *acephala* (DC.) Alef. var. *gongylodes*	Kohl rabi
B.o. convar. *acephala* (DC.) Alef. var. *medullosa* Thell + var. *viridis* L.	Marrowstem kale/leaf kale
B.o. convar. *acephala* (DC.) Alef. var. *sabellica* L.	Curly kale
(Syn.: *B.o.* convar. *a.* var. *laciniata* (L.) Schulz)	
B.o. convar. *botrytis* (L.) Alef. var. *botrytis* L.	Cauliflower
(Syn.: *B.o.* var. *botrytis* L. subvar. *cauliflora* (Gars.) DC. ex Thell)	
B.o. convar. *botrytis* (L.) Alef. var. *cymosa* Duch.	Sprouting broccoli/calabrese
(Syn.: *B.o.* convar. *b.* var. *italica* Plenck)	
B.o. convar. *capitata* (L.) Alef.	Head cabbage
B.o. convar. *capitata* (L.) Alef. var. *alba* DC.	White cabbage
(Syn.: *B.o.* convar. *c.* var. *capitata* L.	
B.o. var. *capitata* L. subvar. *sphaerica* DC.f. *alba* DC.)	Pointed headed cabbage
B.o. convar. *capitata* (L.) Alef. var. *conica* DC.	Red cabbage
(Syn.: *B.o.g.* 'Pyramidalis')	
B.o. convar. *capitata* (L.) Alef. var. *rubra* DC.	
(Syn.: *B.o.* convar. *c.* var. *capitata* L.	
B.o. var. *capitata* L. subvar. *sphaerica* DC.f. *rubra* DC.)	

Latin name	Common name in the UK
Brassica oleracea convar. *capitata* (L.) Alef. var. *sabauda* L.	Savoy cabbage, savoy
(Syn.: *B.o.* var. *bullata* DC. subvar. *sabauda* (L.) O.E. Schultz)	
B.o. convar. *oleracea* var. *gemmifera* DC.	Brussels sprouts
(Syn.: *B.o.* var. *bullata* DC. subvar. *gemmifera* (DC.) Levéillé)	
Brassica pekinensis (Lour.) Rupr.	Chinese cabbage
Brassica perviridis (L.H. Bailey) L.H. Bailey	Spinach mustard
Brassica rapa L.	Rape
B.r. var. *rapa*	
(Syn.: *B. campestris* L.)	
B.r. var. *silvestris* (Lam.) Briggs	Turnip rape
(Syn.: *B. campestris* var. *oleifera* DC.)	
Camelina sativa (L.) Crantz	Large-seeded false flax
(Syn.: *Myagrum sativum* L.)	
Lepidium sativum L.	Cress
Raphanus sativus L.	Summer and winter radish
R.s. var. *niger* (Mill.) S. Kerner	Black radish
R.s. var. *oleiformis* Pers.	Fodder radish
R.s. var. *sativus*	Radish
(Syn.: *R.s.* var. *radicula* Pers.)	
Sinapis alba L.	White mustard

APPENDIX II: Records of occurrence of *Alternaria brassicae* on cruciferous crops.

Continent	Country	Host	Reference
Africa	Angola	Cruciferae	CMI (1971)
	Egypt	Cruciferae	CMI (1971)
	Ethiopia	Cruciferae	CMI (1971)
	Ivory Coast	*B. oleracea*	Roger (1937)
	Kenya	Cruciferae	CMI (1971)
	Malawi	Cruciferae	CMI (1971)
	Mauritius	Chinese cabbage	Coombes & Julien (1949)
	Morocco	Cabbage	Malencon & Delecluse (1937)
	Mozambique	Cabbage	Decarvalho (1948)
	Rhodesia	Cruciferae	CMI (1971)
	Senegal	Cruciferae	CMI (1971)
	South Africa	Brussels sprouts	Holtzhausen & Knox-Davis (1974)
	Sudan	Turnip	Tarr (1952)
	Tanzania	Turnip	Wallace & Wallace (1945)
	Zambia	Cruciferae	CMI (1971)
Asia	Afghanistan	Cruciferae	CMI (1971)
	Bruei	Cruciferae	CMI (1971)
	Burma	Cruciferae	CMI (1971)
	Cambodia	Cruciferae	CMI (1971)
	Ceylon	Mustard, cabbage	Bond (1947)
	China	Rape, cabbage	Porter (1926)
	Hong-Kong	Cruciferae	CMI (1971)
	India	Mustard	Chahal (1981)
	Indonesia	Cruciferae	CMI (1971)
	Iran	Cruciferae	CMI (1971)
	Iraq	Cruciferae	CMI (1971)
	Israel	Cruciferae	CMI (1971)
	Japan	*B. rapa*, *B. rapella*	Yoshii (1933)
	Korea	Cruciferae	CMI (1971)
	Laos	Cruciferae	CMI (1971)
	Lebanon	Cruciferae	CMI (1971)
	Malaysia	Cruciferae	CMI (1971)
	Nepal	Cruciferae	CMI (1971)
	Pakistan	Cruciferae	CMI (1971)
	Philippines	Cabbage	Fajardo & Palo (1934)
	Saudi Arabia	Cruciferae	CMI (1971)

Continent	Country	Host	Reference
Asia	Singapore	*Brassicas*	Seow & Lim (1969)
	Taiwan	Cruciferae	Wu (1979)
	Thailand	Cruciferae	CMI (1971)
	Turkey	Cruciferae	CMI (1971)
	USSR	Cabbage	Kikoina (1931)
	Vietnam	Cruciferae	CMI (1971)
Australasia & Oceania	Australia	Turnip	Anon (1959)
	New Caledonia	Cruciferae	CMI (1971)
	New Zealand	Swede	Morton (1964)
	Papua New Guinea	Cruciferae	CMI (1971)
Europe	Bulgaria	Turnip	Khristov (1979)
	Britain	*Brassicas*, oilseed rape	Humpherson-Jones (1983)
	Cyprus	Cruciferae	CMI (1971)
	Czechoslovakia		
	Denmark	Cruciferae	Neergaard (1979)
	France	Rape	Louvet (1958)
	Finland	Cruciferae	Tahvonen (1979)
	Germany	Colza, rape	Raabe (1939)
	Greece	Cruciferae	CMI (1971)
	Italy	Cauliflower	Ferraris (1924)
	Netherlands	Cruciferae	Bolle (1924)
	Norway	Cruciferae	CMI (1971)
	Poland	Oilseed rape	Csyzewska (1958)
	Rumania	Cabbage, cauliflower	Crisan (1976)
	Sardinia	Cruciferae	CMI (1971)
	Spain	Rape	Romeromunog & Jimenediaz (1979)
	Sweden	Oilseed rape	Morner (1980)
	Switzerland	Rape	Winter & Huber (1978)
	USSR	Turnip, radish	Musaev (1979)
	Yugoslavia	Cabbage	Sekulkouska (1959)
North America	Canada	Rape	McDonald (1959)
	Mexico	Cruciferae	CMI (1971)
	USA	Cruciferae	Chupp (1935)

Continent	Country	Host	Reference
Central America & West Indies	Bermuda	Cruciferae	CMI (1971)
	Costa Rica	Cruciferae	CMI (1971)
	Dominican Republic	Cruciferae	CMI (1971)
	Guatemala	Cruciferae	CMI (1971)
	Honduras	Cruciferae	CMI (1971)
	Jamaica	Cruciferae	CMI (1971)
	Nicaragua	Cruciferae	CMI (1971)
	Panama	Cruciferae	CMI (1971)
	Puerto Rico	Cruciferae	CMI (1971)
	Salvador	Cruciferae	CMI (1971)
	Trinidad	Cruciferae	Stell (1922)
South America	Argentina	Radish, turnip	Marchionatto (1947)
	Bolivia	Cruciferae	CMI (1971)
	Brazil	Cauliflower	Arruda (1938)
	Chile	Cruciferae	CMI (1971)
	Colombia	Cauliflower	Toro (1929)
	Guyana	Cruciferae	CMI (1971)
	Peru	Cruciferae	CMI (1971)
	Surinam	Cruciferae	CMI (1971)
	Uruguay	Cruciferae	CMI (1971)
	Venezuela	Cruciferae	CMI (1971)

APPENDIX III: Records of occurrence of *Alternaria brassicicola* on cruciferous crops.

Continent	Country	Host	Reference
Africa	Egypt	Cruciferae	Michail *et al.* (1979)
	Ethiopia	Cruciferae	CMI (1982)
	Gambia	Cruciferae	CMI (1982)
	Ghana	Cruciferae	Ellis (1971)
	Libya	Cruciferae	Ellis (1971)
	Madagascar	Cruciferae	CMI (1982)
	Malawi	Cruciferae	CMI (1982)
	Mauritius	Cauliflower	Coombes & Julien (1949)
	Morocco	Cruciferae	CMI (1982)
	Mozambique	Cruciferae	CMI (1982)
	Nigeria	Cruciferae	CMI (1982)
	Senegal	Cruciferae	CMI (1982)
	Sierra Leone	Cruciferae	CMI (1982)
	South Africa	Cruciferae	CMI (1982)
	Sudan	Cauliflower, turnip	Tarr (1951)
	Tanzania	Cruciferae	CMI (1982)
	Uganda	Cruciferae	Mason (1928)
	Zambia	Cruciferae	CMI (1982)
	Zimbabwe	Cruciferae	CMI (1982)
Asia	Brunei	Cruciferae	CMI (1982)
	Burma	Cabbage	Su (1934)
	Cambodia	Cruciferae	CMI (1982)
	China	Cruciferae	CMI (1982)
	Hong-Kong	Cruciferae	CMI (1982)
	India	Cabbage	Mason (1928)
	Indonesia	Cruciferae	CMI (1982)
	Iran	Cruciferae	CMI (1982)
	Israel	Cruciferae	CMI (1982)
	Japan	Cabbage	Yoshii (1933)
	Korea	Cruciferae	CMI (1982)
	Malaysia	*B. rapa*	Jamil (1966)
	Nepal	Cruciferae	Ellis (1971)
	Oman	Cruciferae	CMI (1982)
	Pakistan	Cruciferae	CMI (1982)
	Saudi Arabia	Cabbage	Anon (1967)
	Sri Lanka	Cruciferae	CMI (1982)
	Turkey	Cabbage	Gobelez (1956)
	USSR	Cruciferae	Juhans (1934)

Continent	Country	Host	Reference
Australasia & Oceania	Australia	Swede	Anon (1959)
	New Caledonia	Cruciferae	CMI (1982)
	New Zealand	Swede	Morton (1964)
	Papua New Guinea	Cruciferae	CMI (1982)
Europe	Britain	*Brassica* seed crops	Green (1947)
	Cyprus	Cruciferae	CMI (1982)
	Denmark	Cruciferae	Neergaard (1979)
	Finland	CMI (1982)	Tahvonen (1979)
	France	Cabbage	Hasan & Vago (1966)
	Germany	Rape, cabbage	Domsch (1957)
	Greece	Cruciferae	CMI (1982)
	Irish Republic	Cruciferae	CMI (1982)
	Italy	Cruciferae	CMI (1982)
	Netherlands	Cruciferae	Bolle (1924)
	Norway	Cruciferae	CMI (1982)
	Rumania	Cruciferae	CMI (1982)
	Sweden	Cruciferae	Loof (1959)
	USSR	Cruciferae	Nelen (1961)
	Yugoslavia	Cruciferae	CMI (1982)
North America	Canada	Cauliflower	Conners (1935)
	USA	Cruciferae	Chupp (1923; 1935)
Central America & West Indies	Antigua	Cruciferae	CMI (1982)
	Barbados	Cruciferae	CMI (1982)
	Costa Rica	Cruciferae	CMI (1982)
	Cuba	Cruciferae	CMI (1982)
	Jamaica	Cruciferae	Anon (1945)
	Panama	Cruciferae	CMI (1982)
	Trinidad	Cruciferae	CMI (1982)
South America	Chile	Rape	Bertossi (1963)
	Venezuela	Cruciferae	CMI (1982)