RESISTANCE OF WHEAT TO *SEPTORIA NODORUM* BERK.

By

Elizabeth Anne Baker

B.Sc.(Lond.)

A thesis submitted in part fulfilment of the requirements for the degree of Doctor of Philosophy of the University of London

Department of Botany and Plant Technology,
Imperial College of Science and Technology,
Silwood Park Field Station,
Ascot, Berkshire.

October 1975

ABSTRACT

In laboratory tests on detached leaves wheat cultivars infected by S. nodorum showed continuous variation in symptoms through different degrees of necrosis, chlorosis and lesion extent. There was a disappearance of surface wax under the hyphae and appressoria. Although surface growth was similar in resistant and susceptible cultivars, colonisation within the leaf, in the former case, was less and restricted to the area beneath the infection droplet. No structural barriers were observed and staining reactions for lignin were negative. The exact relationship of the hyphae to the host cells could not be ascertained. At low inoculum concentrations lesion development was delayed, and in the resistant cultivar lesions often failed to develop at all. The intensity of the browning/necrotic reaction was partly suppressed in the dark and coalescence of lesions in the susceptible cultivar was prevented at 25°C. Liquid cultures of S. nodorum failed to yield any phytotoxic principles.

In the field inoculations at later growth stages resulted in higher overall % infection compared to the earlier inoculations. On resistant plants fewer lesions developed and less of these spread than on the susceptible ones. High yielding cultivars had a lower % infection and lower infection rate on the flag leaf and ear compared to the lower yielding cultivars, although in the former case a high % infection of the lower leaves resulted in an apparently higher total % infection.

Levels of the antifungal benzoxazolinone derivatives were shown to decrease with age; these substances could not be detected in plants over 2-3 weeks old. Other compounds, which remain to be identified, but which were active against S. nodorum, were extracted from plants at all growth stages. There were no qualitative differences between resistant

and susceptible cultivars, but generally speaking the extracts from the resistant cultivar were more active. Infected tissue extracts were usually more inhibitory to S. nodorum than the extracts from healthy control material.

CONTENTS

	Page
ABSTRACT	2
INTRODUCTION AND LITERATURE REVIEW	8
MATERIALS AND METHODS	II6
A. Fungi	I6
B. Culture media	I6
C. Plant material	I8
D. Inoculation procedure	20
E. Observation of leaf surfaces	22
F. Preparation of material for light microscopy	22
G. Preparation of material for stereoscan electron microscopy	24
H. Extraction of plant material	25
J. Separation and purification techniques	25
K. Location and estimation of substances	27
L. Field experiments	3I
M. Statistical analyses	33
EXPERIMENTAL RESULTS	
SECTION 1: Varietal reactions on detached leaves and factors affecting their development	36
Varietal reactions	37
Macroscopic and microscopic development of the resistant and susceptible reactions	4I
Effect of centrifugation of spores on subsequent lesion development	56
Effect of spore concentration on lesion development	59
Effect of light and temperature on lesion development	62
Investigation of the possible toxic effect of S.nodorum	65
DISCUSSION	72

SECTION 2:	Development of S.nodorum infection on winter and spring wheat in the field	77
	Field experiment 1A: The effects and possible interactions of inoculation date, variety and leaves on development of S.nodorum infection on winter wheat.	78
	Development of S.nodorum infection	8I
	Analysis of variance (whole plants)	88
	Meteorological conditions 1974	90
	Microclimate of the crop canopy	93
	Differences between parts of plant	98
	Analysis of variance (parts of plant)	I00
	Apparent infection rates	I04
	Summary of infection data	I08
	Field experiment 1B: A detailed study of infection in naturally infected and artificially inoculated plants of winter wheat.	II0
	Increase of lesion number and %infection for flag and second leaves of winter wheat	II2
	Meteorological conditions 1975	I20
	Statistical analysis	I23
	Summary	I25
	DISCUSSION	I27
	Field experiment 2A: The effects and possible interactions of inoculation date, variety and leaves on development of S.nodorum infection on spring wheat	I32
	Development of S.nodorum infection	I34
	Analysis of variance (whole plants)	I40
	Meteorological conditions	I42
	Differences between parts of plant	I43
	Apparent infection rates	I45
	Summary of infection data	I48

	Field experiment 28: A detailed study of infection in naturally infected and artificially inoculated plants of spring wheat	I50
	Increase of lesion number and %infection for flag and second leaves and ears of spring wheat	I5I
	Meteorological conditions	I6I
	Statistical analysis	I62
	Summary	I64
	DISCUSSION	I66
SECTION 3:	Studies on pre- and post-formed antifungal compounds in winter wheat resistant and susceptible to S.nodorum	I69
	I.The presence of pre- and post-formed inhibitors of S.nodorum in crude and partially purified extracts of wheat tissues of varying ages	I70
	1.Healthy week old seedlings	I70
	2.Healthy and inoculated 30 day old glass-house grown plants	I72
	3.Healthy and inoculated 7 week old glass-house grown plants	I77
	4.Healthy and inoculated and naturally infected 6 month old field grown plants	I83
	Summary	I88
	II.Chromatographic separation and assay of pre- and post-formed antifungal compounds in winter wheat of varying ages	I9I
	1.Preformed inhibitors present in healthy intact wheat plants	I92
	2.Potential antifungal compounds in healthy intact wheat plants	I95
	3.Antifungal compounds in artificially inoculated wheat	202

4.Antifungal compounds at various growth stages in healthy and naturally infected field grown winter wheat 219

Summary 215

III. The disappearance of the benzoxazinone compounds in winter wheat resistant and susceptible to S.nodorum 219

A.1.Glasshouse grown plants 219

2.Cooled cabinet grown plants 220

3.Field grown plants 220

B.1.Assessment of ferric chloride reaction by optical density 221

2.Assessment of benzoxazinones by UV spectra 223

C. The levels of benzoxazinones in healthy wheat plants 223

DISCUSSION 228

FINAL DISCUSSION 232

BIBLIOGRAPHY 235

ACKNOWLEDGEMENTS 242

APPENDICES 243

1A. Weekly mean % S.nodorum infection, winter wheat 1974 244

1B. Detailed study of infection, winter wheat 1975 263
Summary of data for 1974 270
Summary of data for 1975 271

2A. Weekly mean % S.nodorum infection, spring wheat 1974 272⅞

2B. Detailed study of infection, spring wheat 1974 293

3. Spore droplet bioassays 300

INTRODUCTION AND LITERATURE REVIEW

"Septoria - the lurking threat to wheat yields"

This headline appeared in Farmers Weekly in 1973, and although Septoria disease of wheat has been recognised in England since 1845 (Berkeley) only recently has the extent of yield loss been appreciated. A three year (1970-1972) study by the Agricultural Development and Advisory Service, covering 500 acres on 300 farms showed that Septoria spp. caused worst diseases of wheat and were far more serious nationally than mildew or yellow rust. Yield reductions of up to 65% have been recorded (Jenkins and Morgan, 1969; Brönnimann, Sally and Sharp, 1972). Also, since grain quality is adversely affected and quality requirements for selling wheat for denaturing are strict, the importance of the disease should not be underestimated. Septoria disease is important in the wheat growing areas of over 50 countries (CMI Plant Disease Distribution Map No. 397).

Why has Septoria suddenly become so important? Disease build-up has resulted from a succession of mild winters and cool damp summers, combined with an increasing wheat acreage, particularly of the more susceptible dwarf cultivars (Scott, 1973). Emphasis on non-ploughing techniques which result in less efficient stubble cultivations is another factor.

The two most prevalent species are Leptosphaeria (Septoria) nodorum Berk., and Septoria tritici Rob. ex Desm. The common names are leaf and glume blotch, and leaf spot respectively. Both species are specialised facultative parasites, which infect Triticum spp. to a high degree but can be made to weakly infect a number of other cereals and grasses (Weber, 1922; Schmiedeknecht, 1967; Dereveyankin, 1969; Shearer and Zadoks, 1972).

So far no physiological races are known, only differences in mycelial growth behaviour and sporulation ability (Brönnimann, 1968a; Scharen and Krupinsky, 1970a).

The symptoms of Septoria diseases are difficult to distinguish and identify, particularly in the early stages of infection when sporulating bodies are absent, and can be confused with natural senescence.

The species Septoria nodorum has been used in this research. It can attack all aerial parts of the plant, although appreciable effects on yield only occur when the flag leaf and ear become heavily infected (Brönnimann, 1968a).

Penetration is by pycnidiospores, directly through the cuticle, after which hyphae enlarge slightly and grow intercellularly before entering the cells (Weber, 1922). However the exact relationship of the fungal hyphae with host cell walls remains to be determined, since it has proved impossible to stain hyphae satisfactorily within the leaf (Morgan, 1974).

Lesions on the leaves occur most usually near the tip in natural infections, and appear as linear light brown spots with a yellow margin. In susceptible cultivars the lesions spread and coalesce, but they remain limited necrotic flecks in non-compatible reactions. On the glumes, lesions appear as dark brown/purple blotches. S. nodorum also causes a seedling blight when it is seed transmitted (Keitreiber, 1961). An early infection by the pathogen results in stunting of plants and reduced tiller number, which leads to reduction in grain quantity and quality. Sutton (1920) reported that on infection of the nodes, mycelium entered the vessels and plugged them up, so cutting off the upward flow of sap. Node infection results in lodging of plants which causes losses at harvesting. Greatest yield losses however arise from a shrivelling of the grain (Becker, 1963) due to a reduction in assimilatory area (Scharen and Taylor, 1968; Brönnimann, 1968a, 1969a; Scharen and Krupinsky, 1970b). The percentage crude protein of grain is unaffected (Cooke and Jones, 1970a) as is the respiration of infected plants. Since there is an increase in yield loss over and above that of the loss of photosynthetic area, particularly in the more susceptible

cultivars, a toxic effect of the pathogen has been suggested (Brönnimann, 1969a). However neither Thomas (1962) nor Baker (1969) were able to demonstrate toxin production by S. nodorum. But Bousquet and Skajennikoff (1974) have isolated an active compound from culture filtrate of S. nodorum which damaged four wheat varieties with different reactions to the fungus to a different extent.

The epidemiology of Septoria diseases has received more attention in recent years. There are several sources of primary inoculum: the seed (Becker, 1963; Burhardt, 1954) and infected chaff, plant debris and volunteer plants, upon which the fungus can overwinter (Weber, 1922; Obst, 1971; Cooke and Fozzard, 1973). The spores, which are released cyclically, only remain viable in pycnidia in debris on the soil surface (Scharen, 1964, 1966; Von Wechmar, 1966). Infection is greatly influenced by air temperature the optimum being 18-25°C (Shipton, Boyd, Roseille and Shearer, 1971), and by the period of high humidity after inoculation (Renfro and Young, 1956; Scharen, 1964, 1966; Shearer and Zadoks, 1974; Holmes and Colhoun, 1974). The necessary dew period for infection varies with cultivars, being less for the more susceptible ones (Brönnimann et al., 1972).

Plant breeders are at present searching for wheat varieties which are resistant. No present ones possess immunity to S. nodorum, all becoming infected but to differing degrees (Baker, 1970), and the amount of infection is often not positively correlated with yield loss (P.B.I. Report, 1968; Brönnimann, 1968a, 1968b; Cook and Jones, 1971; Sharp, Brönnimann and McNeal, 1972). Cultivars whose yield loss is minimal are termed tolerant, irrespective of the degree to which they become infected. The terms "resistance" and "susceptibility" can be applied to success of leaf infection, but give no idea of the effect of the pathogen on yield and are therefore not usually used when categorising cultivars.

Because of the small correlation with damage, estimation of attack cannot be used as a criterion for selection. Tolerance is best estimated by the thousand grain weight of the infected line expressed as a percentage of the non-infected control. Difference in grain characteristics of infected and healthy plants may also be used (Brönnimann, 1968a). Lupton (1971) found inoculation at growth stage 10.5 and infection assessment at growth stage 11.1 a good indication of yield loss.

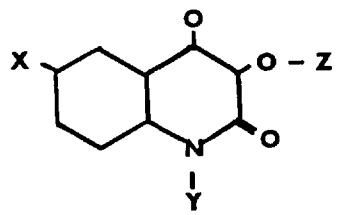
Both the genetic and physiological basee of tolerance are largely unknown. That resistance of leaves to infection is heritable was demonstrated by Laubscher, von Wechmar and van Shalkwyk (1966). Subsequent investigations indicate a linear and additive gene effect with respect to thousand grain weight (Brönnimann, 1970). Short stem cultivars are generally more heavily injured than normal ones. This is true for both genetically short forms and those treated with stem shortening agents such as CCC (Brönnimann, Künzli and Hani, 1972). Short forms are even more severely injured before heading than after, while in normal plants there is a marked increase in susceptibility at heading and flowering. Early maturing cultivars tend to be less affected than later ones (Lupton, 1971; Brönnimann, 1968b), and hard wheats are more resistant than soft wheats (Lebedeva, 1960). Since spread of the disease is upwards by rain splash, the taller cultivars are able to some extent to 'escape' infection. Symptom expression is influenced by both leaf age and position of the infection site; although Pirson (1960) found colonisation less in older than younger leaves, Morgan (1974) reported younger tissue to be more resistant. Scharen (1963) could demonstrate no effect of leaf age and concluded that environment was more important in symptom development. Shipton et al. (1971) suggested differential wettability of leaf surfaces as the factor underlying leaf response at different ages.

Research in the fifties suggested a role for preformed antifungal compounds in resistance of graminaceous species to disease. Virtanen and Hietala (1955) isolated an antifungal compound from rye seedlings, which they identified as 2(3H) benzoxazolinone (BOA). Maize and wheat seedlings were later shown to contain a related compound, the methoxy derivative MBOA, (Virtanen, Hietala and Wahlroos, 1957). They subsequently found that both BOA and MBOA were artefacts of the extraction procedure, and were in fact formed from primary benzoxazinoneglucosidic precursors in the intact plant. These, on enzymatic hydrolysis, break down first to the benzoxazinone aglucones, DIBOA and DIMBOA, and then on heating to the benzoxazolinones (see fig. 1 overleaf) (Virtanen and Hietala, 1960 ; Hietala and Virtanen, 1960). The two latter groups of compounds are highly antifungal, while the glucosides have a negligible inhibitory effect (Virtanen, 1958, 1961; Wahlroos and Virtanen, 1958, 1959; Loomis, et al 1957; Elnaghy and Linko, 1962).

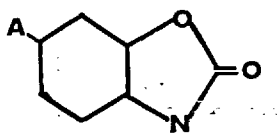
The free aglucone, DIMBOA, was thought to be a natural constituent of plant tissue and the factor responsible for resistance against moulds (Wahlroos and Virtanen, 1964). However, Hofman and Hofmanova (1969, 1971) demonstrated that only the glucoside occurred in intact plants, and that there was no free aglucone. Other 1,4 benzoxazolinone derivatives have been isolated from maize (Gahagan and Mumma, 1967; Hofman and Hofmanova, 1971) and it is suggested that these are possible intermediate compounds in the biosynthesis of the glucoside.

Thus the presence of these compounds is well documented, although their role in resistance remains to be determined. Release of aglucones may be important in resistance of maize to the European corn borer (Klun and Robinson, 1969). Elnaghy and Linko (1962) reporting on the role of DIMBOA-glucoside in resistance of wheat to stem rust showed a decrease in the compound after infection by the avirulent race. They suggested that on infection, injury to the cells releases aglucone which causes their

Figure 1:- The 1,4 - benzoxazolinone derivatives of cereals



Benzoxazinone skeleton



Benzoxazolinone skeleton

Key:	X	Y	Z	Abbreviated Name
1.	H	OH	$C_6H_{11}O_5$	DIBOA - glycoside (2-2,4-dihydroxy-1,4-2H-benzoxazin-3-4H-on-ß-Dglucopyranoside)
2.	H	H	$C_6H_{11}O_5$	HBOA - glycoside (2-2 hydroxy- 1,4-2H- benzoxazin-3-4H-on-ß-Dglucopyranoside)
3.	CH_3O	OH	$C_6H_{11}O_5$	DIMBOA-glycoside (GDMBO) (2-2,4-dihydroxy-7methoxy-1,4-2H-benzoxazin-3-4H-on-ß-Dglucopyranoside)
4.	CH_3O	H	$C_6H_{11}O_5$	H MBOA - glycoside (2-2hydroxy-7methoxy-1,4-2H-benzoxazin-3-4Hon-ß-Dglucopyranoside)
5.	H	OH	H	DIBOA- aglucone (2,4 dihydroxy-1,4-benzoxazinone)
6.	CH_3O	OH	H	DIMBOA - aglucone (2,4 d hydroxy-7 methoxy-1,4-benzoxazinone)

	A	
7.	H	BOA (2-3H- benzoxazolinone)
8.	CH_3O	MBOA (6 methoxy-2-3H- benzoxazolinone)

death, and so prevents development of the obligate parasite. However, Septoria nodorum is a facultative parasite. Elnaghy and Shaw (1966) found that concentrations of DIMBOA-glucoside were highly negatively correlated with the percentage of rust races attacking wheat, and that the line with the Sr 6 temperature sensitive resistance gene had a high content of DIMBOA glucoside at moderate temperatures. That high pre-inoculation temperatures predispose leaves to attack was reported by Shipton et al. (1971). The correlation mentioned above was based on two extremes and after further investigations Knott and Kumar (1972) could provide little evidence that the quantity of the glucoside was involved in rust reeistance of wheat. In addition, although levels of the glucoside are relatively high in seedlings, it has been difficult to demonstrate its presence in older plants in the field (Chigrin and Rozum, 1969).

With regard to the role of the benzoxazolinone derivatives in resistance of wheat to Septoria Morgan (1974) found an increase in antifungal activity in lesions, which may have been due to aglucone formation. He found that glucoside was present only in uninfected control tissue of the resistance cultivar and not in the lesion. However glucoside was not detected in the lesions on the susceptible cultivar, or in uninoculated control tissue. During saprophytic growth the fungus is capable of hydrolysing the glucoside to the aglucone and it is therefore possible this could occur in lesions. In this respect the close association of the hyphae with the cell wall, which has a bound β-glucosidase enzyme, is of interest (Chkanikov, Tarabrin, Shabanova, Konstantinov, 1969).

Cross protection demonstrations with rust (Johnston and Huffman, 1958) have shown that under challenge wheat leaves are capable of an induced resistance response. Unlike the phytoalexin response in leguminous hosts (Cruickshank, 1966) there were no significant differences in total or

individual phenolic compounds in healthy and diseased plants, with or without the gene for resistance to rust (Seevers and Daly, 1970). Baker (1969) concluded that S. nodorum did not stimulate phytoalexin production since pycnidiospores of the fungus germinate in drops on leaves irrespective of the fact that spores of the same fungus had previously germinated there. However S. tritici does induce a phytoalexin response (Baker, 1969; Morgan, 1974) which inhibits both S. tritici and to a lesser extent S. nodorum. This would account for varietal differences in susceptibility in some cases, but there are anomalies. The response is affected by temperature, age of tissue and time. Morgan (1974) found a 72h incubation period necessary before inhibition was detected in droplets on leaves. There are no other reports of phytoalexin production in Triticum spp., although they have been found in barley in reaction to Erysiphe graminis (Oku et al. (1974) and oats in reaction to Puccinia coronata

The aim of this research was to investigate in detail the varietal reactions of wheat to S. nodorum at the field, plant, lesion and biochemical levels. Initially the exact relationship of the hyphae within host tissue needed to be determined. Then the role in resistance of pre- and post-formed antifungal compounds, in diseased and healthy wheat plants, with particular reference to the group of 1, 4 benzoxazolinone derivatives.

MATERIALS AND METHODS

A. Fungi

1. An isolate of Leptosphaeria nodorum Muller. (Septoria nodorum Berk.) (S20/72) was obtained from the Plant Breeding Institute, Cambridge. Stock cultures were kept on potato dextrose agar slopes under oil in McCartney bottles, while sporulating cultures were maintained on oatmeal agar under white light.

2. Cladosporium sp., isolated by Dr. I.M. Smith, Imperial College, London, was maintained on potato dextrose agar under white light.

B. Culture Media

1. Oatmeal Agar was prepared by boiling 30g rolled oats (Scots Porridge) in 600 ml distilled water for 20 min. After filtration through one layer of muslin the filtrate was combined with 400 ml distilled water containing 20g Standard Agar (Davis Gelatine Ltd.).

2. Potato Dextrose Agar was prepared by bringing to the boil 39g potato dextrose agar (Oxoid CM1 39) in 1 litre distilled water.

50-75 ml aliquots of the above media were placed in 150-250 ml Erlenmeyer flasks which were then fitted with cotton wool plugs and aluminium foil caps and autoclaved at 121°C (15 p.s.i.) for 15 min.

Subculturing was carried out by placing about a ml of sterile distilled water in the flasks and suspending in this spores of the respective fungi collected on a sterile needle from already sporulating cultures. Cultures were maintained in an illuminated incubator (Gallenkamp 1H280) at 20°C. Sporulating cultures of Septoria and Cladosporium were obtained after 7 and 4 days incubation respectively.

3. Nutrient Solution of the following composition was used in bioassays:-

$NaNO_3$ (GPR)	2g	made up to one litre, autoclaved for 15 min. at 121°C
KH_2PO_4 (Analar)	1g	
$MgSO_4.7H_2O$ (BDH lab reagent)	0.5g	
Sucrose (BDH lab reagent)	15g	
D(+) Glucose (GPR)	0.5g	
Trace element solution	10ml	

The Trace element solution had the following composition:-

$FeSO_4.7H_2O$	0.02g
$ZnSO_4.7H_2O$	0.10g
$Na_2MoO_4.2H_2O$	0.002g
$CuSO_4.5H_2O$	0.002g
$MnCl_2.4H_2O$	0.002g

made up to one litre, autoclaved at 121°C for 15 min. and stored in the dark.

4. Liquid Media used in toxin studies

(i) Modified Fries Medium (after Luke and Wheeler, 1955)

$(NH_4)_2\ C_4H_4O_6$ (Ammonium tartrate)	5g
NH_4NO_3	1g
K_2HPO_4	1g
$MgSO_4.7H_2O$	0.5g
$CaCl_2$	0.13g
NaCl	0.1g
$C_{12}H_{22}O_{11}$	30g

made up to one litre in distilled water.

(ii) Wheat kernel extract I

100g untreated seed of the cv Joss Cambier were boiled up in one litre distilled water and then filtered through Whatmans No. 1 filter paper.

(iii) Wheat kernel extract II

100g untreated seed of the cv Joss Cambier were macerated in the Omnimixer homogeniser (Sorvall inc.), at speed 7 for one minute, made up to one litre and filtered through Whatmans No. 1 filter paper.

(iv) Wheat leaf extract

50g of leaves from 6 week old plants of the cv Maris Ranger were macerated in phosphate buffer, pH7, in the Omnimixer homogeniser; filtered first through 2 layers Whatmans No. 50 filter paper and then through a sterile 0.45 grade membrane filter (Oxoid Ltd., Southwark) under vacuum.

20 ml aliquots of the liquid media i) - iv) were added to 150 ml Erlenmeyer flasks (which had previously been sterilised in the case of medium iv) fitted with cotton wool plugs and aluminium foil caps and autoclaved at 121°C for 15 min. (excluding iv)).

The flasks were inoculated in a sterile flow cabinet with 0.25 ml of a sterile 10^{6} spores/ml Septoria spore suspension, delivered from a sterile pipette.

C. Plant Material

Spring wheat (sw) and winter wheat (ww) (Triticum vulgare, Vill.) seed was obtained from the following sources:-

1. Plant Breeding Institute, Cambridge

Maris Ranger (ww)	Cappelle Desprez (ww)	Maris Huntsman (ww)
Petkus (71/64)(sw)	Maris Widgeon (T518)(ww)	Champlein (T387)(ww)
Maris Ploughman (T733)(ww)	Troll (T112)(sw)	Maris Fundin (ww)
Kolibri (T83)(sw)	Cardinal (T270)(sw)	

2. Rothwell Plant Breeders, Lincoln

Zorba (Engelen 565) (10055) (ww)

3. Swiss Federal Research Station for Agronomy, Zürich

71806 (sw)	91442 (sw)	Fortuna (sw)	71807 (sw)
91292 (sw)	B519 (sw)	90951 (sw)	91499 (sw)
Svenno (sw)	90972 (sw)	91451 (sw)	Fletcher (sw)
91414 (sw)	91433 (sw)		

Zenith stem length mutants 1 and 4

4. United States Department of Agriculture

Bounty 208 (CI 15079) (sw)

5. Department of Agriculture, Madrid University

Splendeur (ww)	Florence Aurore (sw)
D104 (Triticum durum)	Champlein (ww)
Orso (ww)	Tres enanitos (ww)
Magali (ww)	Bidi 17 (Triticum durum)
Siete Cerros (sw)	Blue Bird Z (ww)
Mara (ww)	Safarino (ww)
Mexipak (sw)	Cajeme (ww)
Boulmiche (ww)	Inia - 66R (ww)
Pane 2 (ww)	Capitole (ww)
Atys (sw)	Charles Peguy (ww)
Goya (ww)	

Seed was stored in paper or muslin sacks in a dry wooden cupboard at room temperature. The seed was fumigated with 70 µl methyl bromide for 6 hours to prevent weevil contamination.

In the glasshouse, seeds were sown to a depth of 4 cm in John Innes No. 1 compost, 5-10 seeds per 5" pot, which were placed in spore free cabinets (Finney, 1973), on a self-watering sand bench under six 400 watt mercury vapour lamps giving a light intensity at bench level of c. 6,500 lumens/m^2 for 16h per day. Temperature range was 5-25°C.

For plant material up to 10 days old, seeds were placed in plastic germination trays (36 x 22.5 x 12.5 cm., Stewarts Plastics Ltd.), on damp blotting paper supported by a plastic shelf over tap water. Strips of 15 x 2.5 cm grade 90 blotting paper (Whatman) were used as wicks between the water and shelf. Trays were kept in the laboratory at room temperature.

D. Inoculation procedure

1. Preparation of spore suspension

Sterile distilled water was poured from a Mc Cartney bottle on to sporulating cultures, which were then stroked with a bent inoculating needle to disperse the spores. The spore suspension was decanted back into the bottle, agitated on a Fisons Whirlimixer for five seconds and filtered through a layer of muslin. The spore concentration was estimated using an 'Improved' Neubauer glass haemocytometer (Hawksley, London B.S.748) and adjusted to the required concentration by dilution with sterile distilled water. The washing of spores by successive centrifugation and resuspension (Morgan, 1974) was not practised since it was found to have a deleterious effect on subsequent infection. This may have been due to a nutrient leaching effect since centrifuged spores, when re-suspended in a 0.1% glucose solution, were able to infect normally.

2. Inoculation of Plant Material

(i) Detached leaves

Whole leaves were excised at the ligule with a sharp scalpel, passed gently through the thumb and forefinger acropetally, to remove the wax bloom but not to damage the epidermal cells, and then floated, adaxial side uppermost upon 75 ppm benzimidazole solution, in plastic boxes or trays depending on leaf size.

In varietal reaction tests, leaves were cut into 3 cm segments and these floated on 75 ppm benzimidazole in petri dishes.

A 10^6 spores/ml suspension applied from an Agla Micrometer syringe, as 20 µl droplets placed axially at 1 - 2 cm intervals along the leaves, gave relatively large clearly defined lesions. Alternatively inoculum was applied as a spray from an atomiser (ASL Spraymist). This resulted in a number of smaller lesions. Initially a spray gun (Shandon Scientific Co.Ltd.) with a pressurised power unit at 70 p.s.i. was used, but this resulted in poor symptom development, possibly due to spore damage when forced under pressure through a small nozzle.

Control inoculation drops of sterile distilled water were applied in the same manner as the inoculum.

In all cases lids were sealed with sellotape in order to maintain the required high humidity for infection, and then incubated in an illuminated incubator (Gallenkamp 1H280) at $20^{\circ}C$.

(ii) Whole plants

In the field clumps of about ten plants were spray inoculated until run off, and then covered with a polythene bag, sealed at the bottom with sellotape, for a period of 96 hours. This provided the necessary high humidity for infection, independent of prevailing weather conditions.

E. Observation of Leaf Surfaces

1. Necoloidin Strips

A Necoloidin solution was applied to the inoculated leaf surface by drawing a rod lightly over it. After about a minute, when the leaf began to curl, the film was stripped off and stained in 1% cotton blue in lactophenol for a few minutes, rinsed in lactophenol, and then mounted in fresh lactophenol. This technique enabled clear observation of the leaf surface, spore germination and hyphal growth.

2. 'Silcoset 105' Leaf Replication

A mixture of Silcoset 105 mixed with curing agent 'A' was poured over the inoculated surface and peeled off when set. The mould surface was coated with colourless nail varnish, which was removed when dry and mounted in water for examination. Although this technique minimised disturbing the surface growth, the replicas tended to be less clear, and so method E1. was preferred.

F. Preparation of Material for Light Microscopy

Morgan (1974) found difficulty in staining *Septoria* hyphae within leaf tissue using the Shipton and Brown(1962) technique and chloroform and chlorine clearing. A number of other clearing and staining regimes were set up, but none of them proved completely satisfactory.

1. Clearing

$\frac{1}{4}$ - 1 cm. square pieces of leaf tissue were cut and cleared in glass vials (2" x $\frac{3}{4}$" GWS) containing:-

(i) lactophenol

(ii) methanol

(iii) chloral hydrate

(iv) 1:1:1 mixture of methanol: chloroform: lactic acid

(i) resulted in considerable brown discoloration of the material while (ii) and (iii) took up to 4 days. Method(iv) was the more efficient, material being ready for staining after 1-2 days.

2. Staining

After clearing leaf pieces were transferred to glass vials containing stain, and placed in a desiccator which was then evacuated using a bench B95A vacuum pump (Aerosol) to ensure that stain would enter the intercellular spaces.

(i) acetic aniline blue: - 15:1:4, 5% aqueous phenol,
1% aqueous aniline blue,
glacial acetic acid

(ii) lactofuchsin:- 0.1% acid fuchsin in lactophenol

(iii) picric acid (chitin specific)

(iv) thionin and orange G:- 0.1% thionin in 5% phenol differentiated in saturated orange G solution

(v) 1% cotton blue in lactophenol

(vi) 1% aqueous aniline blue

Generally (vi) gave the better staining result.

To ensure that the inability of the hyphae within the leaf to take up the stain was not due to the inability of the stain to penetrate the tissue, thin sections were cut and a further, more specific staining technique tried - that of the histochemical localisation of β-glucosidases which involved the simultaneous azocoupling technique of Ashford (1970). 100 μ sections were cut on a Reichert OMP freezing microtome on a Frigistor thermoelectric stage cooler, washed in distilled water, and then pretreated in a series of buffered aqueous solutions to reduce osmotic damage:- 5, 10, 15, 20% methyl alcohol - 5-10 minutes per solution. These enzymatically active sections were incubated in the substrate solution, 6 brom-2 naphthyl β-D-glucopyranoside (Cohen et al., 1951) after addition of the diazonium salt (Fast Garnet 2 mg/ml solution) and after the solution had been stirred and filtered.

The high background activity of host β-glucosidases rendered this method unsuitable for locating the fungal hyphae within the leaf.

Detection of lignin

Lignin was located using a modification of the chlorinesulphite test (Ride, 1975). Leaf segments were decolourised in hot 70% ethanol and rinsed in water. Chlorine treatment was achieved by suspending leaves above a 1:1 mixture of 1% $KMnO_4$ and conc. HCl for 4h. After transferring to slides, segments were covered with 20% sodium sulphite and gently rolled to infiltrate the solution.

G. Preparation of Material for Stereoscan Electron Microscopy

Where epidermal observations were to be made 9 mm^2 pieces of the leaf material required were cut with a sharp scalpel. Otherwise epidermal strips were removed as far as possible and the remaining tissue used. 2.5% glutaraldehyde in 0.1M phosphate buffer pH 7.0 was preferable as a fixative to osmium tetroxide. Leaf material was placed in fixative in small glass vials (2" x $\frac{3}{4}$" GWS) which were then evacuated in a small desiccator in order that the pieces should sink and the fixative infiltrate all parts of the specimen. 2-4 hours later the specimens were given 3 rinses of 15 minutes in the buffer solution and then taken through a graded ethanol/water series:

10, 20, 30, 50, 70	80, 90, 95, 100%
10 mins each	20 mins

and then 3 changes of 30 minutes each in absolute alcohol.

Initially this was followed either by a graded series of absolute alcohol/amyl acetate solutions:- 6:1, 5:2, 4:3, 3:4, 2:5, 1:6 with a final overnight change in amyl acetate, or by a series of absolute alcohol/acetone solutions:- 1:3, 1:1, 3:1. However, these last two procedures did not give much improvement to the final specimen and their use was discontinued

The specimens were thus taken from absolute alcohol and placed directly into the bomb of the critical point drier. Liquid CO_2 was then passed through this bomb for 4 hours after which the CO_2 was vaporised, so leaving the specimens completely dry, and ready for mounting onto zinc stubs using either Durafix or silver dag. They were then coated with gold-paladium alloy in a Polaron E5000 Diode Sputter Coater and viewed in a Cambridge Stereoscan Mk IIA electron microscope.

H. Extraction of Plant Material

Two basic methods were employed, one to minimise and the other to maximise enzymic hydrolysis.

1. Minimal Hydrolysis

Weighed excised plant material was placed at intervals into a beaker containing boiling 95% ethanol or boiling water (5-10 mls per gram tissue). Once all the tissue was added the solution was boiled for a further 5 minutes. This tissue was then macerated in an omnimixer homogeniser (Sorvall Inc.) at speed 7 for 45 seconds. The slurry was filtered through Whatmans No. 1 filter paper in a Buchner funnel and the residue washed with further aliquots of solvent. All filtrates were combined for rotary evaporation.

2. Maximal Hydrolysis

Weighed excised tissue was macerated as above, but in distilled water and then left to stand at room temperature for 3 hours. Ethyl alcohol was then added until a 60% alcohol solution was reached, and the tissue again macerated, after which it was filtered as in 1.

J. Separation and Purification Techniques

Rotary film evaporation

The filtrates in each case were evaporated almost to dryness at 40°C on a rotary film evaporator (Wright Scientific Ltd.) which was fitted with two additional double condensers in series to ensure condensation of ether.

1. Solvent Partition

The residue in the 250 ml rotary evaporation flask was transferred quantitatively to a 100 ml separating funnel using 60% ethyl alcohol (1 ml per g fresh wt.), and then shaken with an equal volume of petroleum ether, (40-60°C boiling range). The petroleum ether phase was discarded and the partition repeated twice more to remove the bulk of the chlorophyll pigments. The alcohol phase was taken almost to dryness on the rotary film evaporator. The residue was transferred quantitatively in distilled water to a separating funnel and shaken with an equal volume of diethyl ether. The diethyl ether phases were bulked after further partition, and evaporated to dryness. If any water droplets remained in the ether phase they were absorbed by the addition of about a gram of anhydrous sodium sulphate, after which the ether could be decanted quantitatively into dry glass vials which were stored at -20°C until required for further purification. Aliquots of total crude extract and alcohol fractions were removed when desired and stored in the deep freeze. The aqueous phase from the ether partition was either discarded, or partitioned a further three times against n-butanol. The bulked butanol phases were evaporated almost to dryness and then transferred quantitatively to glass vials for storage, while the aqueous phase was discarded.

2. Chromatographic Techniques

Extracts were applied to pre-coated (20 x 20cm) thin layer chromatography silica gel plates, either glass (Camag, Muttenz, Switzerland) or aluminium (Woelm Eschwege, Germany) or aluminium rolls $60F_{254}$ (Merk, Darmstadt) or plastic sheets Polygram $SilG/UV_{254}$ (Camlab, Macherey-Nagel & Co., Germany) layer thickness 0.2 mm, using drawn out Pasteur pipettes. Samples were loaded at a rate of 0.25-1.0g fresh weight/cm, 2 cm from the edge of the plate and developed to a distance of 10-15 cm from the origin, in Shandon S.P. Chromotanks containing 100 ml freshly prepared solvent.

The following solvent systems were used:-

(a) 3%, 5%, 10% methanol in chloroform

(b) 5%, 25%, 50% acetone in chloroform

(c) 50:50 cyclohexane : ethyl acetate

(d) 4:1:1 butanol : acetic acid : water

(e) 50:30:5:10 ethyl acetate : methyl ethyl ketone: formic acid : water (Francis, Millington and Bailey, 1967)

Sephadex Fractionation

This technique was tried to purify the glucoside. 1 ml aliquots of the crude 60% ethyl alcohol extract (containing 5 g fresh weight/ml) were layered onto a 16 x 3 cm column of Sephadex LH - 20 (Pharmacia Fine Chemicals, Uppsala, Sweden). The flow, 2 ml/min, from the column passed through a Uvicord II ultraviolet absorptiometer detector unit, type 8303A, with a Uvicord II Control unit 8300 attached to an LKB flat bed potentiometer recorder 6500 (LKB Productor AB Bromma I, Sweden) before collection in Pyrex test tubes in 5 ml aliquots on a Radi-Rac Fraction collector, with Rotator type 3401B and turntable disc type 34068 (LKB productor).

Sephadex G-10, with water as the eluant was also tried.

Both of these processes were time consuming and neither gave good separation of the glucoside.

K. Location and Estimation of Substances

1. Ultra violet absorption

Dried plates were observed directly under an ultraviolet lamp for absorbing and fluorescent bands or spots which were then outlined in pencil.

2. Spray Reagents

These were applied (in the fume cupboard) using a Shandon chromatography spray gun.

(i) Ferric chloride (K. Fink and R.M. Fink, 1949)

A 5% solution of $FeCl_3.6H_2O$ in 95% ethanol was prepared and made 0.1 normal by the addition of concentrated HCl. Heating on a hotplate to 50°C was necessary for the salt to dissolve in the alcohol. This reagent was used in the location of benzoxazolinone substances, with which it gave a violet-blue reaction.

(ii) Permanganate (O.B. Maximor and L.S. Panthinkhina, 1965)

An alkaline solution was prepared by mixing equal volumes of 1% and 5% aqueous solutions of potassium permanganate and sodium carbonate respectively. This general reagent gives a yellow reaction with reducing compounds and aromatic polycarboxylic acids.

3. Cladosporium bioassay (Bailey and Burden (1973))

In order to locate antifungal compounds directly on TLC plates this bioassay was used, using a non-pathogen of wheat - Cladosporium. The pathogen, Septoria was unsuitable in this case since growth on TLC was poor and the transparent hyphae difficult to see, unlike the pigmented green of Cladosporium.

A dense spore suspension in sterile nutrient medium B(3) from 4-7 day old Cladosporium P.D.A. cultures, was filtered through one layer of muslin and then applied from a Shandon spray gun, directly onto strips of developed T.L.C. plates which had been dried to remove any traces of solvent. The plates were allowed to dry and then given a further spray with the spore suspension to ensure even cover. Surface moisture was allowed to evaporate and the damp plates then incubated face down, at room temperature, supported by rubber bungs $1\frac{1}{2}$" above damp Kimwipe paper in plastic trays (Stewarts Plastics Ltd.). Plates developed in butanol-acetic acid-water mixtures could not be assayed in this way since acetic acid residues which remain even when the plate is dried, were completely inhibitory.

The plates were viewed after 2 days, and if spore distribution was uneven, given another spray. After 4 days there would be thick green growth of Cladosporium except in those areae where inhibitory substances occurred, which remained white.

1-2 cm wide strips, cut from aluminium TLC plates were used in these bioassays, while the remainder of the plate was stored in the deep freeze in order to minimise decomposition which might occur in light at room temperatures. The inhibitory bands on the bioassay strips were matched up with the stored part of the TLC plates and these areas eluted for further chromatography, spectral analysis and Septoria droplet bioassay.

4. Elution

The area for elution was scraped from the TLC plate into sintered glass funnels 2 (BTL) containing a circle of Whatmans hardened 50 filter paper, and then washed with five ml of 95% ethyl alcohol (per 5 gm tissue). Elution in this manner prevented any fine particles of silica gel entering the eluate and causing light scatter in the spectrophotometer.

5. Spectral Analyses

Spectra were measured on a Beckman SB spectrophotometer with a Beckman Hydrogen Lamp power supply and Beckman Recorder (Beckman Instruments Ltd., Glenrothes, Fife) in 1 ml silica cuvettes, as 1 gm fresh weight/ml 95% ethanol solutions. Measurements in the UV below 240 nm were not taken since considerable error could arise as a result of the interference of co-extracted impurities and scattered light. Errors were reduced where possible using blanks on extracted clean silica gel taken from a point at the same migration distance from the start as the sample.

Photometric determinations of the benzoxazolinone substances was tried after reaction with $FeCl_3$. However since the ratio of reagent concentration to the amount of benzoxazolinone being estimated in the middle of the band or spot differs from that at the edges; and on the upper surface of

the layer from that on the underside, the colour reaction varied, particularly in the case of blanks due to uneven background colour. Also 2-3 times the volume of eluent was required to elute the coloured product, as compared to the original substance. Thus benzoxazolinones were estimated directly from their UV absorption following elution by ethanol.

6. Septoria Bioassay

The Standard Droplet bioassay of Purkayasta and Deverall (1965) was used. After the method of Deverall (1967) glass slides were washed firstly in very hot water containing the liquid detergent Divo-Lab No. 1 (Diversey Ltd., Barnet, Herts). The slides were then rinsed in distilled water, stood overnight in 2% acetic acid, thoroughly rinsed in distilled water, washed in alcohol and stood for a few hours in absolute alcohol, before drying in a warm oven. The cleaned dried slides were then supported on glass rods above water in plastic sandwich boxes ($7\frac{1}{2}$ x $4\frac{1}{2}$ x $2\frac{1}{2}$") (Stewarts Plastics Ltd.) 6 slides per box. The equivalent of 1 gm fresh weight of the eluted inhibitor, which had been evaporated to dryness in a stream of cold air from a hairdryer (Philips Type HP410 av) was taken up in 0.5 ml sterile casamino salts medium, and 3 x 20 µl droplets applied from a 0.1 ml glass pipette to the first slide. This gave a concentration of inhibitor twice that in the original tissue. A serial dilution was then made, removing 3 x 20 µl droplets at each step, giving 1, x $\frac{1}{2}$, x $\frac{1}{4}$, x 1/8 concentrations. On the remaining control slide were placed 3 x 20 µl drops of sterile nutrient medium. To the middle of each of the drops was then added a 2 µl droplet of 5 x 10^6 S. nodorum spore suspension delivered from an Agla micrometer syringe. The lids of the boxes were replaced finally, sealed with Sellotape and placed in an illuminated incubator (Gallenkamp 1H 280) at 20°C for 20 hours. Germination percentage and germ tube lengths were then recorded on ten randomly selected spores in each droplet (giving a total of 30 replicates per concentration) using an Olympus microscope (x 40 objective fitted with an eyepiece micrometer (eyepiece x 15).

K. Field Experiments

1. 1973-74 Season

During cultivations the seedbed was given a 15:40:40 units per acre ($N:P_2O_5:K_2O$) application and then top dressed by hand in early May with a further 40 units per acre of nitrogen.

A split-split plot design was employed. Each of three replicate blocks was divided into main plots for varieties (three winter wheat and four spring wheat cultivars). These main plots were divided into six treatment subplots. Two 12" diameter clumps of 10 seeds were sown in each subplot. This clump size had been found convenient to inoculate and assess in the field (Cooke and Jones, 1970). All sub plots were separated from each other by 2' wide guard rows of winter oats. (cv. Peniarth). The whole area was surrounded by a $\frac{1}{2}$" mesh wire fence, dug into a depth of 18" and three feet high to exclude rabbits. In order to prevent bird damage, a fruit cage (Agriframes Ltd. , East Grinstead, Sussex) was erected over the plots. The main frame of the cage was constructed of 12' long, $\frac{3}{4}$" Flo-coat steel tubing which had a special galvanised finish giving long protection against rust. All uprights were 7'6" overall, and when the cage was erected it stood 6'6" out of the ground, so enabling recording to be carried out underneath. A 'Netlon' $\frac{3}{4}$" plastic ultra-violet treated net was used on all sides while the roof was in two pieces of 1" mesh, also UV treated. Agrihooks were used to hold the nets down to the ground.

The following micrometeorological equipment was used:-

(1) A thermohygrograph T9154 (Cassalla, London). The chart moved 1.6 mm/hour and was changed at weekly intervals.

(2) A leaf surface wetness recorder (Meteorological Office) with a sensitivity of 2g.

(3) A Grant Miniture Temperature recorder, Model D. This was fitted with 20type B thermister probes.

(2) 1974-75 Season

In early October the seedbed was given a 15:30:10 units per acre ($N:P_2O_5:K_2O$) and in mid-April was top dressed with a further 40 units per acre nitrogen.

The design was that of a split plot, the six main varietal plots being divided into four subplots. The latter were twelve feet square, with seeds sown in rows to a 7" spacing. The area was surrounded by a wire mesh fence to eliminate rabbits. Since yields were not required, no attempt was made to prevent bird damage.

M. Statistical Analysis

In analysis of Standard droplet and toxin bioassays the following were calculated:-

1. The Standard error of a mean was calculated by:-

$$\frac{s}{\sqrt{n}}$$

when 'n' is sample size, and 's' is an estimate of the standard deviation calculated by the working formula:-

$$s^2 = \frac{\sum_i x_i^2 - \frac{\left(\sum_i x_i\right)^2}{n}}{n - 1}$$

2. Confidence limits were attached to means by:-

mean $\pm$ 5%, 1% or 0.1% value 't' (with error degrees of freedom) X standard deviation divided by square root sample size:-

$$\bar{x} - + \frac{ts}{\sqrt{n}}$$

3. Students 't' test to compare means:-

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}} \qquad \text{d.f. } n_1 + n_2 - 2$$

The following programs were used on the Wang advanced programming calculator, 720B (Wang Laboratories Inc.) in the analysis of field data.

1. Anovar:- analysis of variance for a complete factorial design

2. Linreg:- linear regression analysis, fitting given data points (x_1, y_1) to a linear equation:-

$$\hat{y} = b_o + b_1 X$$

3. Mulreg:- multiple linear regression analysis for a set of independent variables and one dependent variable. Selection of different sets of independent variables and designation of dependent variable could be made as often as desired

In the case of large volumes of data the CDC 6600 computer was employed using the BMD0 2V (-analysis of variance for factorial design - version of July 22, 1965)

A comparison of regression coefficients was made by using the following formula:-

(for this 't' is distributed as Students 't' with $(n_1 + n_2 - 4)$ degrees of freedom)

$$t = \frac{b_1 - b_2}{\sqrt{s^2 \left(\frac{1}{\sum_i X_{1i}^2} + \frac{1}{\sum_i X_{2i}^2} \right)}}$$

Where quantities b_1 and $\sum_i X_{1i}^2$ are the regression coefficient and sum of squares for X from the first sample, and similarly for the second sample, and

$$s^2 = \frac{\left\{ \sum_i Y_{1i}^2 - \left[\left(\sum_i X_{1i} Y_{1i} \right)^2 / \sum_i X_{1i}^2 \right] \right\} + \left\{ \sum_i Y_{2i}^2 - \left[\left(\sum_i X_{2i} Y_{2i} \right)^2 / \sum_i X_{2i}^2 \right] \right\}}{n_1 - 2 + n_2 - 2}$$

is the best estimate of variation about regression

SECTION 1.

VARIETAL REACTIONS ON DETACHED LEAVES AND FACTORS AFFECTING THEIR DEVELOPMENT.

Varietal Reactions on Detached Leaves

Varietal reaction tests were set up as described in Section D2(i) Materials and Methods. Leaf pieces were inoculated with 3 x 20µl droplets of 10^6 spores/ml suspension of S.nodorum, and lesion development asseesed after 5 days incubation at 18°C in an illuminated incubator. The relative amounts of necrosis, chlorosis and lesion extent were recorded, and the cultivars allotted on arbitrary disease rating. See Table 1. The range of leaf reactions is illustrated in Plate 1.

Throughout the laboratory work cultivars were selected which demonstrated the most susceptible and most resistant reactions. Maris Ranger was used as the susceptible variety throughout. Initially Engelen 565 (Zorba) was used for resistance, but owing to difficulty in obtaining seed, Maris Huntsman was subsequently used.

A range of cultivars was used in field trials.

Plate 1:- Varietal reactions of detached leaf segments to Septoria nodorum , 5 days after inoculation with 20µl droplets of 10^6 spores/ml suspension.



ENGELEN 565 J.CAMBIER C.DESPREZ HYBRID 46 M.RANGER CONTROL

Table 1 Varietal Reactions on Detached Leaves

Cultivar	Necrosis	Chlorosis	Lesion Extent	Disease Rating
Engelsn 565	+	-	-	1
Marie Huntsman	+	-	-	1
91451	+	-	-	1
71806	+	-	-	1
Goya	-	+	-	1
B519	+	+	-	2
Champlain (PBI)	+	+	-	2
Kolibri	++	+	-	2
Petkus	+	+	-	2
71807	+	+	-	2
Capitole	+	+	-	2
Mara	+	++	-	2
Maris Widgeon	++	+	-	3
91292	++	+	-	3
91414	++	+	-	3
Svenno	++	+	-	3
Bounty 208	++	+	-	3
Troll	++	++	+	4
Maris Ploughman	++	++	+	4
Fletcher	++	++	+	4
91433	++	++	+	4
91499	++	++	+	4
Champlain (Spain)	+	++	+	4
Atys	+	++	+	4
Boulmiche	++	+	+	4

Table 1 (Cont'd) Varietal Reactions on Detached Leaves

Cultivar	Necrosis	Chlorosis	Lesion Extent	Disease Rating
Splendour	++	+	+	4
Orso	++	+	+	4
Charles Peguy	++	+	+	4
Florence Aurora	+	++	+	4
Pane 2	++	+	+	4
Cappelle Desprez	++	+++	++	5
Joss Cambier	+++	++	++	5
Hybrid 46	+++	++	++	5
Maris Fundin	++	++	++	5
90951	++	++	++	5
Safarino	++	+	++	5
Tres Enanitos	++	+	++	5
Cajeme	+	+++	++	5
D104	+++	+	++	5
INIA - 66R	+	+++	++	5
Bluebird 2	+	+++	++	5
Magali	+++	+	++	5
Maris Ranger	+++	+++	+++	6
90972	+++	+++	+++	6
Fortuna	+++	+++	+++	6
Siete Cerros	+++	++	+++	6
Mexipak	+++	++	+++	6
Bidi 17	+++	+++	+++	6

Key + indicate relative amounts

Disease rating 1 - 3 Resistant

4 - 6 Susceptible

Macroecopic and Microecopic Development of the Reeietant and Sueceptible Reactions on Detached Leavee

Hypotheeee about the biochemical proceeee of reeistance can only be evaluated effectively when coneidered in relation to obeervable biological evente. The following hietological etudy was an attempt to investigate in detail the infection proceee and eubeequent leeion development in reeietant and eueceptible cultivare infected with S. nodorum. Studiee on the biochemical proceeeee are preeented in Section III.

Leaf piecee of the cultivare Engelen 565 and Marie Ranger were eet up ae described in Section D2(i) Materiale and Methode, and inoculated with 3 x 20µl droplete containing 10^6 eporee/ml S. nodorum. Varioue obeervatione were made:-

(a) Necoloidin etripe (E(i) Materiale and Methode) were prepared to aeeeee germination and eurface hyphal growth at 2, 4, 6, 8, 24, 26, 28, 30, 48, 54 and 120 houre after inoculation. See Table 2.

(b) The leaf pieces were obeerved for macroscopic symptom development and then cleared and etained in aqueoue aniline blue for light microecopy (F1(iv) and F2(vi) Materials and Methods) 1, 2, 3, 4, 6 and 10 daye after inoculation. Four day lesione were cleared and etained for lignin (F2, Materiale and Methods).

(c) Transverse sections of lesions were cut and mounted in aqueoue aniline blue for light microecopy.

(d) Entire lesione, and those with the epidermie removed were prepared for stereoscan electron microscopy (G. Materials and Methods).

Table 2 Surface Development of S. nodorum on Detached Leaves of Resistant and Susceptible Cultivars

Hours after inoculation	% Germination (mean of 3 lesions)	
	Maris Ranger (Susceptible)	Engelen 565 (Resistant)
2	3	5
4	5	5
6	6	4
8	-	6
24	98	-
26	98	97

Hours after inoculation	Distance of hyphal growth from centre of infection site (mm)	
	Maris Ranger	Engelen 565
2	2.16	2.16
4	1.58	1.46
6	1.88	1.63
8	-	1.88
24	1.79	-
26	1.91	2.00
28	2.04	2.29
30	2.00	2.29
48	1.79	2.08
54	2.16	1.81
120	4.25	3.66

Thus germination and surface growth are similar for both cultivars.

The Resistant Reaction on Detached Leaves

24 hours after inoculation

There were no visible macroscopic symptoms. Most spores had germinated producing a single, generally unbranched, hyphae of up to 300 µm. Stereoscan electron microscope (SEM) observations showed disappearance of the waxy layer of the cuticle directly under the hyphae (Plate 2), which ramified irregularly over the leaf surface. Appressoria (Plate 3) formed usually at the junction of epidermal cell walls. In light microscope observations the appressoria were surrounded by a blue halo indicating a greater penetration of the stain at this point. Disappearance of the wax was also evident under appressoria (Plate 3).

48 hours after inoculation

There were no visible macroscopic symptoms. There was now a large amount of branching hyphal growth. Blue haloes, indicating penetration points were numerous. Some epidermal and subepidermal cells had completely absorbed the blue stain, while in others only the walls appeared darkly stained.

72 hours after inoculation

Necrotic flecks were visible under the infection droplet. There was no chlorosis.

Surface hyphal growth was increasing. The inter-cellular spaces of subepidermal cells were beginning to show a necrosis, while the epidermal cells remained intact.

96 hours after inoculation

The necrotic flecks were coalescing to some extent, but the necrotic area was still limited to the area under the infection droplet. There was no chlorosis.

Plate 2:- Scanning electron micrograph of surface of leaf piece of wheat cv. Engelen 565, 24 hours after inoculation showing disappearance of the waxy layer of the cuticle beneath the hyphae of Septoria nodorum.

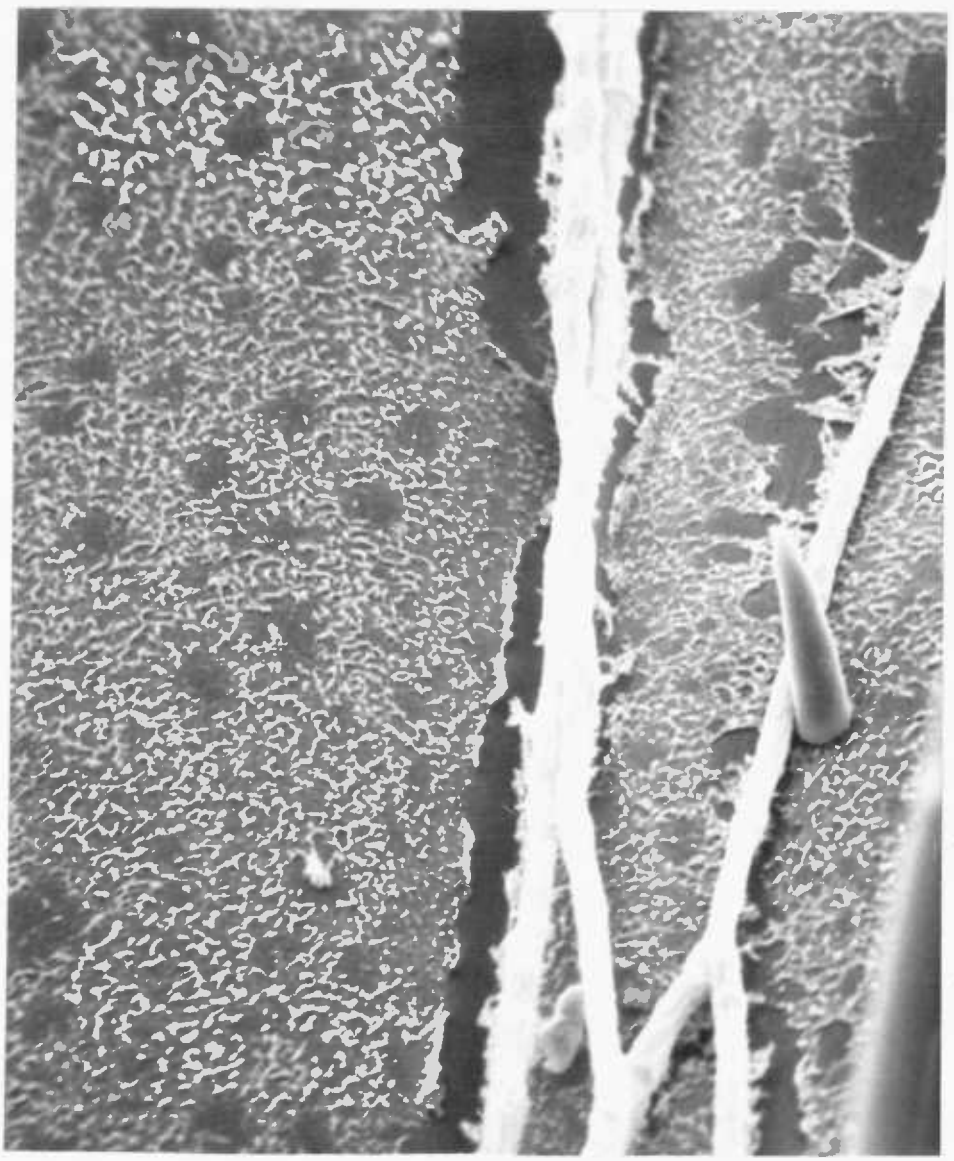
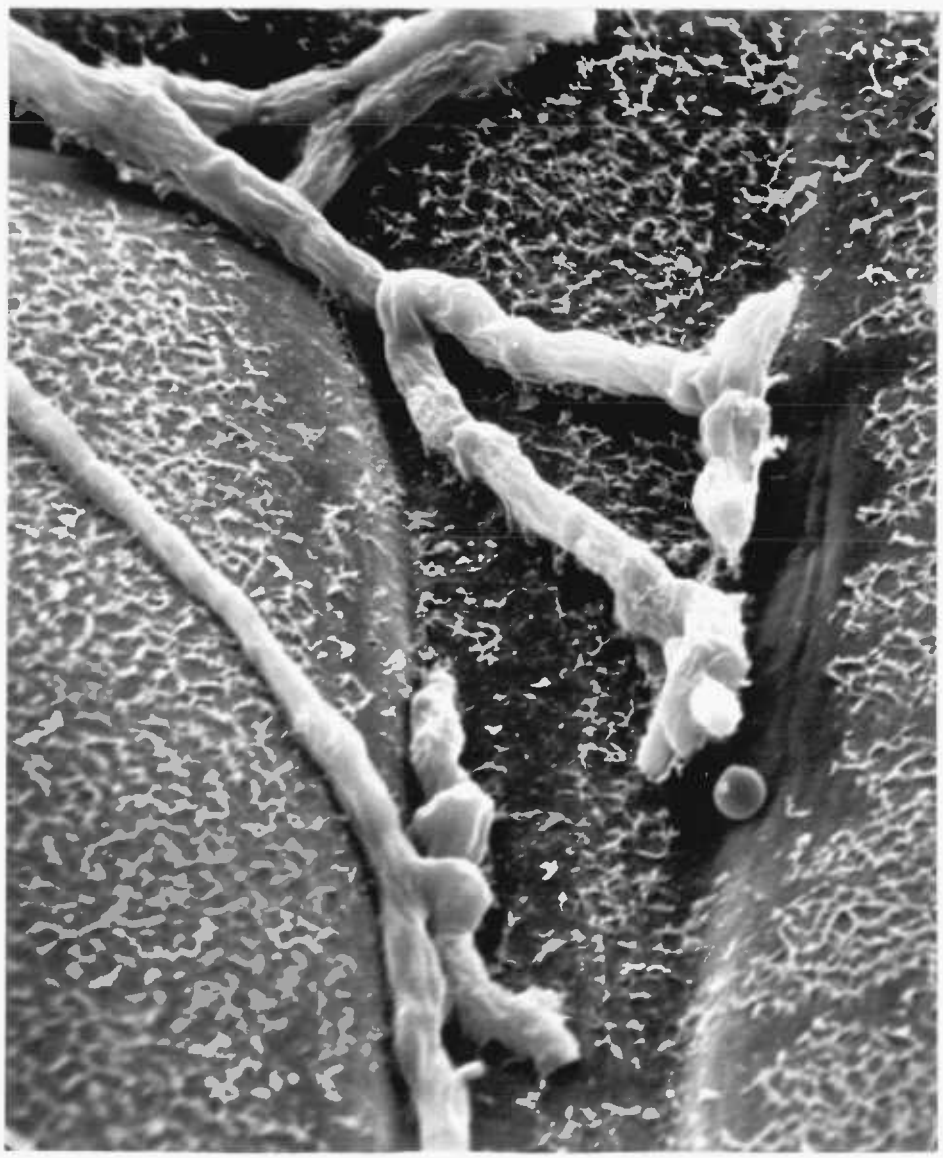


Plate 3:- Scanning electron micrograph of surface of leaf piece of wheat cv. Engelen 565 showing appressorial formation, and associated disappearance of the wax, at the junction of the epidermal cells.



Discrete areas of intercellular necrosis were common particularly in association with the veins. However thin sections showed the vascular bundles *per se* to be unaffected. Owing to necrosis and deep blue staining, hyphae could not be distinguished in the centre of the infection site, but single hyphae, rather thicker than those on the surface, were visible in the epidermal cells and beneath at the edge of the lesion (Plate 4). The hyphae tended to ramify in a generally longitudinal orientation with the long axis of the leaf (Plate 5). The hyphal colonisation was not extensive. (Plate 6). Lesions stained specifically for lignin gave a negative reaction.

144 hours after inoculation

Almost the whole area under the infection droplet was necrotic. There was a small chlorotic halo in some lesions.

Intercellular browning was extensive, particularly in association with the veins, and the mesophyll was disorganised. It was not possible to detect hyphae either within the lesion or ahead of it.

24 hours after inoculation

The dark necrotic lesion was still limited and had not extended beyond the area under the infection droplet.

The whole area was darkly stained and no hyphae were visible.

Plate 4:- Septoria nodorum hyphae in epidermal cells and beneath, at the edge of the lesion on Engelen 565, 96 hours after inoculation.

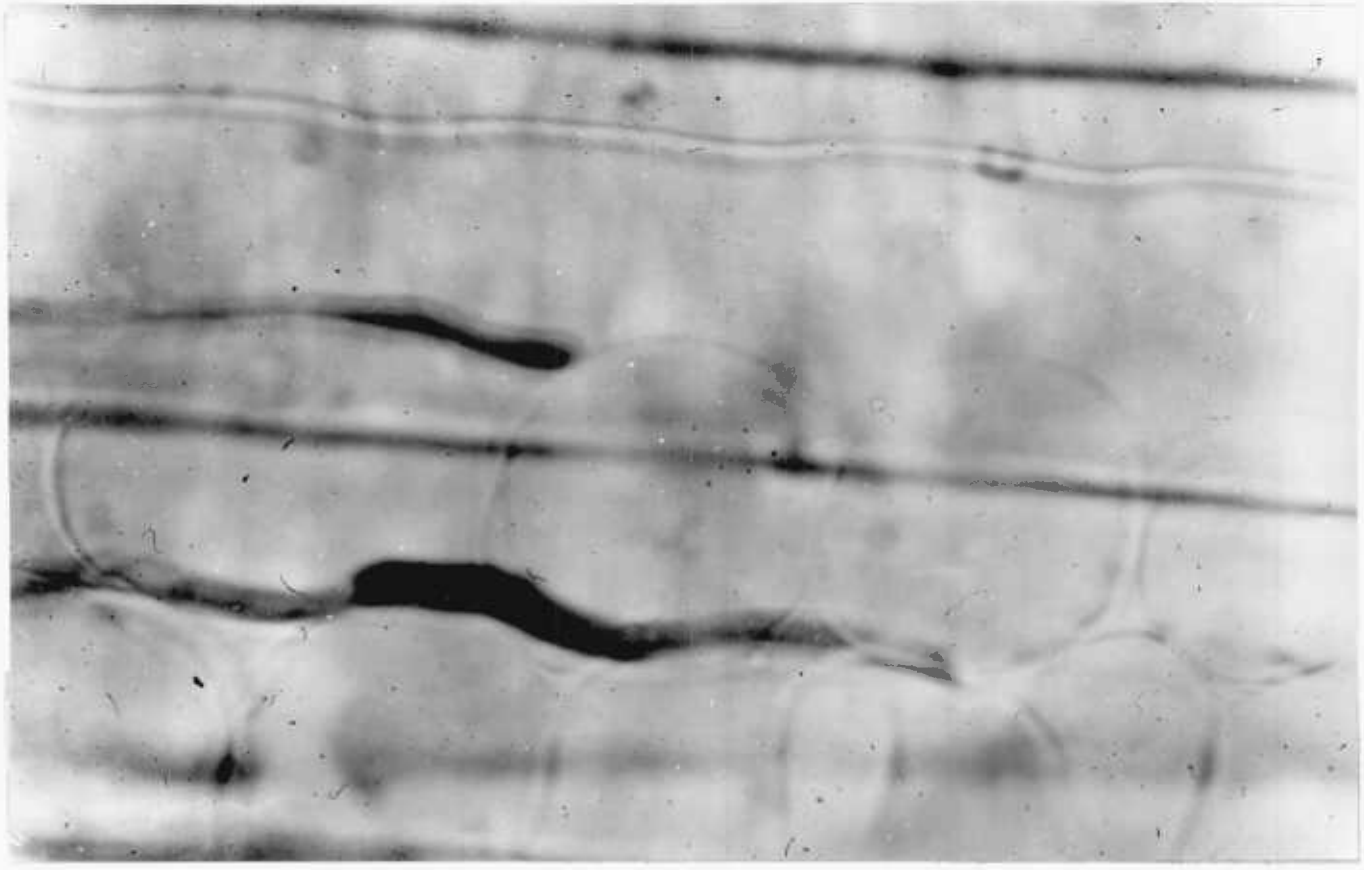


Plate 5:- Stereoscan electron micrograph of leaf piece of wheat cv. Engelen 565, 96 hours after inoculation. Epidermis has been removed to expose the hyphae of S.nodorum ramifying in a longitudinal orientation in the palisade layer.

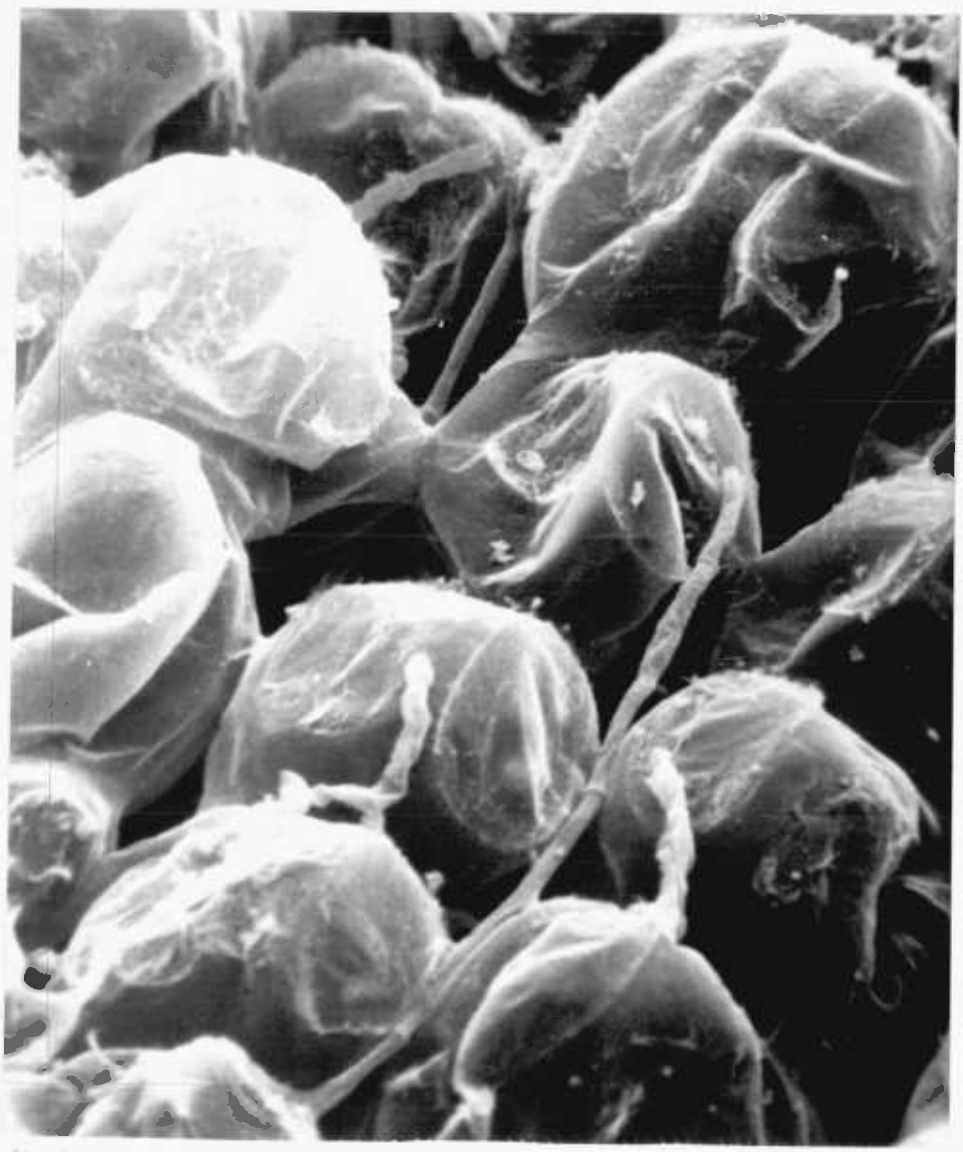


Plate 6:- Stereoscan electron micrograph of cross section of leaf piece of wheat cv. Engelen 565, 96 hours after inoculation, showing hyphae within the epidermal cell and sparce sub-epidermal colonisation by S.nodorum hyphae.



The Susceptible Reaction on Detached Leaves

24 hours after inoculation

There were no visible macroscopic symptoms. Spore germination was almost 100% and there were numerous penetration points. Large numbers of epidermal cells had become completely blue.

48 hours after inoculation

Some chlorosis was visible under the infection droplet, but there was no necrosis.

Surface hyphae growth was extensive, as was penetration. This was evident from the large numbers of blue cells both in the epidermis and beneath.

72 hours after inoculation

The chlorosis was extending to the edge of the infection droplet, but there was still no necrosis.

Little change had occurred since the previous observation. It was almost impossible to detect hyphae within the leaf by light microscope means, however SEM observations showed extensive colonisation within the leaf, both inter - and intracellular (Plate 7). The hyphae were branched and often closely associated with the cell wall (Plate 8).

96 hours after inoculation

The centre of the infection site was a beige/brown colour with a spreading irregular chlorotic halo extending beyond the edge of the infection droplet.

The whole infection site was staining darkly blue. Complete cells rather than the intercellular spaces were going dark brown and again these were particularly associated with the veins. Owing to the deep staining reaction hyphae could not be veiwed in the affected area, although single thick hyphae could be seen ahead of this. A transverse section showed

Plate 7:- Stereoscan electron micrograph of leaf piece of wheat cv. Maris Ranger, 72 hours after inoculation. Epidermis has been removed exposing the underlying cells and showing extensive colonisation of the leaf by S.nodorum.



Plate 8:- Stereoscan electron micrograph of leaf piece of wheat cv. Maris Ranger. Epidermis has been removed exposing the palisade and showing the close association of the hyphae with the cell walls.



Plate 9:- Light micrograph of transverse section of leaf piece of Maris Ranger wheat, 96 hours after inoculation showing the necrosis to have extended through the leaf. Note the apparent resistance of the vascular bundles.

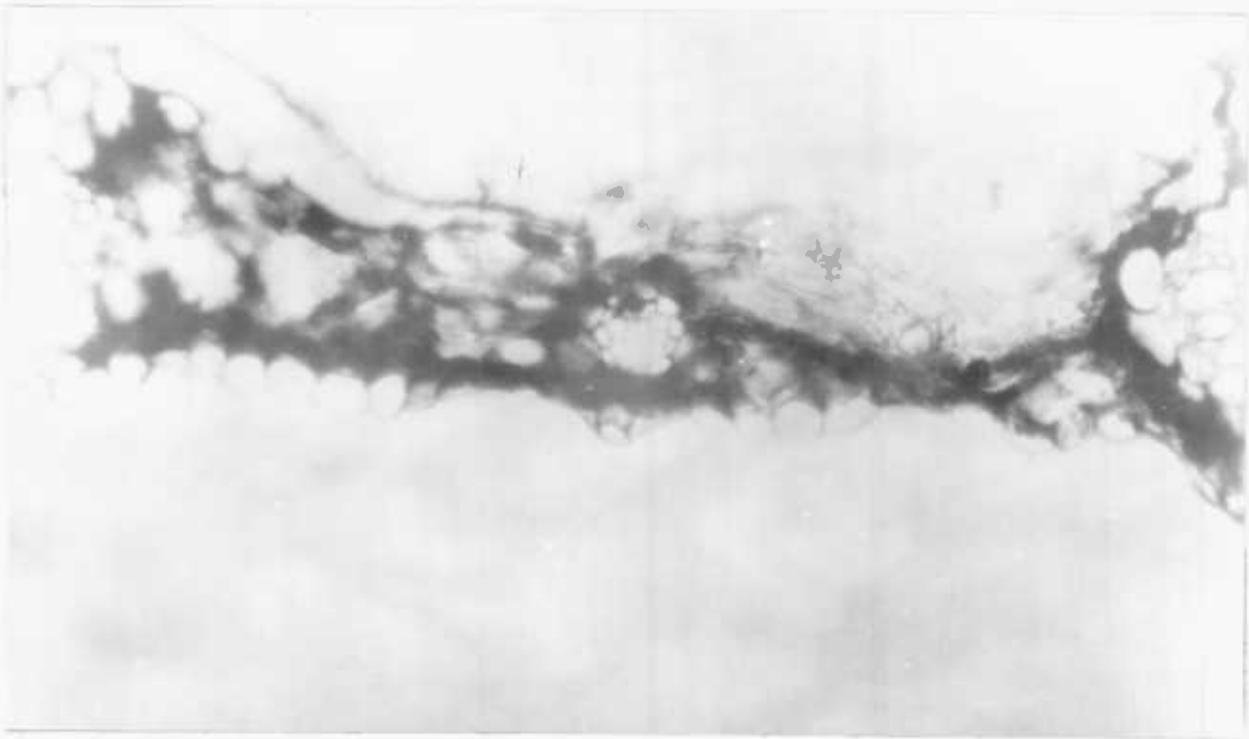
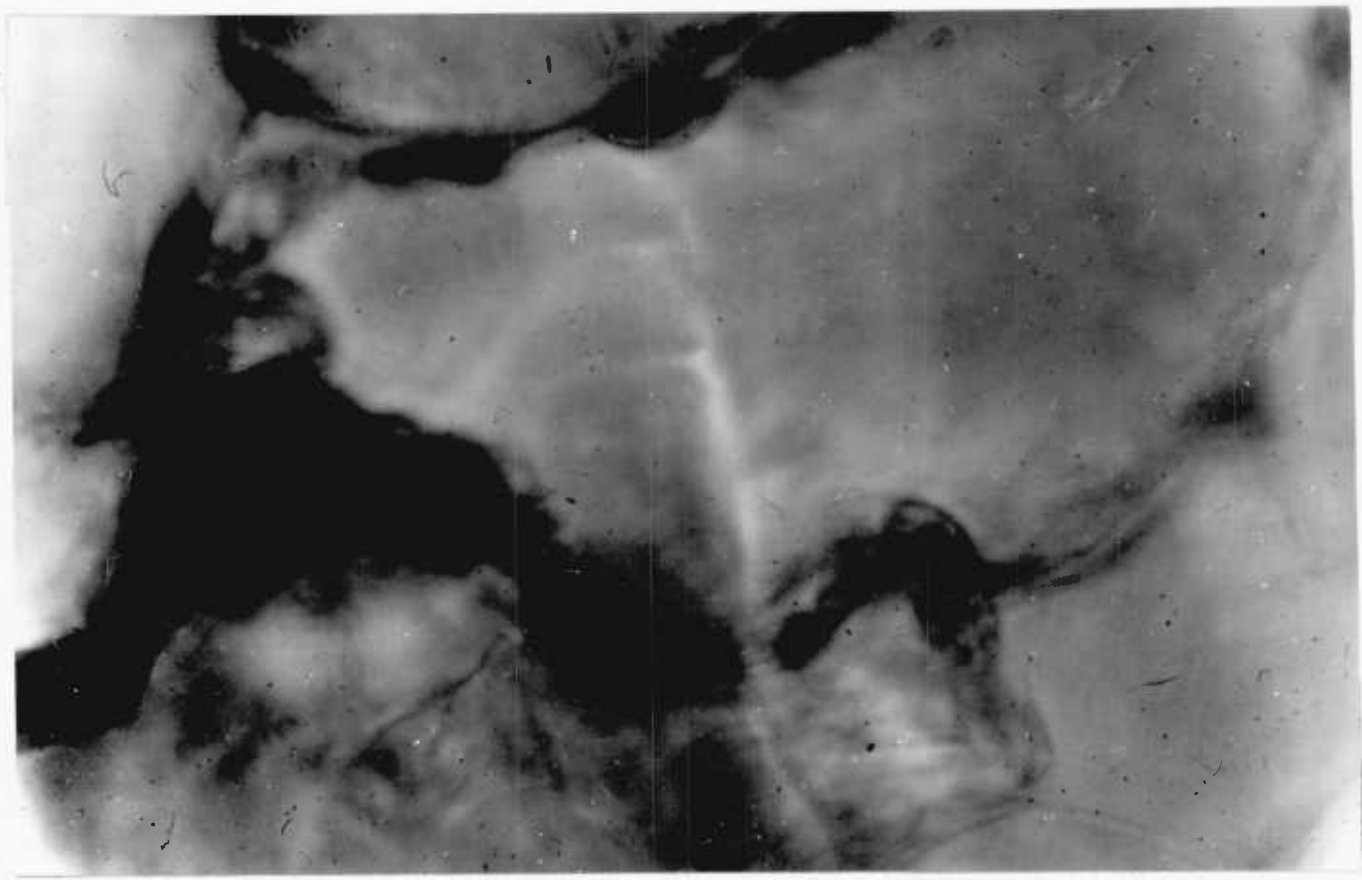


Plate 10:- Light micrograph of transverse section of leaf of Maris Ranger wheat, 144 hours after inoculation with S.nodorum showing disorganisation of cell wall and deposition of blue staining material.



the necrosis to have extended right through the leaf to the lower epidermis. (Plate 9). The vascular bundles appeared resistant in that no reaction occurred in them and also no hyphae were visible there. There was no positive reaction for lignin in specific staining tests.

144 hours after inoculation

The large irregular light brown necrotic lesions with wide chlorotic haloes were beginning to coalesce along the middle section of the leaf pieces.

Observation of the infection site was difficult owing to extensive necrosis and browning, cell disorganisation and excessive stain retention. Leaf sections tended to break on cutting. Hyphae could still be seen ahead of the affected area. In some cells which were not yet necrotic a degradation of the wall was occurring. The cell wall seemed disorganised and there were depositions of blue staining material. (Plate 10).

240 hours after inoculation

The lesions were now all coalescing and the leaf pieces completely chlorotic. There was some surface mycelial growth and pycnidia had formed in the substomatal cavities.

The cell walls ahead of the necrosis had taken up the aniline blue stain, but only in some cases could hyphae be seen in association with them. Where it was possible to see hyphae in the epidermal cells of the affected area, they appeared granular and more branched. The veins still remained largely unaffected. Hyphae were still visible just ahead of the affected area.

Factors Affecting the Development of Varietal Reactions on Detached Leaves

(a) The Effect of Centrifugation of Spores on Subsequent Lesion Development

During preparation of inoculum (Morgan, 1974) washed the spore suspension twice by centrifugation and resuspension. This process is time consuming and was thought to damage the spores and so affect their resultant infecting ability. The following experiment was set up to determine the possible detrimental effects of centrifugation on spores.

Spore suspensions of 10^6 spores/ml, prepared as described in Section D1 Materials and Methods, were centrifuged 0, 1, 2, 3, 4 times for 5 min at 1220g and resuspended either in distilled water or 0.1% glucose solution. Leaf pieces of the cultivars Maris Ranger and Engelen 565 were then inoculated in the usual way. Lesion development was observed. See Table 3.

The successive centrifugation of spores delayed ultimate lesion development, particularly in the resistant cultivar. This effect was overcome by addition of glucose to the infection droplet indicating a nutrient leaching effect by the centrifugation.

It was therefore decided not to centrifuge spores during preparation of suspensions.

Table 3 Effect of Centrifugation of Spores on Subsequent Lesion Development

CV	Days after inoculation	Spores suspended in	0 and x 1	x 2	x 3	x 4
MARIS RANGER	4	Glucose*	Necrotic Lesions with chlorotic halo coalescing	As x 1	Less necrosis and spread	As x 3
		Water	Discrete necrotic lesion with chlorotic halo	As x 1	Lesion more chlorotic	As x 3
	7	Glucose	All lesions coalescing	Large necrotic/ chlorotic lesions	As x 2	As x 2
		Water	Discrete lesions	As x 1	Lesion more chlorotic	As x 3
	13	Glucose	Lesions coalesced No green tissue remaining	As x 1	As x 1	As x 1
		Water	Lesions beginning to coalesce. Green margin almost gone	As x 1	Green margin otherwise as x 1	As x 3

cont'd ....

Table 3 (Cont'd) Effect of Centrifugation of Spores on Subsequent Lesion Development

CV	Days after inoculation	Spores suspended in	0 and x 1	x 2	x 3	x 4
ENGELEN 565	4	Glucose *	Discoloration under droplet	As x 1	Paler green under droplet	None
		Water	None	None	None	None
	7	Glucose	Discrete necrotic lesion	Less necrosis than x 1	As x 2	Less necrosis than x 2
		Water	Necrotic flecks and pale green	As x 1	Pale green under droplete	None
	13	Glucose	Necrotic lesion limited	As x 1	As x 1	As x 1
		Water	" "	Less necrosis than x 1	Smaller lesion than x 2	As x 3

* Glucose suspension 0.1% in Distilled Water.

(b) The Effect of Spore Concentration on Lesion Development

The following experiment was set up in order to determine the effects of different inoculum concentrations on the development of varietal reactions, and to see for instance, if the susceptible cultivar would be more resistant when challenged with a lower spore concentration. The optimum inoculum level for lesion development could also be found.

Leaf pieces of the cultivars Marie Ranger and Engelen 565 were inoculated with 20 µl droplets of spore suspensions of S. nodorum in a 10-fold dilution series from 10^7 - 10^2 spores/ml. Distilled water was used as the control. Symptom development was observed at intervals. See Table 4.

Thus with decreasing spore concentration there was a delay in lesion development. This was more pronounced in the case of the resistant cultivar in which at the lower dilutions necrotic lesions failed to develop at all. In the susceptible cultivar lesions developed at all spore concentrations. Optimum lesion development occurred in 4-6 days with a 10^6 spores/ml suspension. There is no added advantage in using a higher concentration of spores.

Table 4. The Effect of Spore Concentration on Lesion Development

		- Spores per ml -					
	Days after inoculation	10^7	10^6	10^5	10^4	10^3	Control 0
MARIS RANGER	4	Large necrotic lesion + chlorotic halo. Beginning to coalesce	Similar 10^7 but less necrosis.	Some necrosis under few drops.	Discoloration beneath some drops..	As 10^4	None
	6	All lesions coalescing	As 10^7 but less necrosis. Leaf margin green.	Lesion size increasing. Less necrosis than 10^6	Large coalescing lesion.	As 10^4	None
	7	Very little green tissue remaining.	Lesions coalescing but leaf margin green.	Chlorotic lesion coalescing margin green.	"	"	None
	13	All leaf piece brown pycnidia + mycelium.	as 10^7	No pycnidia	As 10^5		Some general chlorosis.

Table 4 (Cont'd) The Effect of Spore Concentration on Lesion Development

	Days after inoculation	- Spores per ml - 10^7	10^6	10^5	10^4	10^3	Control 0
ENGELEN 565	4	Few necrotic flecks under some drops.	As 10^4	Paler green under some drops.	As 10^5	None	None
	6	Limited necrotic spots Very little chlorosis.	Less necrosis than 10^7	Necrotic flecks in some	"	None	None
	7	Necrotic lesion with pale green haloes.	Less necrosis than 10^7	Necrotic flecks with pale green haloes.	As 10^5	Pale green no necrosis	None
	13	Necrotic lesion with chlorotic halo.	As 10^7	Less necrotic than 10^7	Necrotic lesion with chlorotic halo.	Pale green slight necrosis	None

(c) The Effect of Light and Temperature on Lesion Development

It was of interest to determine whether the differential varietal reaction would be sustained at varying temperatures and in the absence of light.

Leaf pieces of Maris Ranger and Engelen 565, floated on 75 ppm benzimidazole in Petri dishes, were inoculated with 20 µl droplets of a 10^6 spores/ml suspension of S. nodorum spores suspended in distilled water or 0.1% glucose. Half of the Petri dishes were covered with silver foil to exclude light. Four covered and 4 uncovered Petri dishes of each cultivar were then placed in illuminated incubators at 18°C, 20°C and 25°C. Subsequent lesion development was recorded. See Table 5. Thus in the absence of light lesion development is delayed and the necrotic reaction partly suppressed. This effect was overcome to some extent by glucose. Lesions developed more rapidly at 20°C than 18°C but coalescence was prevented at 25°C. Throughout the experiment the control leaves in the light remained dark green, while those controls in the dark tended to fade and in some cases go almost completely chlorotic.

The conditions for optimum symptom development appear to be at 20°C or slightly below and in the light. The absence of light confers some degree of resistance on the leaf pieces. Throughout the experiment the differential reactions of the resistant and susceptible cultivars were sustained.

Table 5. The Effect of Light and Temperature on Lesion Development

(a) Maris Ranger

T[a]	DAI[b]	+ LIGHT	- LIGHT	- LIGHT + GLUCOSE[c]
18°C	4	Necrotic Flecks. Diffuse chlorotic haloes coalescing along midrib.	Discrete light brown lesions, pale diffuse chlorotic haloes.	Discrete necrotic lesions.
	5	Necrotic coalescing lesions with chlorotic haloes.	Discrete lesions with chlorotic haloes.	Discrete pale brown lesions.
	8	Dark coalescing lesions + large chlorotic haloes.	Light, discrete, necrotic lesions.	Light necrotic lesions with chlorotic haloes.
20°C	4	Dark necrotic lesions with coalescing chlorotic haloes.	Discrete light brown lesions with chlorosis between.	Large light necrotic lesions, chlorosis between.
	5	" with less chlorosis than 18°C.	Discrete necrotic lesions, chlorotic band coalescing.	Discrete dark necrotic lesions.
	8	Dark necrotic irregular lesions with coalescing chlorotic haloes.	Discrete light necrotic lesions.	Coalescing light necrotic lesions.
25°C	4	Dark necrotic irregular lesions. Chlorotic haloes not coalescing.	Light brown necrotic lesions. Diffuse chlorotic haloes, coalescing on midrib.	Dark necrotic coalescing lesions, with chlorotic haloes
	8	"	"	"

T[a] = Temperature

DAI[b] = Days after inoculation.

c = 0.1% glucose.

Table 5 (Cont'd)

(b) Engelen 565

T	DAI	+ LIGHT	- LIGHT	- LIGHT + GLUCOSE
18°C	4	Necrotic flecks. No chlorosis.	No reaction	Discoloration under some droplets.
	5	Discrete necrotic lesions. No chlorosis.	Some discoloration few chlorotic haloes.	Necrotic lesions.
	8	Discrete dark necrotic lesions with tiny chlorotic haloes.	Discrete light brown lesions. Vague chlorosis.	Discrete larger light brown lesions.
20°C	4	Discrete necrotic lesions. No chlorosis.	Some discoloration	Discrete dark necrotic lesions. No chlorosis.
	5	"	"	" " with some chlorosis.
	8	Some lesions with vague chlorotic haloes.	Light brown lesions with chlorotic haloes.	" "
25°C	4	Necrotic flecks under some droplets. No chlorosis.	Some discoloration only.	Some discoloration only.
	8	Discrete necrotic flecked lesions with tiny chlorotic haloes.	Discoloration only veinal necrosis.	Discrete necrotic lesions. Veinal necrosis.

To Investigate the Possible Toxic Effect of S. nodorum

The work of Bronnimann (1968a), in which he demonstrated an effect of the pathogen over and above that of defoliation, suggested that the fungus was producing a toxic principle during colonisation of the host. Since toxins are produced best by actively growing mycelial cultures, a number of liquid media were made up in order to find the most suitable for vigorous growth of the fungus. Subsequently the cell free culture filtrates were introduced into the resistant and susceptible varieties to determine whether the symptoms could be in part or fully reproduced.

The following media were prepared (Materials and Methods 8(4)).

1. Modified Fries medium
2. Above with the addition of Difco yeast 0.5g/ml 500 giving a concentration of 0.1%
3. Wheat kernel extract I
4. Wheat kernel extract II
5. Wheat leaf extract

After inoculation these cultures, 4 replicates of each, were set up in the following conditions:-

(a) 15°C still

(b) 20°C still

(c) 20°C shake

Growth was recorded after five days. The results are summarised overleaf in Table 6.

Thus the shake cultures of Fries medium with yeast grown at 20°C give best mycelial development, while shake cultures of wheat kernel extract grown at 20°C result in greater pycnidial formation. The Fries medium with yeast would be expected to contain the highest yield of the toxic principle if it is produced.

Table 6. To Show Relative Growth of S. nodorum in Liquid Media

Conditions \ Media	Modified Fries + Yeast	Modified Fries - Yeast	Wheat Kernel Extract I	Wheat Kernel Extract II	Wheat Leaf Extract
			Bacterial contamination from kernels.		No Growth
15°C Still	MM	MM	MM	MM	
20°C Still	M	M	M	M	No Growth
20°C Shake	MMM	MMM	MMPP	MMPP	No Growth

KEY M = Mycelium

P = Pycnidia

The 4-6 day old cultures were filtered firstly through Whatman's No. 1, then Whatman's No. 50 hardened filter paper, and finally through a bacteriological membrane filter (0.45 μ) (Oxoid Ltd. London).

The following assays were set up to assess the toxicity of the cell-free culture filtrates.

(a) The youngest fully expanded leaves of 6-week old (Maris Ranger and Engelen 565) glasshouse grown plants were detached and then either bruised or punctured. The leaves were floated on benzimidazole in plastic boxes. The wounds, which were at 1 cm intervals along the leaf, were inoculated with 20 μl droplets of cell free culture filtrate, and then incubated at 20°C in an illuminated incubator. Controls were set up similarly using liquid medium in which no fungus had grown, and also using distilled water.

(b) cm^2 leaf pieces of Maris Ranger and Engelen 565 were placed in glass vials containing the filtrate, and distilled water respectively, and then evacuated in a desiccator until the leaf pieces sank - so ensuring all intercellular spaces were flooded with the respective solutions. The leaf pieces were then removed and floated on 75 ppm benzimidazole in petri-dishes in the incubator.

(c) The cut ends of excised, youngest fully expanded leaves of 6-week old plants were placed in the filtrate, and then placed in a draught from a hairdrier to maximise transpiration for about half an hour. The filtrates were replaced with water and the vials were then placed in the incubator.

Assays (a) - (c) were viewed daily.

(d) Germinated wheat kernels with radicles of 5 mm were placed on blotting paper in petri-dishes, 5 seeds/dish, to which was added 5 mls culture filtrate, control filtrate or distilled water. The lids were sealed with sellotape and the dishes placed in the illuminated incubator at 20°C. Root length and number and shoot length were measured after 72h incubation. Results of (a) - (d) are summarized in Tables 7 and 8.

Table 7:- To show effect of cell-free culture filtrates on susceptible and resistant wheat cultivars.

		- MEDIA -			
	Assay	Wheat kernel medium I 15°C still (MM)	Wheat kernel medium II 20°C shake (MMPP)	Wheat kernel medium I 20°C still (M)	Fries medium + yeast (MMM)
REACTIONS AFTER 7-10 DAYS MARIS RANGER - SUSCEPTIBLE	Damaged leaves	Light brown necrosis under most inoculation droplets, with small chlorotic halo. Leaf edges watersoaked.	Little necrosis, no chlorosis	Little necrosis no chlorosis	No necrotic reaction
		CONTROL - GREEN	CONTROL - SOME CHLOROSIS	CONTROL - GREEN	CONTROL - GREEN
	Evacuated leaf pieces		Water-soaked and some necrosis throughout		
			CONTROL - GREEN		
	Excised leaf tips in toxin		General chlorosis, tip dry. Necrotic flecks at base.		
			CONTROL - SOME CHLOROSIS		

cont'd

Table 7 cont'd : To show effect of cell free culture filtrates on susceptible and resistant wheat cultivars

REACTIONS AFTER 7-10 DAYS

ENGELEN 565 - RESISTANT

Assay	Wheat kernel medium I 15°C still	Wheat kernel medium II 20°C shake(MMPP)	Wheat kernel medium I 20°C still(M)	Friee medium + yeast (MMM)
		- MEDIA -		
Damaged leaves	Few necrotic spots, some chlorosis in middle. Lot mycelial growth.	Some necrosis, some chlorosis; fungal growth.	Very little necrosis, fungal growth, chlorosis.	No necrotic reaction. Some chloroeis
	CONTROL-Green with some chlorosis	CONTROL- Green	CONTROL- Yellow	CONTROL- Some chlorosis
Evacuated leaf pieces		Only necrotic around edges		
Excised leaf tips in toxin		Tip darker and necrotic		

Seedlin root bioassay	(cv. Maris Ranger)	Septoria filtrate	Culture medium	Water control
		5.6cm	5.1cm	3,8cm

(value are means of 60 roots)

TABLE 8. Seedling Toxin Bioassay

(Means of 30 Seedlings) (length in cm)

Exp. I.	Septoria Filtrate (Fries medium + yeast)	Control Filtrate	Control Water
Root Length	6.0	5.4	3.4
Root Number	4.2	5.4	4.9
Shoot Length	5.0	5.8	5.1
Exp. 2	(Wheat kernel extract I)		
Root Length	6.8	4.7	5.9
Root No.	5.6	6.0	4.8
Shoot Length	7.4	7.7	6.3

Septoria Filtrate & Tapwater Control $\overset{***}{>}$ Control Filtrate

Exp. 3	(Wheat kernel extract II)		
Root Length	8.4	5.8	5.1

Despite vigorous mycelial growth of the fungus, the Fries medium culture filtrate failed to initiate any symptoms in either cultivar.

More success was apparent with the wheat kernel extract media; however in no cases were the symptoms, particularly chlorosis, satisfactorily reproduced within the time of normal lesion development of 4-5 days.

The susceptible cultivar did appear to be more sensitive than the resistant cultivar with respect to degree of browning and necrosis when infiltrated with the culture filtrates.

In the root bioassays a stimulation of growth occurred even with the filtrate which was toxic to leaf pieces. This reflects the relative insensitivity of the assay.

DISCUSSION

The varietal reactions of wheat to S. nodorum show continuous variation with regard to degrees of necrosis, chlorosis, and lesion extent. No varieties tested possessed immunity to the pathogen. Moreover different parts of the plant vary in susceptibility; thus Baker (1969) showed that although Svenno and Troll were susceptible to leaf infection, they exhibited some degree of resistance towards ear and seedling infection. This heterogeneity of reaction makes the work of the plant breeder in selecting cultivars very difficult.

Surface development of the pathogen on resistant and susceptible leaves is analagous. Germination is almost 100 per cent in both cases and subsequent extent of growth across the leaf similar, although on the whole, hyphae on the resistant leaf surface tend to branch less.

The disappearance of the surface wax occurred in both cultivars. This may arise from preesure or extracellular enzyme production by the hyphae. There are no reports of wax-degrading enzyme production by S. nodorum.

Visible macroscopic symptoms first became apparent in the susceptible cultivar as a discoloration of the tissues directly beneath the infection droplet. This is a general chlorosis indicating chloroplast disintegration and chlorophyll breakdown. The apparent photosynthetic rate has been shown to have a sharp initial decrease during the first day after inoculation, before symptoms become obvious. (Krupinsky, Scharen and Schillinger, 1973).

The difficulties of staining the hyphae within the leaf tissue made any appraisement of the exact relationship of the fungus and host unfeasible. That S. nodorum produces enzymes capable of cell wall degradation was demonstrated by Baker (1969). In culture the pathogen produced amylases, pectic methyl esterase and polygalacturonase. However, the role of enzymes in pathogenicity remains to be elucidated. From light

microscopy observations the cell walls appeared disorganised. At times hyphae were observed in close association with the cell wall, and this may be one reason why they are difficult to stain. In some cases the dark staining cell walls may in fact have been a cell wall and hyphae in close association. Transmission electron microscopy may be of value in this respect since it should allow a precise determination of the association to be made.

A necrotic and browning reaction was first visible in the resistant cultivar, and although it developed in the susceptible cultivar also, the reaction was never as intense in the latter case. Further, the position of the browning differed; tending to be intracellular in the susceptible and intercellular in the resistant cultivars. The necrotic reaction tended to be suppressed in the absence of light and at higher temperatures. Benedict (1973) showed a direct relationship between decreasing light intensity, increasing peroxidase activity and decreasing numbers of mature pycnidia in celery infected by Septoria apiicola. That seedling infection is reduced at high temperatures was reported by Baker (1969). The larger the inoculum concentration the faster was the development of necrosis. This more intense reaction at higher spore concentrations is consistent with Brönnimann's (1968a) result that higher inoculum concentrations result in greater yield loss.

The role of the browning reaction in resistance to S. nodorum remains open to speculation. Typhula incarnata can grow in wheat tissues which are discoloured deep brown (Hirai, 1956) while in other host/parasite systems there is a direct relationship between browning and resistance (Wood, 1967). This tends to be evident in the Septoria/wheat system, since resistance is associated with a dark necrotic reaction. For this facultative parasite the duration of symbiosis required for the establishment of a parasitic relationship is of primary importance. The rapid

response with browning within this duration, as in Engelen 565 ; may be the cause of resistance. Resistance appears to depend on the relationship between the spread of pathogenic activity of the parasite and the speed of host response.

The rapidity of lignification in wheat in response to the non-pathogens Botrytis cinerea and Mycosphaerella pinodes and its close association with the approach of fungal hyphae, Ride (1975) suggests that lignin plays some part in the restriction of these fungi. He demonstrated a comparative delay in lignification in response to S. nodorum and postulated that the ability of S. nodorum to counter host defences could be due to the breakdown of lignified cell walls by specific enzymes produced only by the pathogen. It was not possible to demonstrate lignification in response to infection in the cultivars in the present research using Ride's staining technique.

Apart from cell wall degrading enzymes the pathogen can affect the host by production of toxic compounds. Various work has indicated the possibility of toxin production; Brönnimann (1968b) demonstrated an effect of the pathogen over and above that of defoliation. The extensive chlorosis of more susceptible varieties and electrolyte leakage (Morgan 1974) also point towards this. The fact that hyphae were observed ahead of any reaction tends to indicate that the toxin, if produced, is not translocated.

Assays did not give conclusive evidence of toxin production by the fungus in culture. The direct application of the filtrate to leaves which had been damaged did to some extent reproduce symptoms, but only 5-7 days after the time of normal lesion development. The necrotic reaction was erratic while the chlorotic reaction was hardly ever reproduced. However the greater sensitivity of the susceptible cultivar did demonstrate a differential effect of the cell free culture filtrates.

In seedling root bioassays the filtrates both from fungal and sterile cultures gave a stimulation of growth over the water controls. A stimulation at low concentrations by non-specific toxins has been reported previously (Bottalico, 1969; Brian, Dawkins, Grove, Hemming, Lowe and Norris, 1961; Kern, Naef-Roth and Defago, 1971). The cell free filtrates used in the assays reported here were already rather viscous and were not further concentrated. Some purification in the form of partition might have been advantageous. The root bioassay is considered by some to be rather insensitive. With the Helminthosporium victorae toxin a tenfold greater concentration was needed to inhibit roots over that needed to demonstrate electrolyte leakage.

The differential growth of the fungus in the various liquid media reflects the sensitivity to culture conditions, and possibly an effect on toxin production. Toxins have been shown to be produced best by active growth of mycelium. However the culture with most mycelial growth (Fries medium + yeast) induced no reaction in the leaf assays. This is of interest since Krupinsky, et al (1973) showed that while mycelial type cultures were more pathogenic to heads and peduncles, pycnidial type cultures were more pathogenic to flag leaves.

Not only do culture conditions (aeration, temperature, C and N sources, metal ions) affect toxin production; but the state of the host plant used for assay and the subsequent conditions of the assay (length of uptake time of toxin, seasonal and diurnal variations in plants, tissue age, light intensity and type, nutrition) affect toxin effect. While these conditions were standardised throughout this work, they may have been unsuitable for toxin production.

The one reported case of toxin production by S. nodorum in culture comes from France. (Bousquet et al 1974) and was published subsequently to this work. They also used modified Fries medium with yeast, but in the dark. Cultures were harvested after 21 days growth, which is rather

old. Before assays they purified the filtrates by ethyl acetate partition and chromatography. This enabled them to obtain inhibition of coleoptiles and roots at 50-200 ppm toxin concentration.

Other Septoria spp have been shown to produce toxins; S. avenae (Poole and Murphy, 1952) and S. linicola (Covey, 1962). In the latter example older cultures proved less toxic, thus showing that the toxin was not a product of staling.

SECTION 2 :

DEVELOPMENT OF *S.NODORUM* INFECTION ON WINTER AND SPRING WHEAT IN THE FIELD

Field Experiment 1A

The experiment was set up to investigate the effects and possible interactions of inoculation date, variety and leaves on development of S. nodorum infection on winter wheat

Details of the split-split plot design used are given in Materials and Methods section L. The cultivars used were Maris Ranger, Cappelle Desprez and Maris Huntsman: susceptible, intermediate and resistant respectively to infection in detached leaf tests (Section I).

The clumps of plants were inoculated at the following stages of growth:-

Growth stage Feekes scale (Large, 1954)	Description
6-7	Stem extension; 2nd node visible
8-9	Last leaf and ligule just visible
10	In boot
10.5	Flowering (i) whole plant inoculated
	(ii) ears only inoculated

There were also uninoculated controls.

The ten main culms in each clump were selected and a visual assessment of the amount of photosynthetic area affected by lesions of S. nodorum on the top four leaves and ear was made each week after inoculation, using the keys of Brönnimann (1968a). Duration of leaf surface wetness, maximum and minimum temperatures and humidity were recorded.

A measurement of temperatures within the canopies of the three cultivars was also made in order to obtain some idea of the microclimate. Temperatures were recorded hourly for certain periods throughout the season, at three levels within the crop:-

(1) at ground level

(2) midway, between the 2nd and 3rd leavee

(3) at the top, level with the flag leaf

and also at the correeponding levels in the open air. The ecale on the Grant recorder wae set to 5-20°C, and although, at times readinge did not fall within thie range, it was thought to be the most practicable.

One replicate in the winter wheat trial wae abandoned due to poor growth.

The data collected was subjected to an analysis of variance on a whole plant and individual leaf basis. The raw data is presented in the Appendix 1A, Tables 1-18.

The apparent infection rate, 'r' wae calculated for whole clumps and individual variety/inoculation stage/leaf combinations, after the data had been transformed using the van der Plank (1963) $\log_e \left(\frac{x}{1-x}\right)$ transformation. Here 'x' represents the proportion of foliage infected; thue where 18% of the leaf is infected $x = 0.18$. '(1 - x)' is a correction factor to allow for a decreasing proportion of tissue left for infection as the season progreeses. The apparent infection rate 'r' ie defined so that if 'r' is constant, $\log_e \left(\frac{x}{1-x}\right)$ plotted against time gives a straight line. Any deviations from straightness show 'r' has varied. Values of 'r' are calculated as the linear regression coefficients of $\log_e \left(\frac{x}{1-x}\right)$ on time.

Mean values of 100% were omitted from the analysis since on transformation they become almost infinity. This unavoidably tends to distort the data since a leaf giving a value of 90% one week and 100% to the following reeults in an apparent decrease in mean infection for the whole plant. Generally leaf 4 reached 100% by 40 and leaf 3 by 63 days after inoculation, and thus the anomaly is the same for all varieties.

Further anomalies in the data arise from the rabbit and mouse damage which occurred in spite of netting the experimental area. Damaged culms were replaced by nearby culms, but the level of infection could not always be reproduced. Thus where a culm with a lower level of infection was substituted, the mean % infection would apparently decrease.

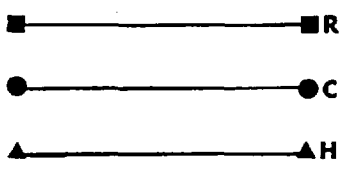
The mean weekly % infection results for each treatment/variety combination are given in Figures 2-7. These are followed by the analysis of variance F-values for the main effects and interactions of treatments, cultivars and leaves in Table 9.

Field Experiment 1A

Winter wheat 1974

The Development of S.nodorum infection

Key to Figs. 2-7



R Maris Ranger

C Cappelle Desprez

H Maris Huntsman

Treatment No.	Inoculation at growth stage
1	6-7
2	8-9
3	10
4	10.5 (whole plant)
5	10.5 (ears only)
Control	uninoculated

Each point on the graph is an average % infection of 3 or 4 leaves and the ear for up to twenty plants.

Figure 2:- Mean weekly % infection; whole plants; winter wheat, 1974

TREATMENT 1

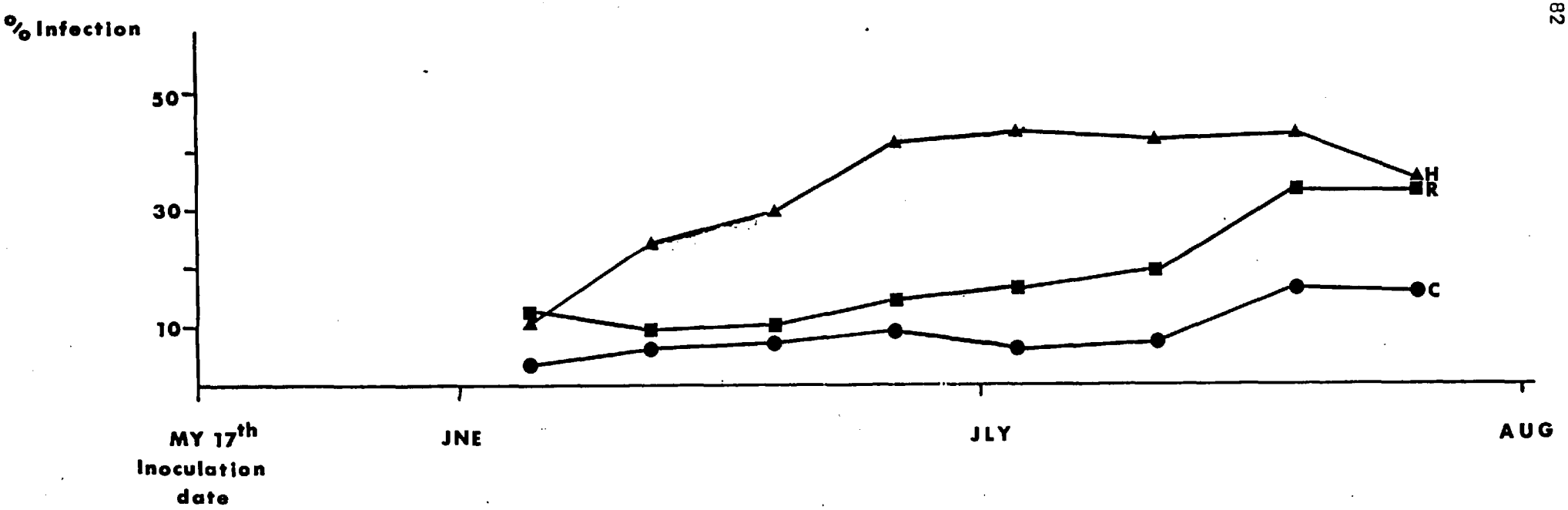


Figure 3:- Mean weekly % infection; whole plants; winter wheat, 1974

TREATMENT 2

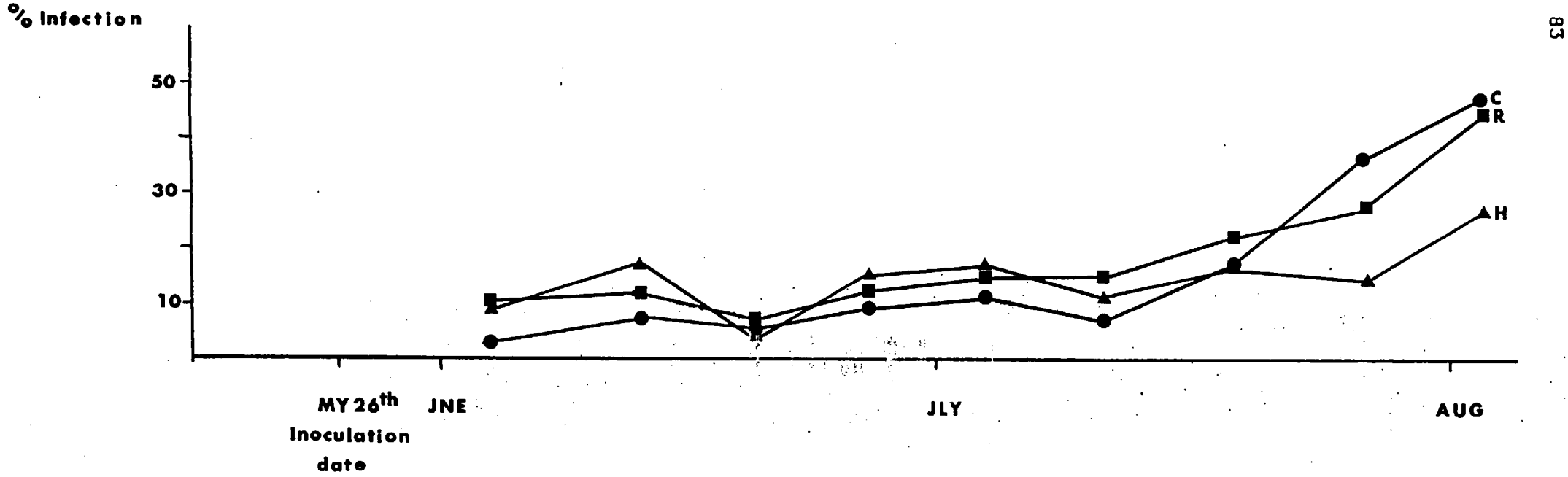


Figure 4:- Mean weekly % infection; whole plants; winter wheat, 1974

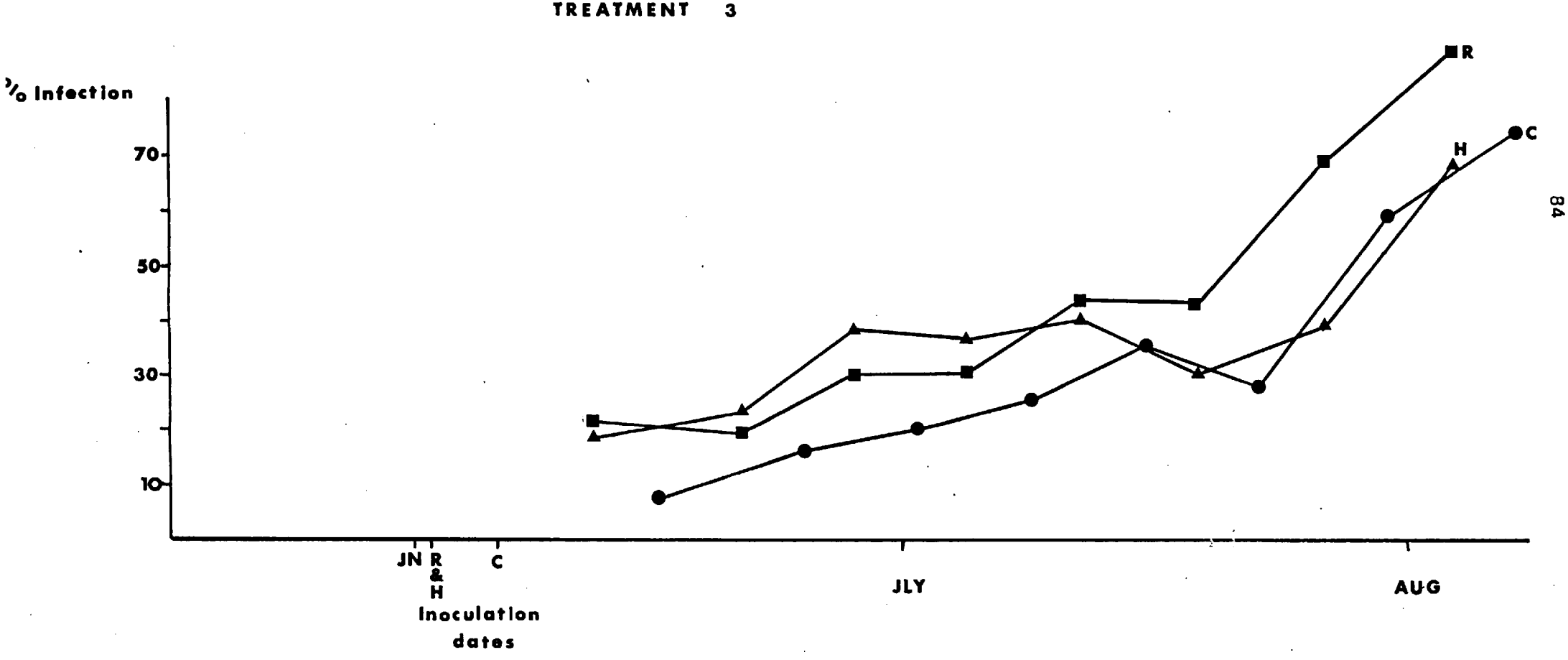


Figure 5:- Mean weekly % infection; whole plants; winter wheat, 1974

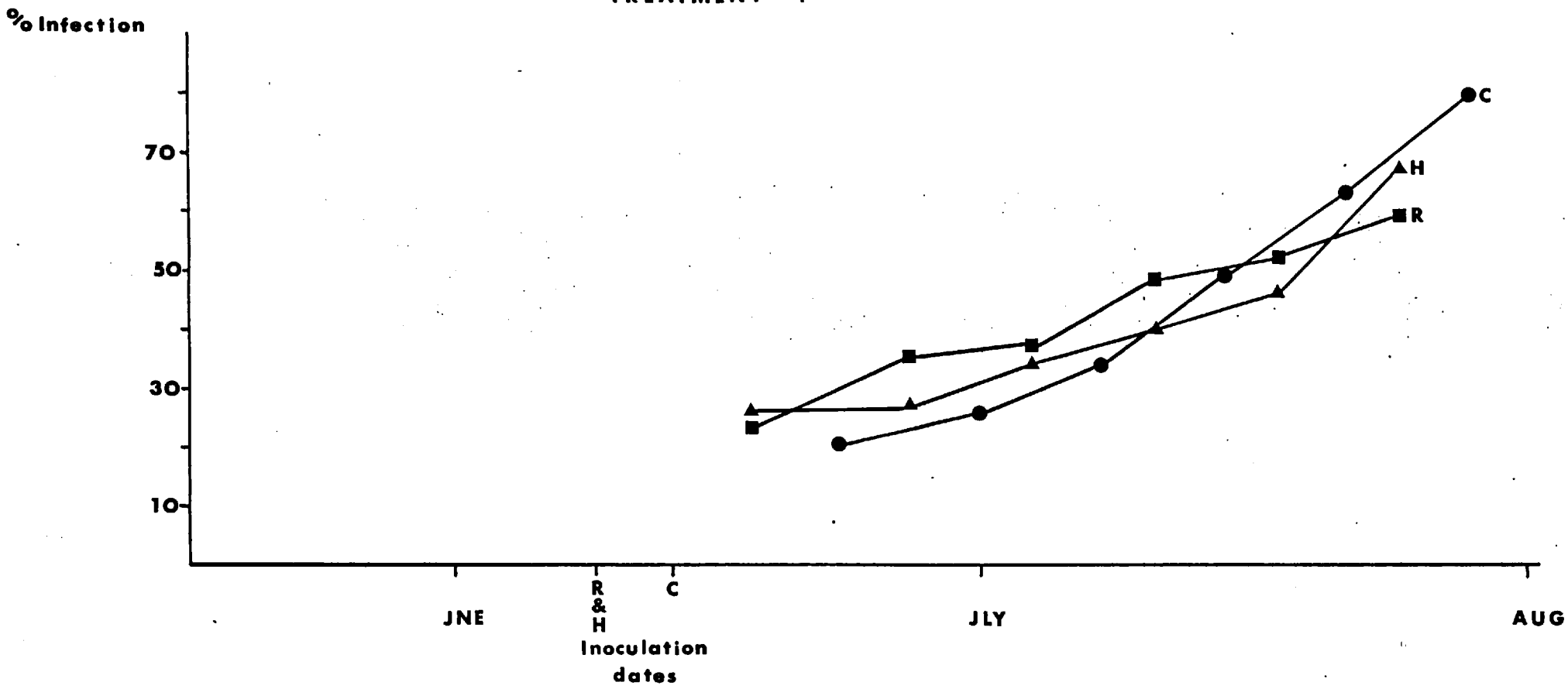


Figure 6:- Mean weekly % infection; whole plants; winter wheat, 1974

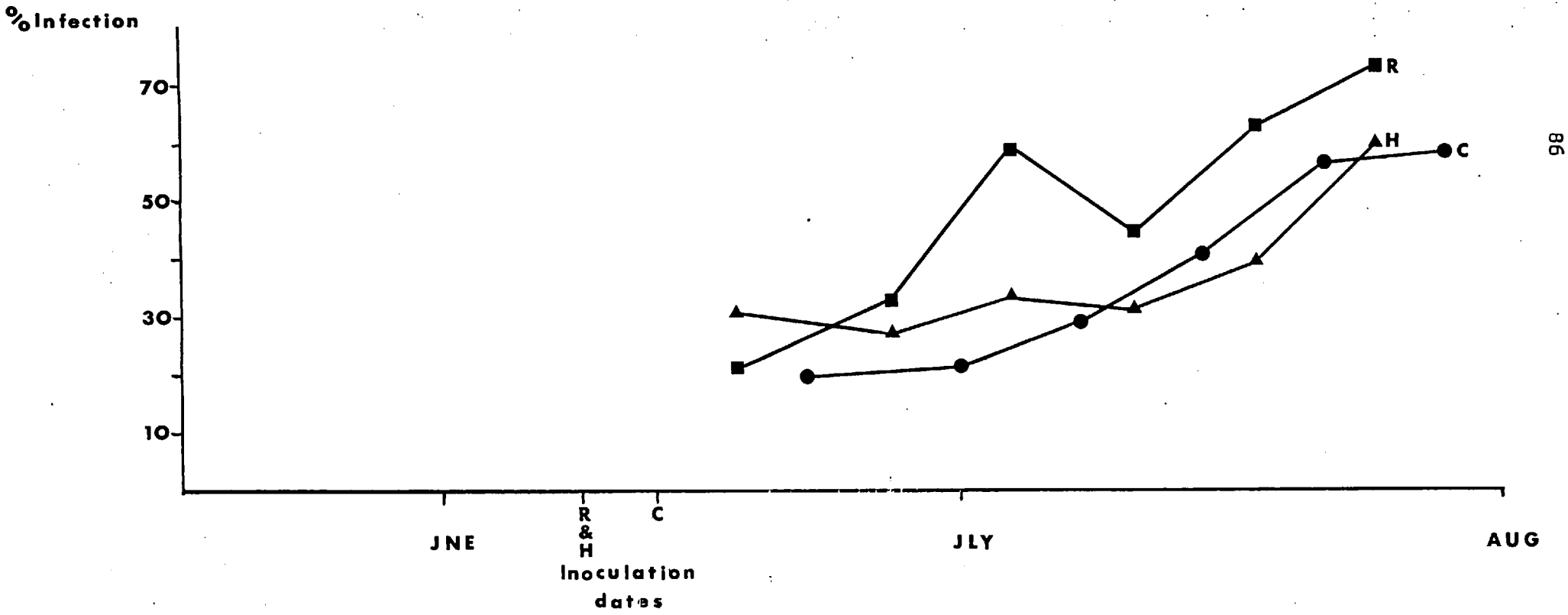


Figure 7:- Mean weekly % infection; whole plants; winter wheat, 1974

UNINOCULATED CONTROL

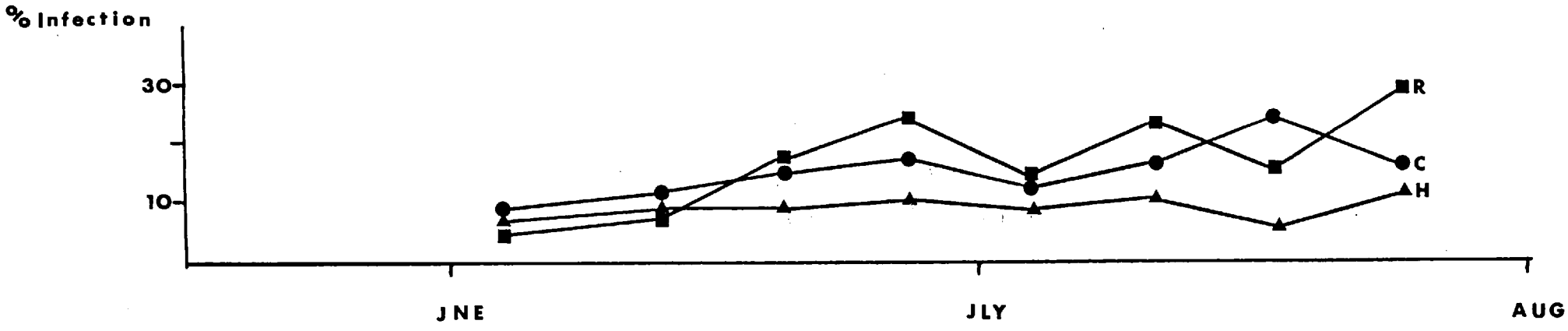


Table 9. Field Experiment 1A

Winter Wheat 1974

The Effects and Interactions of Inoculation Date, Variety and Leaves on Development of S.nodorum infection in Winter Wheat

Analysis of variance - F values (whole plants)

Days after inoculation	Variable		
	Treatment (nos.)	Cultivar	Leaf (nos.)
10	247.85** (2-5)	1.62^{ns}	86.23** (1-4,ear)
19	13.55** (1-6)	2.47^{ns}	97.34** (")
26	20.28** (")	2.07^{ns}	89.28** (")
33	16.63** (")	0.46^{ns}	88.79** (")
40	19.29** (")	0.56^{ns}	143.74** (")
47	34.56** (1-3,6)	0.96^{ns}	153.99** (1-3,ear)
55	23.96** (")	4.78**	74.43** (")
63	2.73^{ns} (1,2,6)	1.80^{ns}	19.92** (")
70	47.96** (")	4.28*	109.40** (1,2,ear)

	Treatment x Cultivar	Treatment x Leaf	Cultivar x Leaf
10	0.40^{ns}	3.60*	1.58^{ns}
19	0.63^{ns}	2.82^{ns}	3.60*
26	1.26^{ns}	2.39^{ns}	0.82^{ns}
33	1.26^{ns}	2.79^{ns}	1.47^{ns}
40	2.68^{ns}	3.21^{ns}	2.09^{ns}
47	2.27^{ns}	7.16**	2.86^{ns}
55	6.43**	7.25**	1.58^{ns}
63	4.69*	11.60**	0.66^{ns}
70	2.11^{ns}	15.49**	2.95^{ns}

* = significant at 5% level ns = not significant

** = " 1% "

Infection in the early inoculation treatments 1 and 2 (Figs 2-3) and the control (Fig. 7) follows a similar pattern which appears relatively constant and analagous for the three cultivars (except Maris Huntsman, treatment 1) until early July and then rises gradually afterwards. Levels of infection do not exceed 40% and the apparent infection rates (Table 14) are relatively low - up to 0.06 units day $^{-1}$.

On the other hand the later inoculation treatments 3-5 (Figs. 4-6) show a steady increase in infection with a sharper rise in mid-July reaching values of 80% infected tissue. The apparent infection rates are generally greater than for the early inoculations - up to 0.09 units. day $^{-1}$. For Maris Ranger the levels of infection are generally higher in treatment 5 (Fig. 6) where only heads were inoculated, in comparison to treatment 4 (Fig. 5) where the whole plant was inoculated. The converse is true for the other varieties.

There is a significant difference between treatments for all times after inoculation (Table 9), except 63 days. Not all treatments are included at each time (as is indicated in Table 9) and this probably accounts for the lack of significance at 63 days. At this time only treatments 1, 2 and the control are being compared. The significant difference at 70 days after inoculation may be due to the higher value for treatment 2, compared with treatment 1 and the control, which was not apparent at 63 days.

As was stated above, the varieties had similar levels of infection up to about 47 days after inoculation, and only at the later stages of growth, after heading, were there varietal differences in infection. This is supported by the presence of a significant effect of variety on infection at 55 and 70 days after inoculation (Table 9), when only treatments 1-3 and control, and treatments 1-2 and control respectively are being compared. This is rather surprising considering the relatively

high values throughout for Maris Huntsman treatment 1 and Maris Ranger, treatment 5, and is possibly a reflection of the insensitivity of the statistical test.

On the whole the varieties reacted in a similar manner to the varying dates of inoculation. This is substantiated by the absence of a cultivar/treatment interaction for up to 47 days after inoculation (Table 9). The rather high level of infection on Huntsman for treatment 1, in comparison with treatments 2, 3 and the control may be the cause of the significant interaction apparent at 55 and 63 days after inoculation. (at which times only these treatments were being recorded), Although, as can be seen in Fig. 2, this Huntsman effect is more pronounced earlier on, it does not become significant until 55 days.

The Prevailing Meteorological Conditions 1974

The establishment of a successful infection and its subsequent speed of development is affected by the prevailing meteorological conditions. The polythene inoculation bag provided the necessary conditions for the initial establishment of infection independent of the weather. The leaf surface wetness conditions following the inoculations are given in Fig.8.

For treatments 1 and 2 the inoculation bags were removed in dryweather conditions, and similarly for Maris Huntsman and Maris Ranger, treatments 4 and 5. In contrast for treatment 3 and forCappelle Desprez, treatments 4 and 5, the inoculation bags were removed in wet conditions. Thus for the early inoculations conditions following treatment application were unfavourable to S. nodorum, while for the later treatments leaf surface wetness conditions following inoculation were more advantageous to infection and spread of the pathogen.

There does not appear to be a well defined correlation of infection with the temperature and humidity data presented in Fig. 9. However, throughout July the increase in % infection is steepening for

Figure 8:- DURATION OF LEAF SURFACE WETNESS 1974

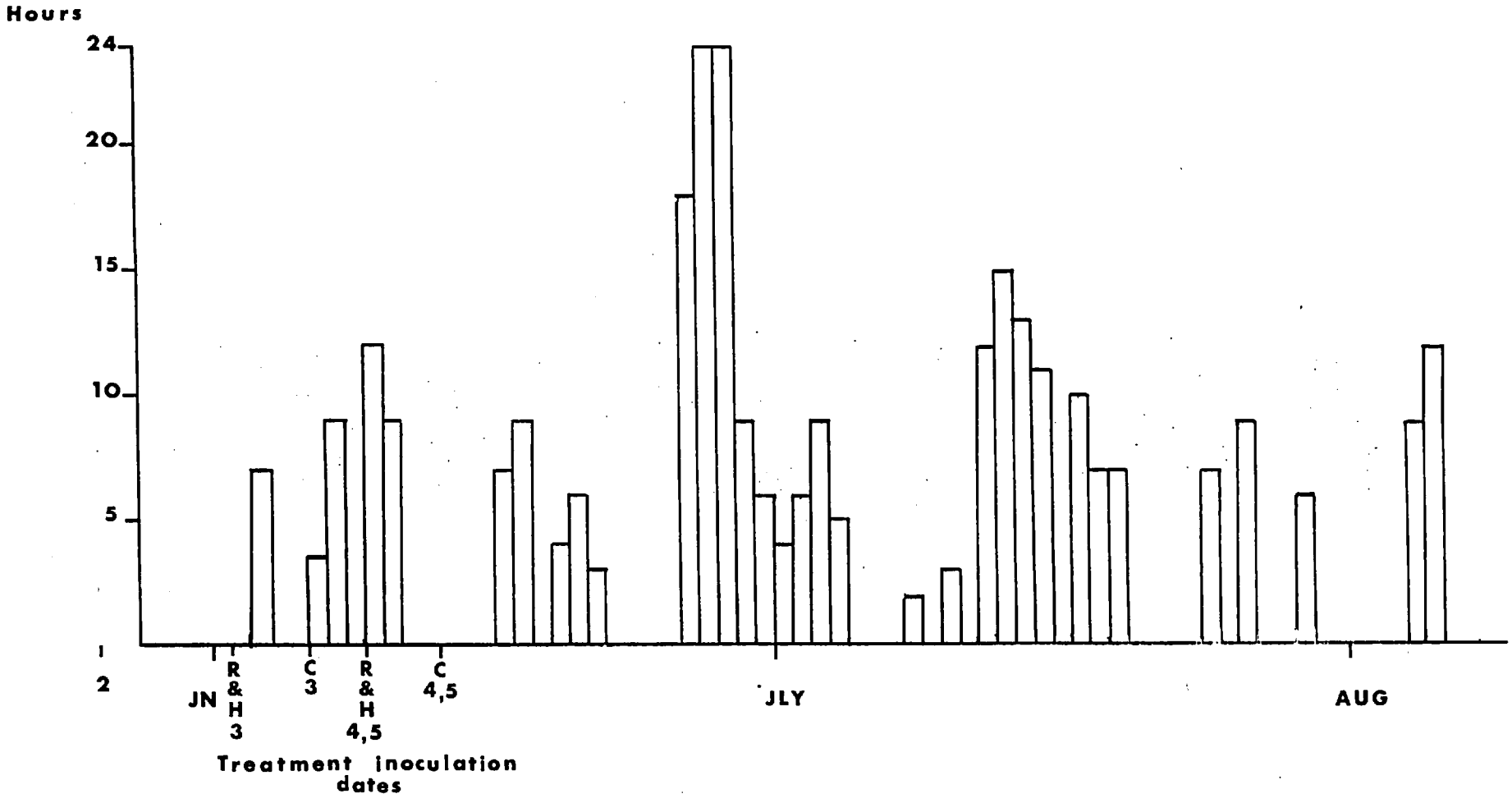
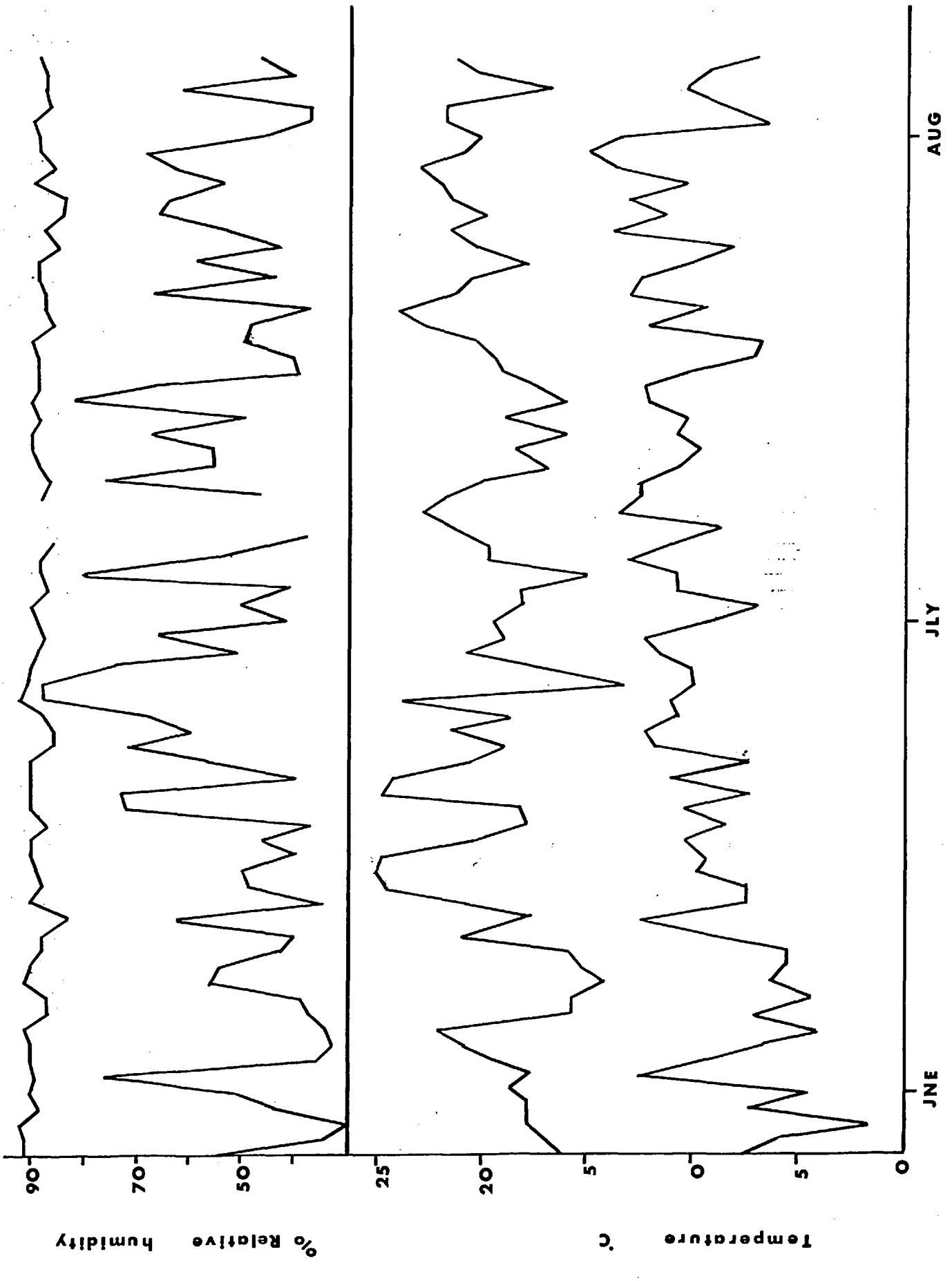


Figure 9:- Temperature & Relative Humidity ; Maxima & Minima 1974



most cultivar/treatment combinations, and this coincides with the long periods of leaf surface wetness, high minimum humidities and lower temperatures of this month.

The Microclimate of the Crop Canopy

The mean hourly day temperatures for a week early and later in the season are presented in Figs. 10 and 11 and 12 and 13 respectively.

There is apparently less variation in the temperature within the canopy of the susceptible cultivar, Maris Ranger, and temperatures are slightly lower in comparison with the resistant Maris Huntsman, while Cappelle Desprez is intermediate.

Early in the season (Figs. 10 and 11) the effect is most apparent in the mornings around 1100 hrs. when there is about a $4^{o}C$ difference between the ground and flag leaf level in the Maris Huntsman canopy, but only about $1^{o}C$ for Cappelle Desprez and less than $1^{o}C$ for Maris Ranger.

Later in the season, when the canopy is senescing the difference is diminished (Figs. 12 and 13) there being less than $1^{o}C$ variation within all the canopies. However the general relationship of variation is the same as for earlier on.

The more uniform microclimate of Maris Ranger may be a reflection of the canopy structure since the cultivar has rather larger leaves (average leaf area = 32 cm^2) which form a more dense canopy in comparison to Huntsman (average leaf area = 26 cm^2). Of greater interest than the temperature readings per se would be the corresponding humidity levels within the canopy. However, due to lack of equipment, this parameter could not be recorded.

Figure 10:- Temperatures at three levels within the wheat canopy; means for May 29th – June 5th 1974

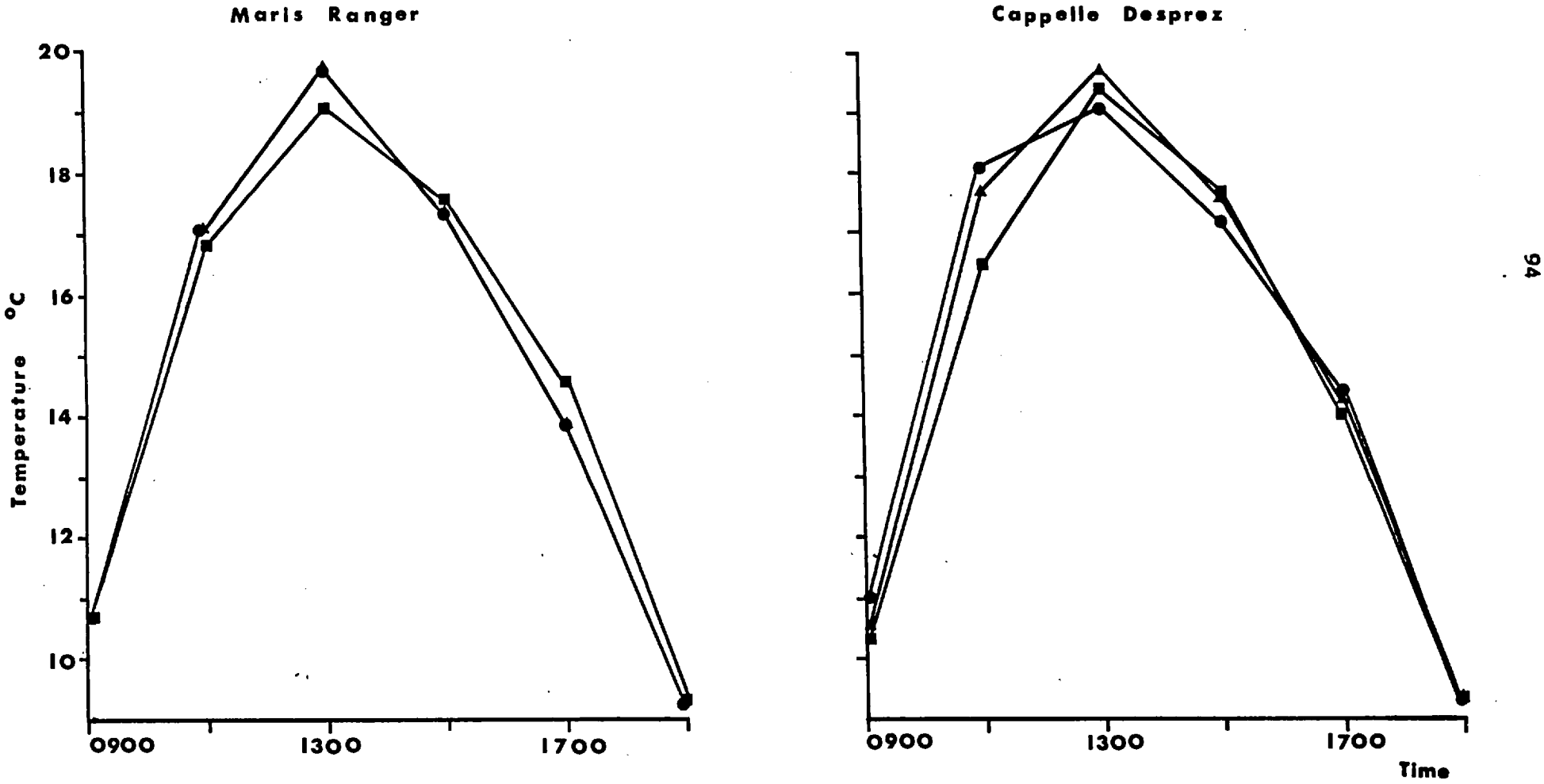


Figure 11:- Temperatures at three levels within the canopy; means for May 29th_ June 5th 1974

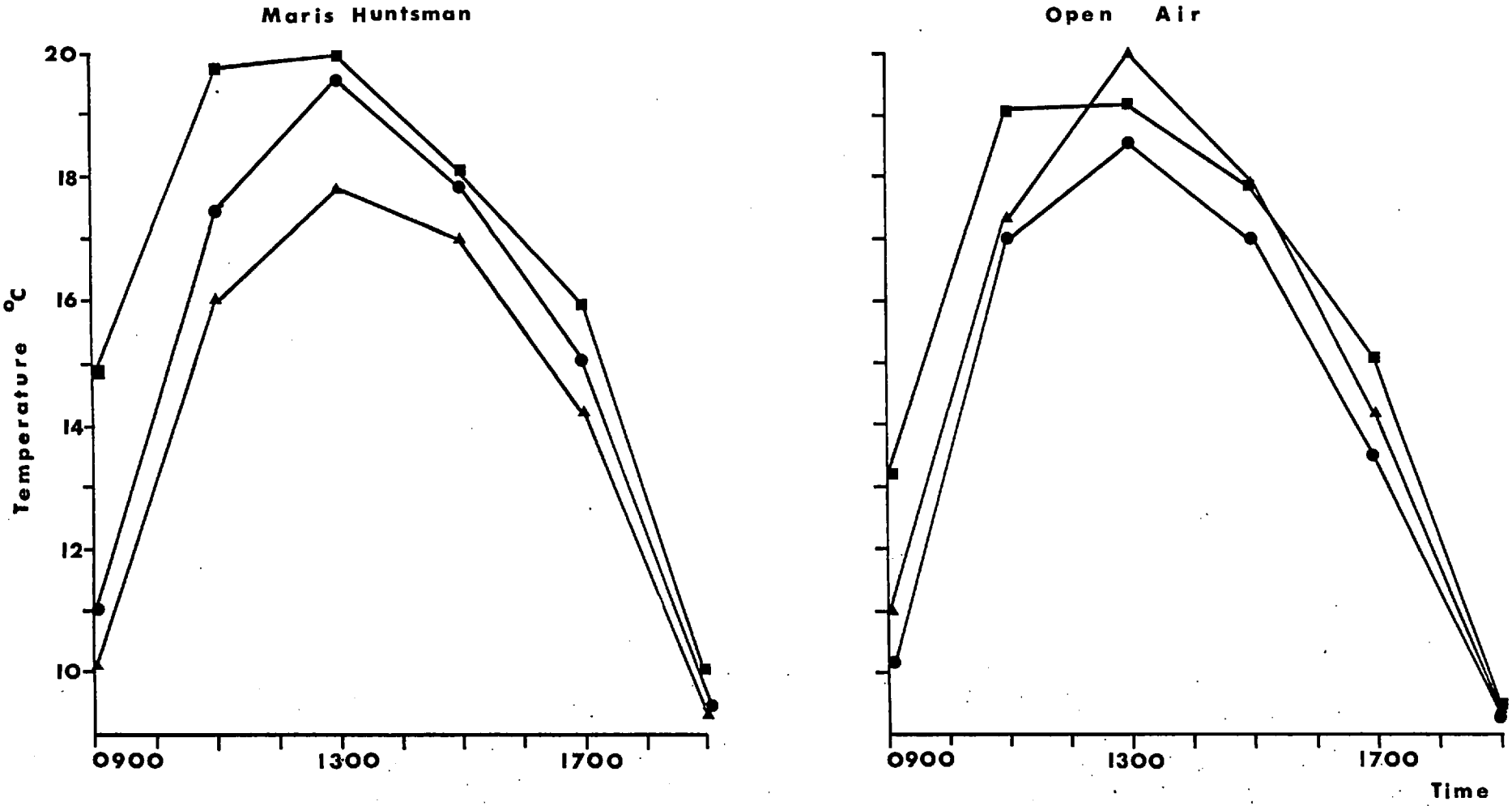


Figure 12:- Temperatures at three levels within the wheat canopy; means for July 25th - Aug. 2nd 1974

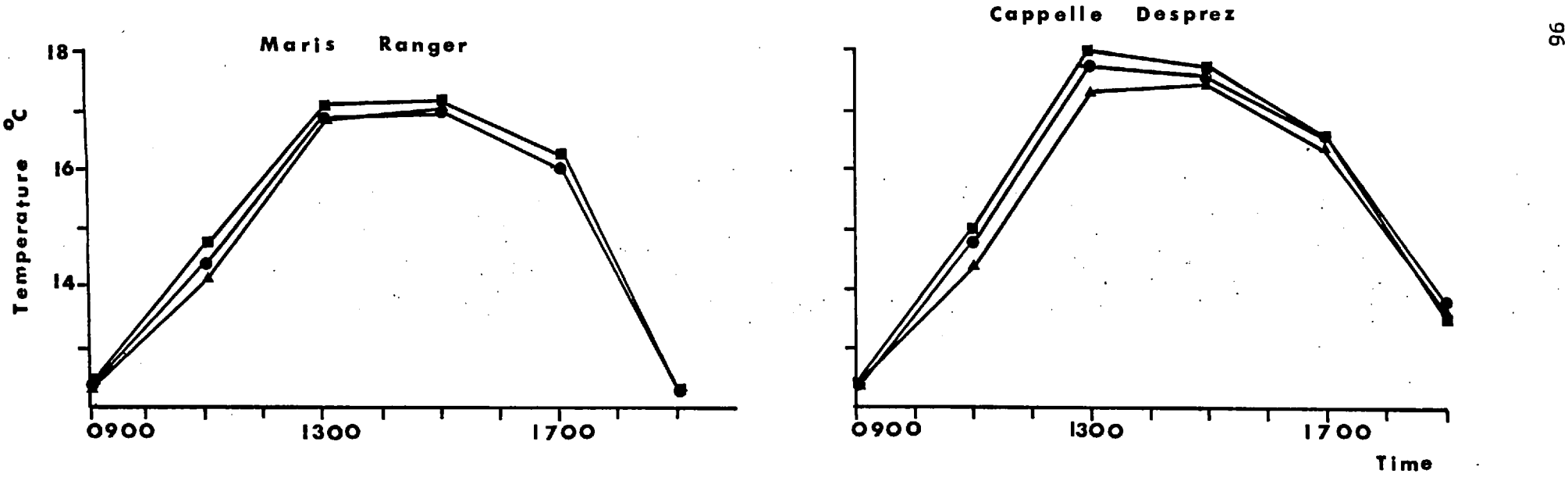
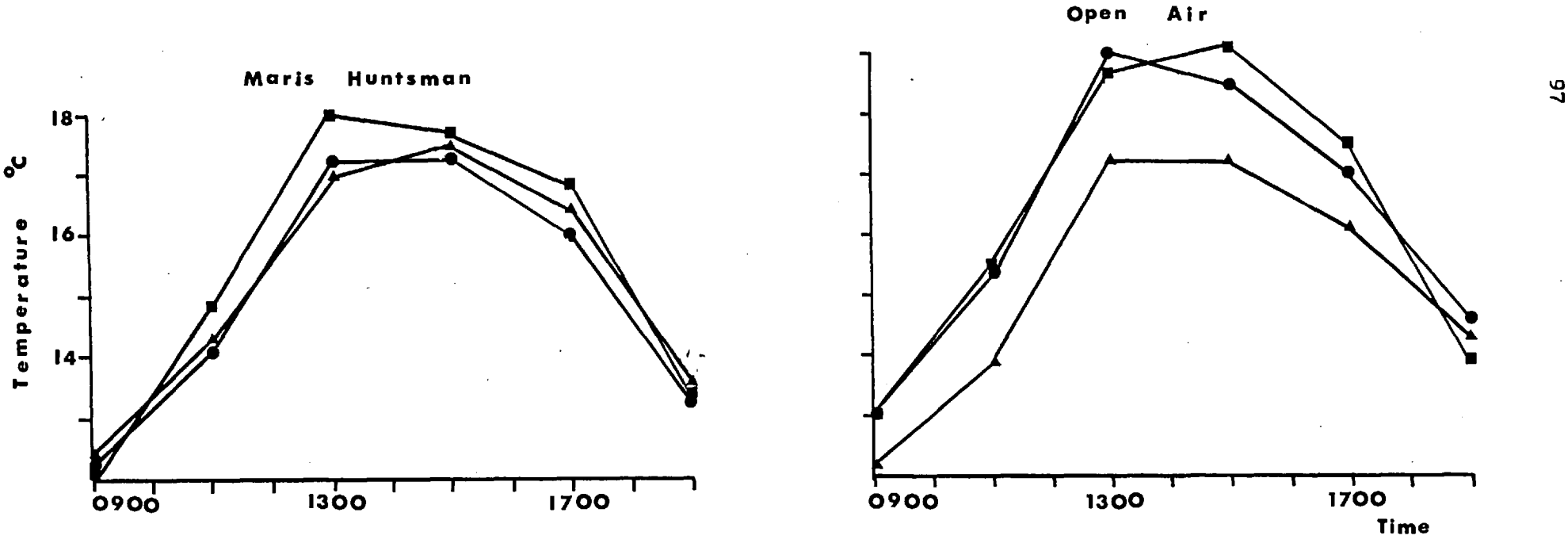


Figure 13:- Temperatures at three levels within the wheat canopy; means for July 25th - Aug 2nd 1974



Differencee between parts of plant (averaged over cultivars and treatments.)

The progress of infection on different leaves and on the ear following inoculation is given in Fig.14. All the leavee and the ear show a eimilar pattern (which parallels that of whole plante Fige. 2-7) which ie relatively low for the firet 33-40 days after inoculation (except leaf 4) and then a riee in % infection eubsequently. However the absolute % infection valuee are very different, there being a gradient of increasing infection down the plant; the ear showe the leaet and leaf 4 the moet infection. Thie is verified by the significant differenoe between leavee for all times after inoculation shown in Table 9.

In order to obtain further information on the effects of inoculation date and variety on development of infection on individual leaves, which wae not given in the whole plant analysis (Table 9), a further analysie of variance was carried out for each leaf. The F values and significance levels are presented in Table 10. The time of inoculation can be eeen to have a eignificant effect on % infection for ell parts of the plant for all times after inoculation.

Figure 14:- Mean weekly % infection of parts of the culm after inoculation.

each point is a mean of 180–360 plants, covering 3-6 treatments and 3 cultivars

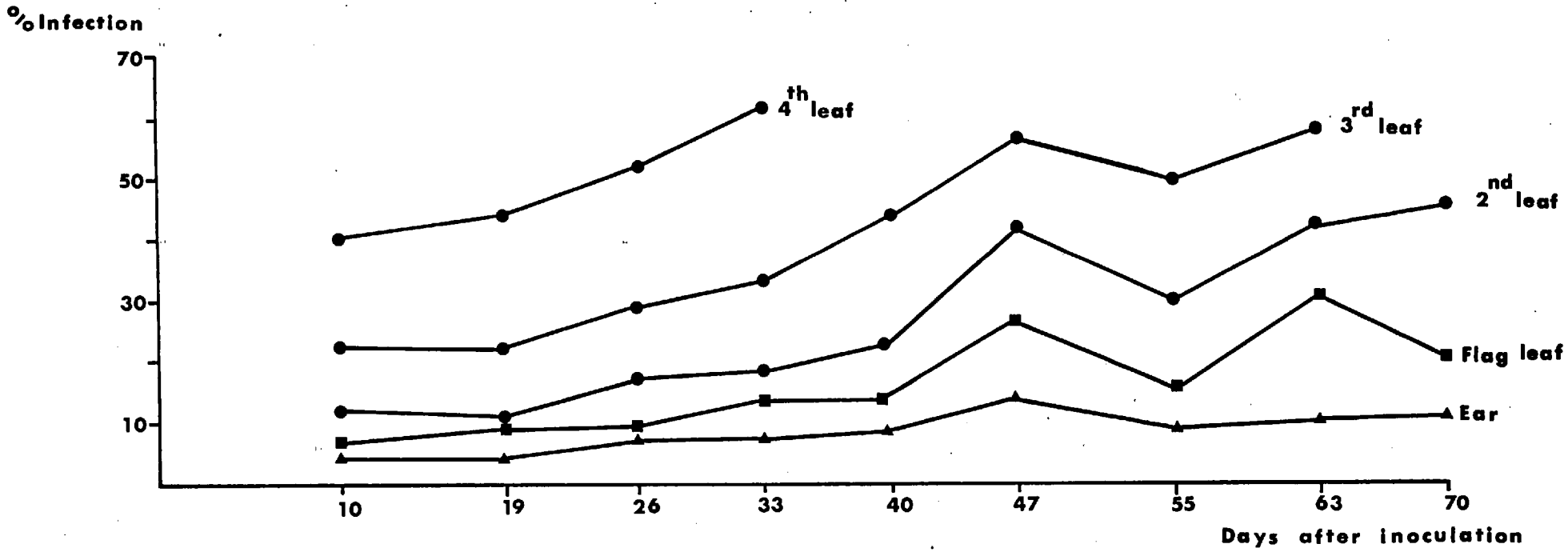


Table 10. Field Experiment 1A

Winter Wheat 1974

The Effects of Inoculation Date and Variety on Development of S. nodorum Infection on the Ear and Top 4 Leaves of Winter Wheat

Analysis of Variance F values (with significance levels)

(a) Inoculation Date

Days after inoculation	Leaf				
	4	3	2	Flag	EAR
10	17.34**	13.94**	17.03**	63.22**	15.54**
19	5.93**	5.81**	20.80**	9.38**	66.79**
26	14.94**	9.50**	5.95**	3.82*	5.49*
33	14.63**	10.65**	4.52*	8.69**	8.51**
40		23.27**	4.00*	3.48NS	12.10**
47		42.93**	57.17**	48.32**	3.66NS
55		19.30**	34.10**	4.86*	6.70*
63		11.95**	45.44**	45.67**	7.44**
70			91.16**	4.03NS	2.11NS

(b) Variety

Days after inoculation	Leaf				
	4	3	2	Flag	EAR
10	1.56NS	3.32*	2.33NS	0.29NS	2.47NS
19	3.38*	4.60**	2.52NS	6.29**	2.74NS
26	1.75NS	0.76NS	2.44NS	1.14NS	5.30**
33	0.79NS	0.83NS	0.23NS	0.44NS	8.22**
40		0.73NS	0.30NS	4.52**	20.55**
47		2.74NS	3.01NS	0.93NS	3.50**
55		0.40NS	0.31NS	14.13**	22.63**
63		0.86NS	0.51NS	4.31*	17.09**
70			0.16NS	15.45**	8.02**

Continued/

Table 10 (Cont'd)

(c) Inoculation Date and Variety Interaction

Days after inoculation	Leaf				
	4	3	2	Flag	EAR
10	1.04^{NS}	0.70^{NS}	1.54^{NS}	0.56^{NS}	3.69^{*}
19	1.61^{NS}	0.55^{NS}	0.63^{NS}	2.45^{NS}	1.60^{NS}
26	0.84^{NS}	1.45^{NS}	0.96^{NS}	1.64^{NS}	2.32^{NS}
33	1.47^{NS}	2.37^{NS}	0.82^{NS}	0.18^{NS}	2.55^{NS}
40		3.17^{NS}	2.23^{NS}	1.67^{NS}	4.99^{**}
47		2.55^{NS}	4.43^{*}	2.60^{NS}	1.13^{NS}
55		1.54^{NS}	6.20^{**}	9.05^{**}	6.02^{**}
63		0.68^{NS}	4.61^{NS}	2.37^{NS}	4.10^{*}
70			2.08^{NS}	2.01^{NS}	1.83^{NS}

* significant at 5% level

** significant at 1% level

The differential reaction of cultivars to infection at 55 and 63 days after inoculation, apparent in Teble 9, can be seen in Table 10, to be due largely to the reactions of the ear and flag leaf. In fact the ears of the varieties have significantly different levels of infection from 26 days after inoculation onwards, while the flag leaves are significantly different at 19, 40 and 55 days onwards. The nature of thie difference can be ascertained from Table 11 in which the progress of infection for parts of the plant for each cultivar is given.

For the flag leaf (Table 11) the ranking order is Maris Ranger > Cappelle Desprez > Maris Huntsman in all cases of significance, but the first, and in non-significant cases as well. For the ear Maris Ranger infection levels exceed those of Cappelle Desprez and M. Huntsman throughout, except the first, and in most cases Cappelle Deeprez infection exceeds that of Maris Huntsman. These rankings are not apparent on leaves 2, 3, and 4. This contrast would have been expected to show up as a significant cultivar/leaf interaction in Table 9. Its presence may be obscured by the lack of interaction within leaves 2-4 and the flag leaf and ear. Thus bulking the data for leaves 2-4 and contrasting this with the flag leaf and ear may give a significant interaction.

One might expect the flag leaves in treatment 4 (inoculation of the whole plant) to be noticeably more affected than those in treatment 5 (inoculation of ears only). The effect however seems small and only up to 19 days after inoculation. (see Appendix 1A, Tables 4 and 5, 10 and 11, 16 and 17). This implies that there is a large movement of inoculum downwards from the ear to the flag in treatment 5.

Table 11.

Field Experiment 1A
Winter Wheat 1974.

The Progress of % Infection for the different Parts of the Plant of each Variety

(averaged over treatments)

Days after inoculation	Leaf	4			3			2			Flag			EAR		
	Cultivar	R	C	H	R	C	H	R	C	H	R	C	H	R	C	H
10		34.5	37.6	51.3	30.5	12.0	25.8	16.5	5.7	15.1	7.1	5.8	9.0	5.1	2.0	6.4
19		36.4	43.5	53.0	31.3	13.8	22.2	16.1	5.4	12.1	6.0	5.4	2.5	7.8	1.6	5.0
26		48.1	49.1	61.3	34.5	24.5	30.1	22.3	9.6	19.6	14.0	6.1	9.4	8.6	1.8	12.3
33		59.1	60.3	67.8	31.6	31.6	38.1	20.5	17.1	19.2	11.1	10.3	7.9	15.3	3.5	4.2
40					43.8	43.2	47.2	27.2	29.0	16.8	19.3	16.8	5.7	19.3	4.9	4.1
47					57.0	51.8	63.0	38.7	39.8	47.9	31.8	23.8	28.2	23.2	13.1	7.1
55					51.6	46.7	54.2	35.5	32.2	23.1	28.9	17.4	3.7	19.5	3.9	5.7
63					64.2	64.3	47.3	42.5	46.7	41.5	39.6	28.4	28.4	25.3	8.4	5.4
70								51.1	46.8	42.9	34.6	23.8	6.8	21.6	8.3	5.1

Key: R = Maris Ranger C = Cappelle Desprez H = Maris Huntsman

Apparent Infection Rates for Individual Leaves

The apparent infection rates, 'r' for parts of plant/variety/treatment combinations are given in Table 12 and the variety comparisons of rates in Table 13.

The infection rates for ears are based on late observations only, and mostly relate to the increase from 0-20% infection. They will therefore be considered separately. It is prominent in Table 12 that Maris Huntsman has very low, non-significant 't' values in comparison with Maris Ranger and Cappelle Desprez. That these differences are significant is shown in Table 13. Also of note is the fact that the ears for early inoculation treatments 1 and 2 and the control have higher 'r' values than the later inoculation treatments 3-5. This may arise because the plants in the former case were inoculated sometime before the ear emerged. Accordingly the ears score zero until they become secondarily infected under the favourable end of season conditions. In the latter case, however ears were inoculated just before (treatment 3) or after (treatments 4-5) emergence, and the first recorded values are relatively high which will tend to bring the infection curve (and 'r') down. Hence this effect of ears on infection rate is an artefact of the treatments.

The ranking order of 'r' for leaves across all treatments is given below (1 represents the highest rate).

Leaf	Cultivar		
	Ranger	Cappelle	Huntsman
3	3	3	3
2	2	1	2
Flag	1	2	1

The lowest infection rate for all cultivare ie on the 3rd leaf, whilet the highest'r' occurs on the flag leavee of Marie Ranger and Maris Hunteman and on the eecond leaf of Cappelle Desprez.

The overall ranking order of cultivare infection ratee ie Cappelle Deeprez > Marie Ranger > Maris Huntsman. This difference between cultivare is only significant (in all treatmente) for the ear. (Table 13). The other notable significant differencee are between Maris Ranger andCappelle Desprez for treatment 4 in which the former has lower values of 'r'.

The ranking order for leaves accross all cultivars is given below.

Leaf	Treatment					
	1	2	3	4	5	Control
3	2	3	3	2	3	3
2	3	1	2	3	2	2
Flag	1	2	1	1	1	1

On the whole flag leaves have the highest values of 'r' irrespective of inoculation date, and the 3rd leaves the lowest values.

Table 12.

Field Experiment 1A

Winter Wheat 1974

Apparent Infection Ratee, 'r' (unite. day $^{-1}$)

Treatment	Leaf	Cultivar		
		Marie Ranger	Cappelle Desprez	Marie Huntsman
1	3rd	0.0619	0.0600	0.0582
	2nd	0.0586	0.0623	0.0698
	Flag	0.1068	0.0831	0.0901
	Ear	0.0869	0.0927	0.0125 N.S.
2	3rd	0.0325	0.0638	0.0321
	2nd	0.0688	0.0795	0.0822
	Flag	0.0956	0.0777	0.0600
	Ear	0.0988	0.1316	- 0.0105 N.S.
3	3rd	0.0493 N.S.	0.0686	0.0528
	2nd	0.0501	0.0864	0.0367
	Flag	0.1149	0.0654	0.0619
	Ear	0.0699	0.0648	- 0.0108 N.S.
4	3rd	0.0414	0.1287	0.0328
	2nd	0.0339	0.1262	0.0534 N.S.
	Flag	0.0703	0.0904	0.0658
	Ear	0.0485	0.0782	0.0304 N.S.
5	3rd	0.0653 N.S.	0.0729	0.0369 N.S.
	2nd	0.0790	0.1166	0.0420 N.S.
	Flag	0.0972	0.1009	0.0248 N.S.
	Ear	0.0505	0.0523	- 0.0305
Control	3rd	0.0536	0.0408	0.0067 N.S.
	2nd	0.0578	0.0568	0.0462
	Flag	0.0956	0.0524	0.0808
	Ear	0.0727	0.0619	0.0183 N.S.

Table 13. Field Experiment 1A

Winter Wheat 1974

Treatment	Leaf	Cultivar Comparison		
		Maris Ranger and Cappelle Desprez	Maris Ranger and Maris Huntsman	Maris Huntsman and Cappelle Desprez
1	3rd	0.111	0.205	0.093
	2nd	1.472	- 0.626	- 1.618
	Flag	1.361	0.735	- 0.297
	Ear	- 0.341	3.381*	4.611*
2	3rd	- 1.720	0.042	1.847
	2nd	- 0.675	- 1.257	- 0.176
	Flag	0.847	2.044*	0.969
	Ear	- 1.498	4.073*	13.865*
3	3rd	- 0.837	- 0.170	1.260
	2nd	- 1.485	0.699	2.555*
	Flag	1.309	1.275	0.123
	Ear	0.371	4.520*	3.892*
4	3rd	- 6.167*	0.975	5.152*
	2nd	- 7.815	- 1.703	3.599*
	Flag	- 1.066	0.269	3.258*
	Ear	- 2.743*	1.434	2.850*
5	3rd	- 0.205	0.477	0.583
	2nd	- 1.115	0.769	1.484
	Flag	- 0.127	2.881*	3.040*
	Ear	- 0.133	4.458	3.563*
Control	3rd	1.590	3.966*	2.791*
	2nd	0.111	1.160	1.137
	Flag	1.835	0.474	- 0.846
	Ear	0.401	2.161*	2.328*

* Significant at 5% level.

Summary of infection data

The data for the whole season is summarised in Table 14. Cappelle Desprez and Maris Huntsman show lower levels of infection overall, but the former has generally higher infection rates. The rather high mean value for Maris Huntsman is largely due to the very high level of infection for this variety in treatment 1, and this tends to obscure its otherwise general resistance in comparison with the other two cultivars. Thus, although Maris Huntsman, showed a high initial level of infection in most cases, this did not increase greatly. This is reflected in the lower values of 'r' for this variety, particularly for the ear. Significant differences in overall infection rates only occur in treatments 4 and 5. (Table 14).

The low levels of infection for the early inoculations and control are possibly due to a petering out of the initial 'epedemic' following inoculation, while in the later inoculations the 'epidemic' picks up without a break.

Table 14

Field Experiment 1A

Winter Wheat 1974

Mean % S. nodorum infection, and infection rates (r) for the season

Treatment	Cultivar			Mean %
	Maris Ranger	Cappelle Desprez	Marie Huntsman	
1 %	17.8	8.3	32.6	19.6
'r'	0.0646	0.0541	0.0568	
2 %	18.0	15.8	13.7	15.8
'r'	0.0594	0.0663	0.0467	
3 %	42.9	31.8	34.7	36.4
'r'	0.0699	0.0634	0.0536	
4 %	41.5	44.1	38.2	41.3
'r'	0.0495	0.0912	0.0447	
5 %	47.5	35.8	33.2	38.8
'r'	0.0719	0.0780	0.0380	
Control %	16.5	15.5	9.2	13.7
'r'	0.0641	0.0455	0.0512	
Mean %	30.7	25.2	26.9	

Comparison of overall apparent infection rates: t - values

Treatment	M. Ranger & Cappelle D.	M. Ranger & M. Huntsman	M. Huntsman & Cappelle D.
1	0.9140	0.641	- 0.212
2	- 0.578	1.214	1.774
3	0.406	1.133	1.006
4	- 3.062**	0.393	2.971**
5	- 0.434	2.038*	2.272*
Control	1.740	1.061	- 0.485

* significant at 5% level

** significant at 1% level

Field Experiment 18

Within the 1973/4 winter wheat trial (1A) a more detailed study of infection development in naturally infected and artificially inoculated plants was made. The relative importance of increase in lesion number and spread of existing lesions in development of infection was of particular interest.

Ten plants of each cultivar were selected at random: five from treatment 3 (inoculation at G.S.10) and five from the uninoculated control.

The following measurements were made weekly:-

(a) on the flag and 2nd leaves.

(1) Length and breadth (at the midpoint) of the leaf until no further increase.

(2) % area leaf affected by S. nodorum

(3) Total number of lesions caused by S. nodorum

(4) Number of lesions longer than 3 mm.

(5) Internode length of flag and 2nd leaf

(6) " " 2nd and 3rd leaf

(b) On the ear

(1) % glumes affected by S. nodorum

(2) Total number of lesions due to S. nodorum

(3) Number of lesions greater than 3 mm.

(4) Peduncle length.

An approximation of leaf area was made from the product of the length and breadth measurements, and then the number of lesions per unit area estimated. The % of lesions exceeding 3 mm was also calculated, and mean internode lengths, for each week throughout the season.

The experiment was repeated and extended in 1974/75. Instead of isolated clumps as plots a group of plants within a normal stand was used (see Materials and Methods L). In addition to the 3 cultivars mentioned a further 3 were also used:- Marie Fundin (- a semi dwarf - susceptible), Capitole and Goya - two resistant early maturing cultivars. both bred in France but obtained in Spain. Data could not be collected for ear infection owing to extensive bird damage.

The results for both seasons follow broadly similar patterns although absolute values for infection and lesion numbers were considerably greater in 1973/74. Growth in this season was rather poor owing to adverse climatic conditions and soils, while in 1974/5 the crop was far more vigorous. It was felt that the data collected in 1974/5 was more representative than 1973/4 and so it will be presented while summaries of the data collected in 1974 for Maris Ranger, Cappelle Desprez, and Maris Huntsman, and in 1975 for Goya, Capitole and Maris Fundin are presented in the Appendix 1B Tables 25 and 26 respectively.

The increase in lesion number and % infection for naturally infected and artifically inoculated flag and 2nd leaves of Maris Ranger, Maris Huntsman and Cappelle Desprez in 1975 is given in Figs. 15-18 The % lesions exceeding 3 mm is given in Table 15.

The data from which these Figures are taken is presented in Appendix 1B, Tables 19-24.

Field Experiment 1B

Winter Wheat 1975

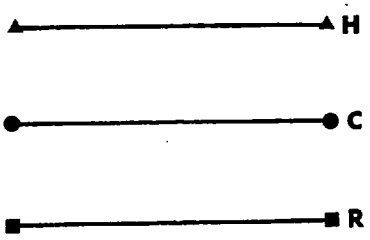
Detailed Study of Infection Development

Key to Figs 15-18

Scale. Vertical axis 2 cm represents 10% infection by *S. nodorum*

1 cm " 0.1 lesions/cm^2

Horizontal axis 3 mm " 1 day



H Maris Huntsman

C Cappelle Desprez

R Maris Ranger

Each point on the graph is an average of five leaves on five plants.

Figure 15 :- Increase in % infection and lesion number for naturally infected flag leaves of winter wheat, 1975

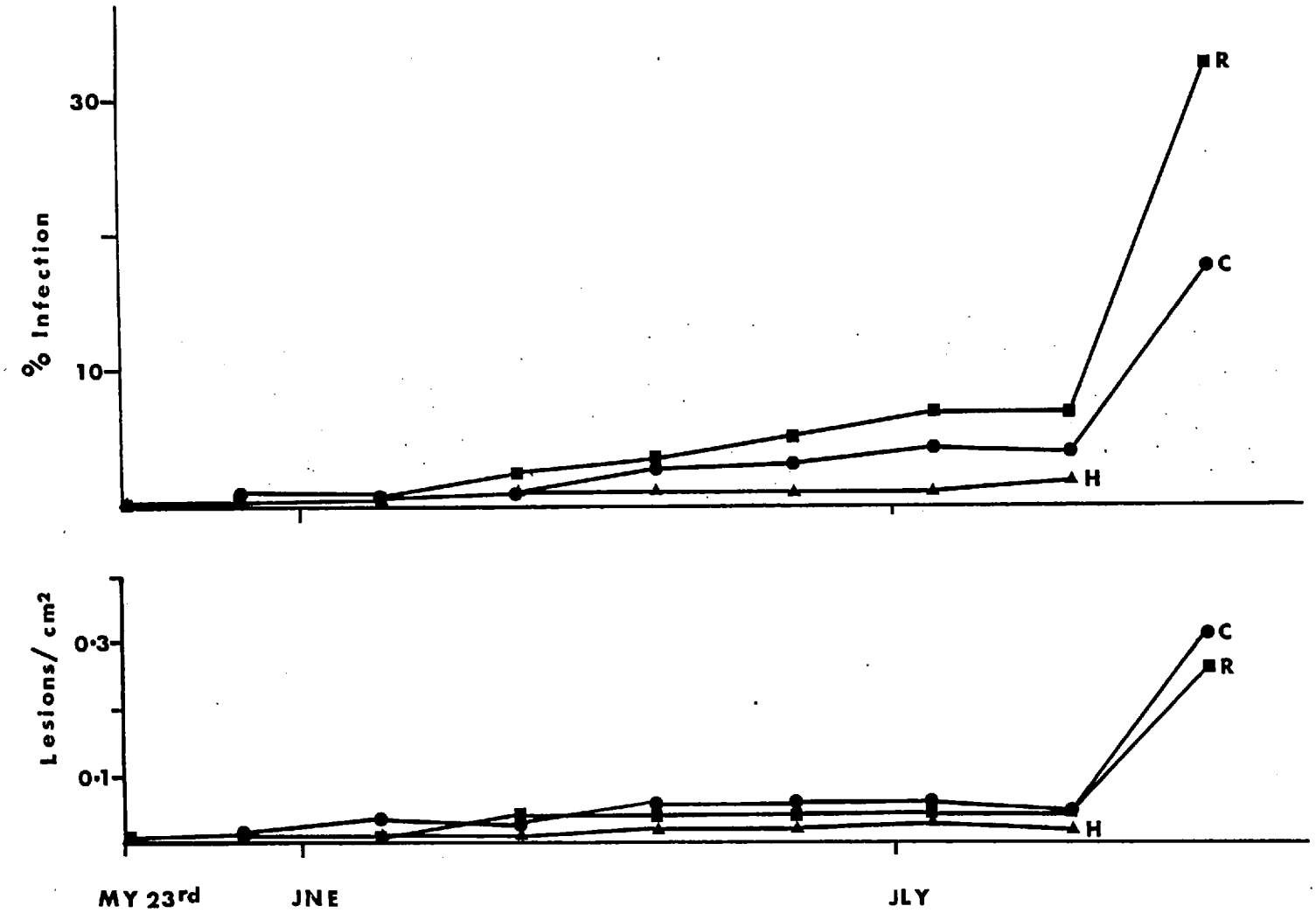


Figure 16 :- Increase in % infection and lesion number for artificially inoculated flag leaves of winter wheat,1975

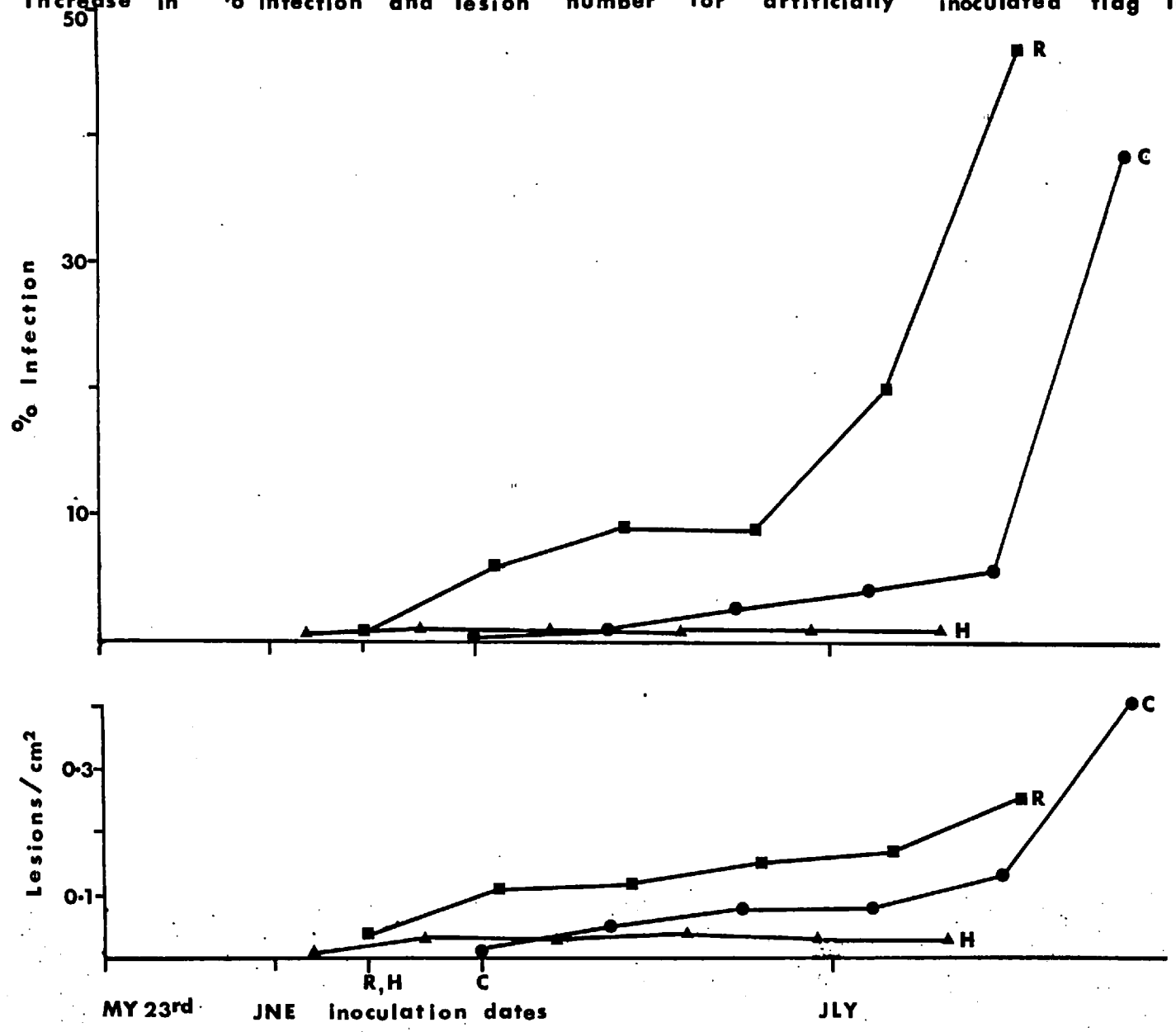


Figure 17 :- Increase in % infection and lesion number for naturally infected 2nd leaves of winter wheat, 1975

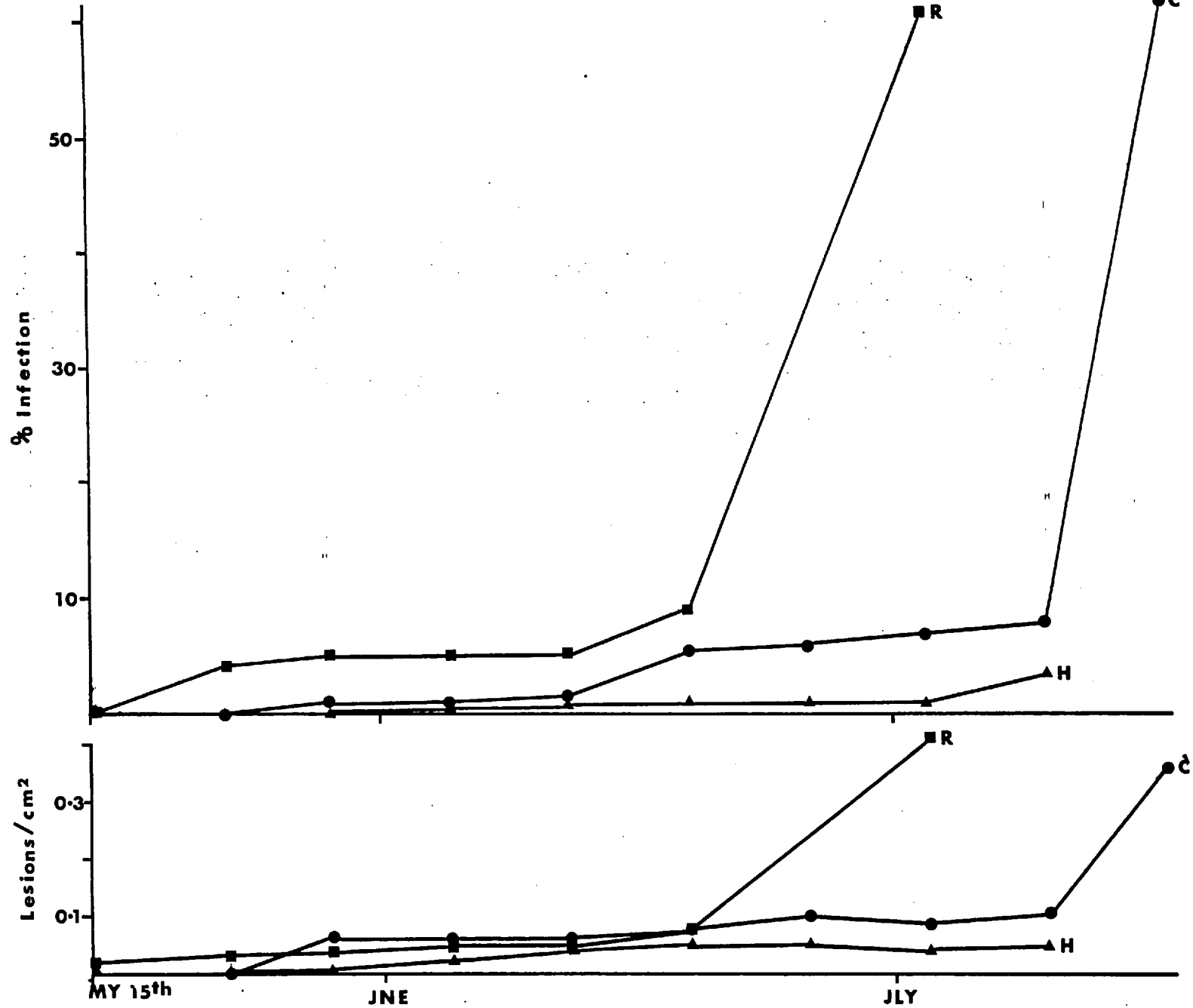
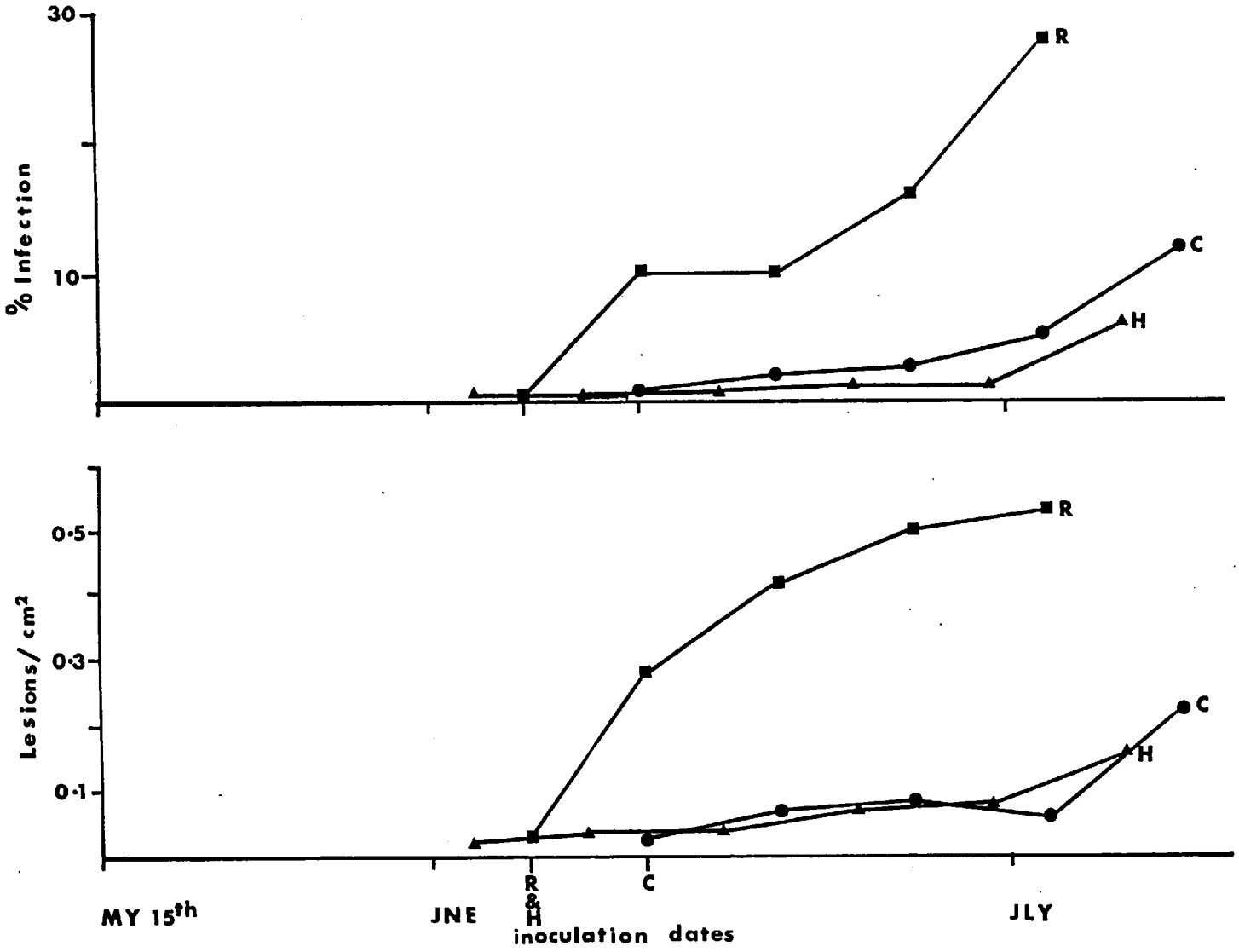


Figure 18:- Increase in % infection and lesion number for artificially inoculated 2nd leaves of winter wheat, 1975



Increase of lesion number

Maris Huntsman showed very little increase in lesion number on either flag or second leaves which were naturally infected. There was a slight rise on artificially inoculated second leaves at the end of the season for this variety.

Cappelle Desprez followed a similar pattern of slow increase initially but always showed a steep rise in lesion number at the end of the season.

The susceptible variety, Maris Ranger, varied in its response to infection; on the flag leaves there were generally more lesions throughout the season and then a slightly less steep rise at the end compared with Cappelle Desprez. On second leaves, however the rise in lesion number on naturally infected plants started about 3 weeks before the other two cultivars, while in artificially inoculated second leaves there was a steep increase in lesion number each week after inoculation.

Increase in % infection

The increase in lesion numbers is closely paralleled by increase in % infection, except that for artificially inoculated Maris Ranger flag leaves the % infection rises before that of lesion number, so indicating an increase in the size of existing lesions. The % of lesions exceeding 3 mm is presented in Table 15 and it can be seen that for Maris Ranger this is greater than for the other cultivars. Further, in Maris Huntsman the commencement of lesion spread is delayed in comparison with Maris Ranger and Cappelle Desprez.

TABLE 15.

Field Experiment 18

Winter Wheat 1975

% lesions exceeding 3 mm

*Time Days	Part + Types of infection	Cultivar		
		Maris Ranger	Cappelle Desprez	Maris Huntsman
0(0)		0	0	0
6(7)	Flag	0	0	0
13(14)	Natural infection	0	0	0
20(21)		18.0	37.3	0
27(25)		57.1	34.8	30
34(35)		73.3	38.2	33.2
41(42)		58.0	41.6	26.4
48(49)		70.3	51.6	56.4
(56)		63.4	-	-
-3(0)	Flag	0	0	0
3(7)	Artificial	39.0	20.6	0
10(14)	Inoculation	42.6	59.2	25.0
17(21)		46.3	57.7	31.0
24(28)		59.5	38.6	36.6
31(35)		83.8	45.0	45.0
0 (0)	2nd leaf	31.6	0	0
8 (7)	Natural infection	60.0	0	0
14		46.6	0	0
21		43.0	0	20.0
28		45.0	24.1	25.0
35		85.3	26.0	23.2
42		85.4	21.2	32.2
49		-	26.4	40.0
56		-	64.3	61.6
70		-	52.3	-

TABLE 15 (Cont'd) Field Experiment 1B

Winter Wheat 1975

% lesions exceeding 3 mm

*Time Days	Part + Type of infection	Cultivar Maris Ranger	Cappelle Desprez	Maris Huntsman
-3(0)	2nd leaf	4	10.6	0
3(4)	artificial	46.6	54.3	0
10(14)	inoculation	33.8	49.1	0
17(21)		57.4	43.3	29.3
24(28)		-	63.6	38.0
31 -		78.2	-	41.3

*Figures in brackets represent time for Maris Ranger and Cappelle Desprez.

The Prevailing Meteorological Conditions 1975

The leaf surface wetness conditions, and maximum and minimum temperatures and humidities for May - July 1975 are given in Figs. 19 and 20 respectively.

The season on the whole was rather dry, with almost no rain between mid-May and mid-July(Fig. 19). This is in sharp contrast with the corresponding period for 1974 (Fig. 8).

Temperatures in May were relatively low, the minimum usually below 7°C and the maximum not exceeding 20°C. (Fig. 20). While in June and July the maximum temperatures were mainly between 20-20°C and the minimum not often below 10°C.

Maximum humidity remained fairly constant around 90% rather higher in May and July, but very low in June around 30-50% (Fig. 20).

In general the increase in lesion number seen to occur at the end of the season (Figs. 15-18) in mid-July coincides with a two week period with some hours of leaf surface wetness and with relatively high minimum temperatures. But in Maris Ranger the rise in the lesion number on second leaves follows very short periods of leaf surface wetness, and coincides with low minimum relative humidity, neither of which are optimal for Septoria.

Figure 19:- DURATION OF LEAF SURFACE WETNESS 1975

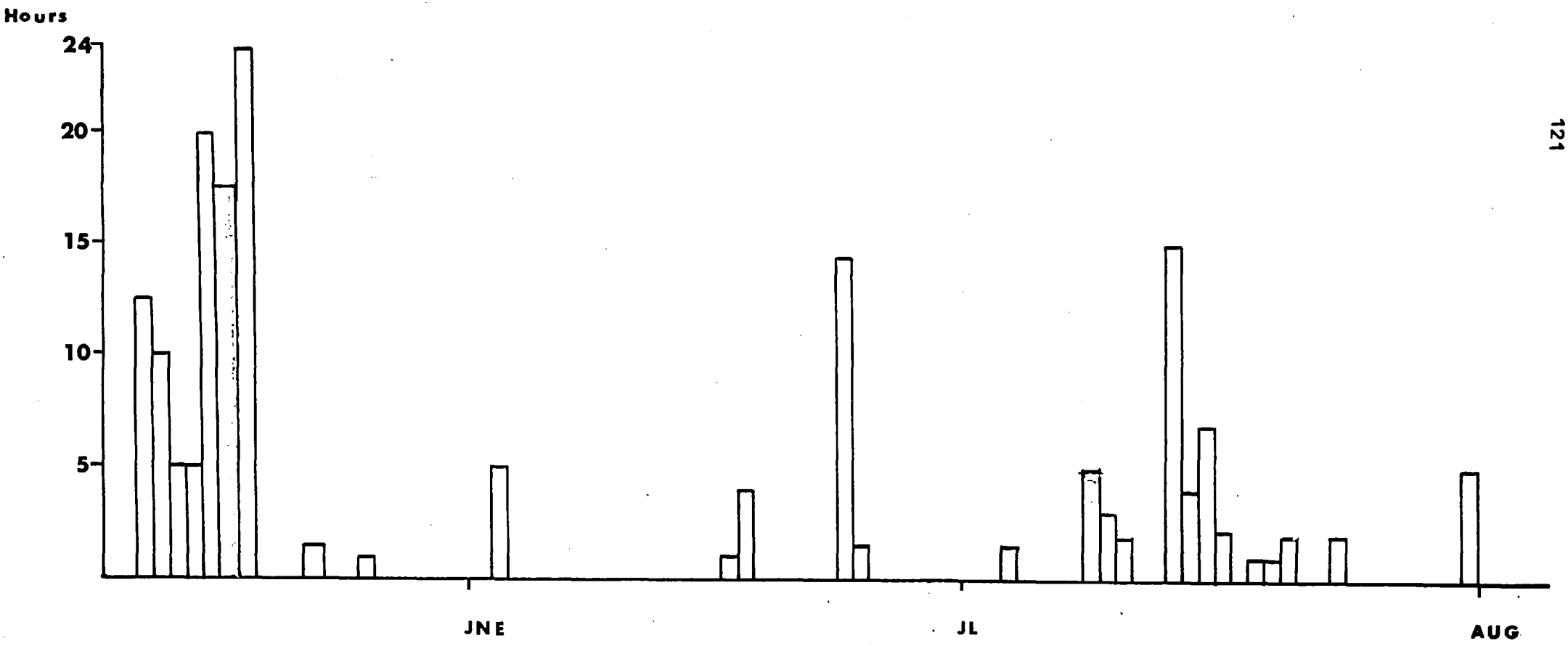
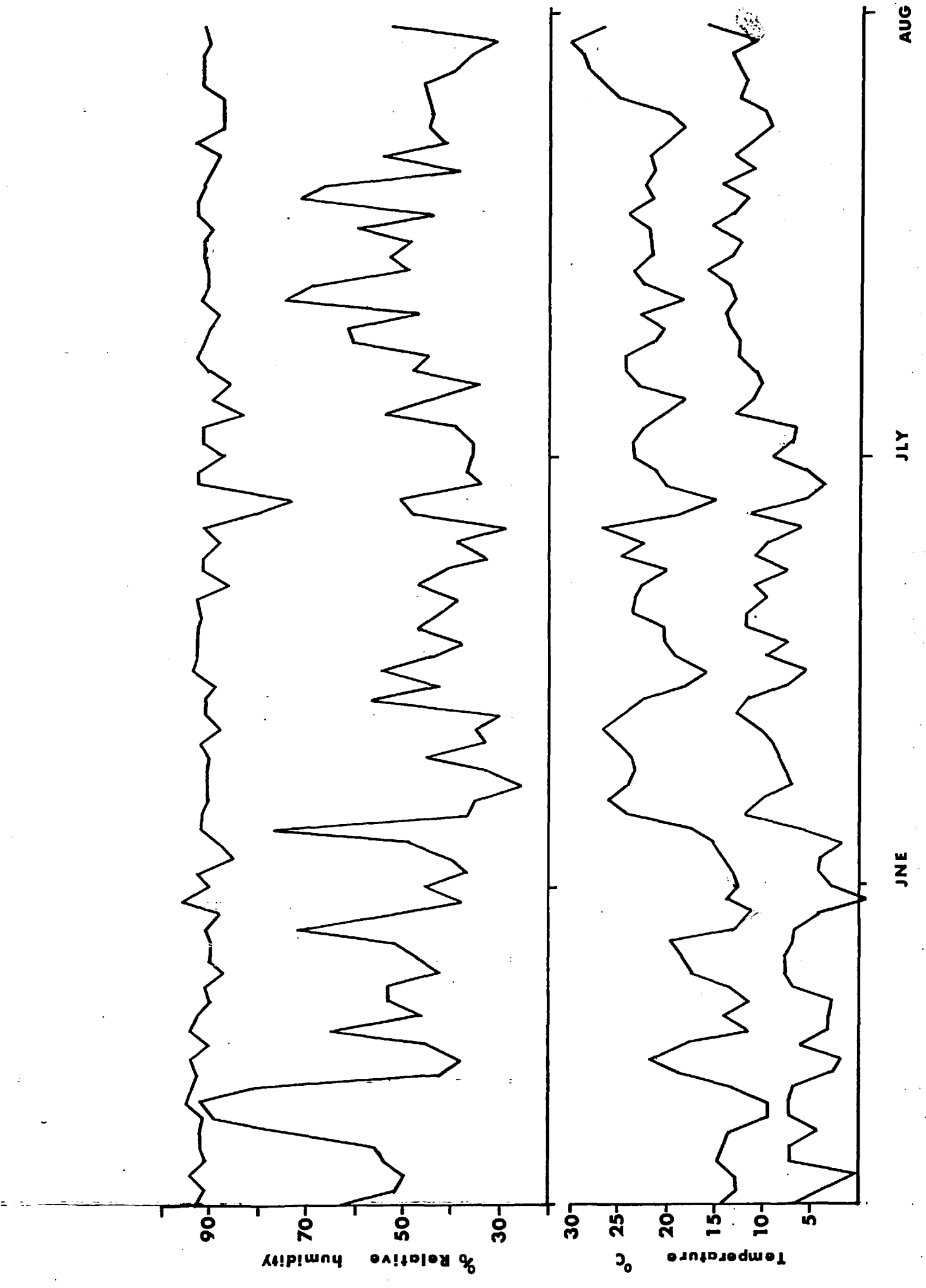


Figure 20:- Temperature & Relative Humidity; Maxima & Minima 1975



Statistical analysis

The data were subjected to a multiple linear regression analysis with % leaf area infected the dependent variable, and lesion number/cm^2, % lesions greater than 3 mm, internode length and time as independent variables.

Where, for example, in the case of the resistant cultivar, there was little variation in infection throughout the season, a regression analysis was considered unjustified.

Homogeneity of variance of the dependent variable was determined by comparing the root error mean square from the regression analysis of variance with the observations.

The partial regression coefficients of lesion number/cm^2, % lesions greater than 3 mm and internode length or plant height, on % infected leaf area are given in Table 16.

In all cases but one the regression coefficient for lesion number on % infection is significant for both flag and second leaves and in all three cultivars. The regression of increase in lesion size on % infection is only significant in artificially inoculated flag leaves of Cappelle Desprez and naturally infected second leaves of Maris Ranger. The independent variable, plant height is significant only in naturally infected second leaves of Cappelle Desprez and artificially inoculated second leaves of Maris Huntsman. In the former case the regression coefficient is negative indicating that taller plants have less infection. The converse is true in the latter case.

The significant regression coefficients for lesion number on infection are largest for Maris Ranger, with Cappelle Deaprez intermediate and Huntsman (where analysed) the smallest. This reflects the larger size of the lesions in the susceptible cultivar, Maris Ranger.

TABLE 16

Field Experiment 18

Winter Wheat 1975

Detailed Study of infection

Partial linear regression coefficients

Dependent variable: % infection

Independent variable	Leaf Inoculation type	Cultivar Maris Ranger	Cappelle Desprez	Maris Huntsman
	Naturally infected flag			
1. Lesion no/cm^2		110.602**	41.856***	Analysis not justified due to lack of variability in dependent variable.
2. % lesions >3 mm		0.034	0.036	
3. internode length		- 0.065	- 0.134	
	Artificially inoculated flag			
1. Lesion no/cm^2		90.702*	23.430**	Analysis not justified
2. % lesions >3 mm		0.087	0.026*	
3. internode length		- 0.011	- 0.641	
	Naturally infected 2nd			
1. Lesions no/cm^2		60.966***	28.234***	12.656
2. % lesions >3 mm		0.035*	0.045	0.012
3. Plant height		0.009	- 0.212**	- 0.025
	Artificially inoculated 2nd			
1. Lesion no/cm^2		12.155	59.043***	36.263
2. % lesions >3 mm		0.051	0.020	0.020
3. Plant height		0.277	-0.042	0.298**

* significant at 5% level
** " " 10% "
*** " " 0.1% "

Summary of infection data

A summary of data from the detailed study of infection is given in Table 17.

As expected from laboratory tests (reported in Section I) Maris Ranger shows the greatest % infection particularly in inoculated plants. Maris Huntsman shows a relatively consistent and much lower % infection whether inoculated or naturally infected. There is a parallel relationship for lesion number in these two cultivars. Maris Ranger shows a 3-6 fold increase in lesion number in artificially inoculated plants over naturally infected ones, compared to only a 2-fold increase in Maris Huntsman. Cappelle Desprez shows more lesions/cm^2 than Maris Ranger in naturally infected plants, but less of these exceed 3 mm. On average about twice as many Maris Ranger lesions exceed 3 mm compared with Maris Huntsman lesions.

The apparent infection rates 'r' given in Table 16 were calculated from the mean % infection data (Appendix 1B Tables 19-24) after transformation, $(\log_e (\frac{x}{1-x}))$ as described in 1A. It is notable that in Cappelle Desprez, and to a lesser extent in Maris Ranger the infection rate is higher in artificially inoculated plants than in the naturally infected ones. This indicates an apparent increase in susceptibility of these cultivars after inoculation. Since both naturally infected and artificially inoculated plants were exposed to exactly the same prevailing meteorological conditions this could not be an important factor in the reaction of leaves to the pathogen. And the increase in 'r' probably does indicate an increase in plant susceptibility (flag leaf and ear) which is particularly apparent at high inoculum levels for Maris Ranger and Cappelle Desprez. The apparent infection rate in Huntsman is unaffected by artificial inoculation and thus this variety "retains its resistance" in the presence of inoculum throughout the season.

TABLE 17

Field Experiment 1B
Winter Wheat 1975

Detailed study of infection

Summary Table (Mean values for whole season)

Variable	Leaf	Cultivar		
		Maris Ranger	Cappelle Desprez	Maris Huntsman
	Flag naturally infected			
% infection ('r' units day^{-1})		5.0 (0.0882)	4.3 (0.0711)	0.9 (0.0193)
Lesions/cm^2		0.043	0.079	0.015
% lesions > 3 mm		36.6	25.4	18.2
Internode length		21.3	24.3	21.7
	Flag artificially inoculated			
% infection ('r')		9.5 (0.0864)	3.5 (0.1421)	0.9 (0.0103)
lesions/cm^2		0.127	0.093	0.028
% lesions > 3 mm		40.8	35.2	23.0
internode length		25.4	28.7	27.3
	2nd naturally infected			
% infection ('r')		6.4 (0.0851)	4.2 (0.0341)	0.9 (0.769)
Lesions/cm^2		0.064	0.075	0.028
% lesions > 3 mm		54.0	19.4	22.4
Plant height		114.0	113.7	107.8
	2nd artificially inoculated			
% infection ('r')		12.9 (0.1155)	5.0 (0.1412)	2.0 (0.0638)
lesions/cm^2		0.351	0.100	0.070
% lesions > 3 mm		44.0	45.6	18.1
Plant height		112.8	116.8	115.2

'r' = apparent infection rate.

DISCUSSION

Varietal effect on infection development

The levels of infection obtained for the three cultivars in the field experiments were as expected from the detached leaf tests reported in Section I. Throughout the season Maris Ranger was the more susceptible to infection, while Cappelle Desprez and Maris Huntsman were more resistant. The surprising lack of statistical significance between varieties may arise from the fact that Maris Huntsman tended to have a rather high initial level of infection which masks its resistance. The resistance of this cultivar is evident when one considers the apparent infection rates, which are lower than for the other cultivars, particularly on the ears. Thus, notwithstanding the high initial level of infection on Maris Huntsman, the levels do not increase greatly over the season. The generally poor growth of the trial in 1974 owing to poor soil and adverse weather conditions may have influenced the varietal reactions. The vigorous crop obtained in 1975 tended to show a much higher level of resistance, although a direct effect of plant nutrition on infection cannot be assumed. Brönnimann (1969b) could demonstrate no effect of high nitrogen fertilisation on damage by Septoria, although ears of the more heavily fertilised plants were more attacked by the pathogen. Another reason for the absence of significance between cultivars, could be, to some extent, due to the lack of sensitivity of the statistical test since there were only 6-12 error degrees of freedom for varieties in the design used.

Effect of inoculation date on infection development

The control plots and early inoculations had low levels of infection which did not increase markedly at the end of the season. In contrast the later inoculations had higher levels of infection which rose steeply

towards the end of the season, even though these plants had been exposed to the disease for a shorter period of time. These high levels of infection in the later inoculations are a result of heavy infection on the flag leaf and ear which were inoculated directly. The epidemic once established picks up without a break. On the other hand, in the early inoculations the initial epidemic peters out possibly due to unfavourable weather and stage of plant growth. Infection of the more susceptible flag leaves and ears occurs naturally and is less effective than for the late inoculations. The high levels of infection for plants in which only the ears were inoculated indicates that an exchange of inoculum was occurring downwards from the ear to the leaves below. Cooke and Fozzard (1973) showed that glume blotch did not contribute greatly to leaf infection since the climax of the former occurred after leaf senescence; but this was for naturally infected plants.

The varieties all reacted in a similar way to the different inoculation dates. This was expected since they all matured at roughly the same time, Cappelle Desprez being a few days later.

% infection on different parts of the plant

There was a gradient of decreasing infection severity up the plant. One would expect leaf 4 which had been exposed to the disease for longest to have the greatest amount of disease. Also ontogenetically older leaves senesce earlier and this is associated with increasing susceptibility. Brünnimann (1969a)showed that infection of the various leaves and ear contributed in varying degrees to yield loss; thus the flag leaf and ear contributed 22.9% and 29% respectively and the 2nd and third leaves 6.1% and 7.1% respectively. Although Jones and Odebunmi (1971) failed to produce a significant reduction in yield by inoculation of the lower leaves only, Scharen, Schaeffer, Krupinsky and Sharpe (1975) demonstrated

a larger loss from a combination of axial flag leaf lesions plus excision of lower leaves indicating that early infection of lower leaves would result in greater yield losses. The flag leaf and the ear still remain the important determinants of yield (Thorne, 1965) and it is for these parts of the culm that real differences between the cultivars exists. Further the difference is consistent throughout the season; Maris Ranger always has the highest % infection and Maris Huntsman usually the lowest. This ranking is not apparent on the lower leaves. In addition the infection rates on the flag leaves and ears have a similar ranking, the low 'r' values on ears of Maris Huntsman are particularly striking.

Components of infection

The overall greater resistance of Maris Huntsman to infection is brought out in the detailed investigation of infection development. Not only do less infections occur on this cultivar, but fewer of them spread compared to the more susciptible varieties.

Inaccuracies in the measurement of parameters in the detailed study were unavoidable. These arise from lesions coalescing and mis-identification of lesions as they were initiated. Necrotic flecks which appeared were not always due to S. nodorum infection. Allowing for the errors tends to decrease apparent susceptibility. However since a real difference between resistant and susceptible cultivars was obtained the error may be largely disregarded. The lack of precision in estimating % infection at the lower end of the scale from 0-5% resulted in an apparent lack of variability in this parameter for the resistant cultivar, even though lesion number and size were varying. As a result no multiple linear regression could be carried out for this cultivar and useful information may be lost. The greater increase in lesion number and

infection rate on artificially inoculated plants over naturally infected ones of Cappelle Desprez and Maris Ranger indicates an apparent increase in the susceptibility of these cultivars following the application of inoculum at heading. That a low level of inoculum causes a variety to 'withstand' infection by having a lower value of 'r' than at a high inoculum level implies that it is acting as a kind of immunisation. However the apparent increase in susceptibility found is more or less coinciding with the stage at which plants tend to become less resistant anyway and the high level of inoculum applied may not be causing the increase in susceptibility, but rather exaggerating the effect. Under natural conditions the flag leaves may be as susceptible, but will have lower levels of infection if the weather conditions are unfavourable to spread of inoculum up the leaf.

In relating the development of infection to the prevailing meteorological conditions the optimum provisions for the fungus should be considered. These may be assumed as those conditions in which the length of the latent period (the time from infection to pycnidiospore release) is minimised. Investigations (Scharen, 1964; Shearer and Zadoks, 1972; 1974; Holmes and Colhoun, 1974) have shown that both temperature and wetness need to be considered.

A period of 6 wet days at 20-22°C was sufficient for pycnidial formation in second and third leaves of the winter wheat, Felix, while a decrease in the durstion of leaf surface wetness of 12 hours (17-5h) prolonged the latent period 7 days. Although infection will occur in dry conditions, no pycnidia will form. Also the minimum temperature is of importance, 7°C being a threshold (for Felix) below which pycnidia do not form.

The period of time for the establishment of a successful infection varies with varieties, tending to be shorter for the more susceptible cultivars (Brönnimann et al, 1972). In the present experiments the

presence of polythene bags for 96 hours following inoculation provided ample time for the spores to infect even the resistant cultivars. The prevailing weather conditions following removal of the bags will affect the development of infection, although these may be buffered to some extent by the crop canopy. The more uniform, slightly cooler and probably more humid, denser canopy of Maris Ranger may contribute towards a faster increase of infection. This effect, if real, would only be a contribution to susceptibility.

In the 1973/4 season optimum ambient temperatures of 20-22°C occurred between June 13-17; 20-22; July 6-9; 18-22; 27- August 3rd, while there were only two periods of 6 days leaf surface wetness, June 26-July 4; July 12-19 (and these were not continuously wet periods). Although it is difficult to extrapolate between varieties, the latent period would be expected to exceed 6 days.

In the 1974/5 season there were no periods of continuous leaf surface wetness and minimum temperatures early in the season were below 7°C. The adverse drier conditions of the second season are reflected in the rates of and total amounts of infection which tend to be much lower. However the % increase in size of existing lesions was greater. This may be a result of the inability of the fungus to produce pycnidia in dry conditions and instead increase mycelial growth.

Field Experiment 2A 1974

An experiment, analagous in design and adjoining the winter wheat trial, was set up using spring wheat cultivars.

The aim was to investigate the effects and possible interactions of inoculation date, variety and leaf on development of S. nodorum infection in spring wheat.

The particular cultivars used had shown variable disease rating/yield loss relationships in the experiments of Sharp et al (1972):-

Cultivar	Disease rating [a]	% Yield loss [b]
Bounty 208	19.0	54.1
B 519	12.4	22.9
Fortuna	22.7	36.8
Svenno	12.3	43.8

[a] Total disease rating based on a scale 1-9 each for lesions on the flag leaves, sheaths and heads.

[b] % yield loss due to disease in terms of grain yield/head, thousand kernel weight and no. kernels/head.

Thus these cultivars show relatively high disease + high yield loss; low disease + low yield loss; high disease + low yield loss; low disease + high yield loss.

It was of interest to know if the development of infection on the various parts of the plant could be related to the differential responses to yield of the cvs used.

Whole clumps of ten plants were inoculated at the following growth stages:-

G.S. Feekes scale (Large, 1954)	Description
6-7	Stem extension, 2nd node visible
8-9	last leaf just visible
10	in boot
10.5	flowering

There was also an uninoculated control.

The ten main culms in each clump were selected and the % of photosynthetic area affected by Septoria nodorum was estimated visually on the top three leaves and ear for each week after inoculation using the keys of Brönnimann (1968a).

Meteorological data was recorded as for the winter wheat trial 1A.

One replicate was abandoned due to poor growth arising from poor soil and a very dry spring. Growth in the remaining plots was not particularly vigorous and so yield data was not recorded.

The raw data (which appears in the appendix 2A Tables 27-46) was subjected to an analysis of variance on a whole plant basis and as for the winter wheat trial the apparent infection rates ('r') for each treatment cultivar/leaf combination were determined on transformed data using the van der Plank (1963) $\log_e \left(\frac{x}{1-x}\right)$ transformation, where 'x' is the proportion of tissue affected by S. nodorum.

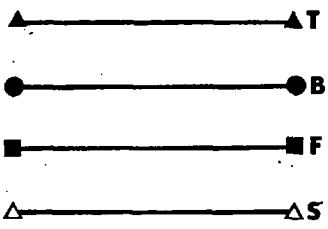
The mean weekly % infections for each variety/treatment combination are plotted in Figs. 21-25. These are followed by the analysis of variance F-values for the main effects and interactions of the variables: cultivar, treatment and leaf. (Table 18).

Field Experiment 2A

Spring Wheat 1974

The Development of S. nodorum infection

Key to Figs. 21-5



T Bounty 208

B B 519

F Fortuna

S Svenno

Treatment No.	Inoculation at G.S.
1	6-7
2	8-9
3	10
4	10.5
Control	uninoculated

Figure 21:- Mean weekly % infection; whole plants. Treatment 1, spring wheat 1974

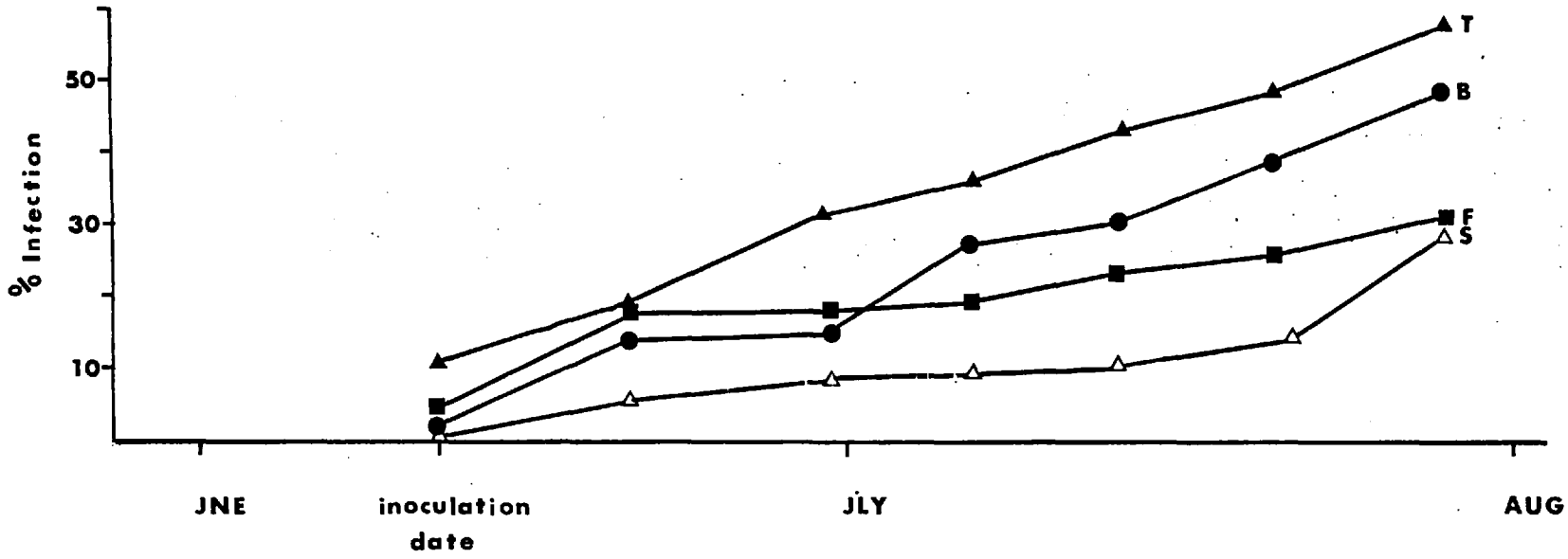


Figure 22:- Mean weekly % infection; whole plants. Treatment 2, spring wheat 1974

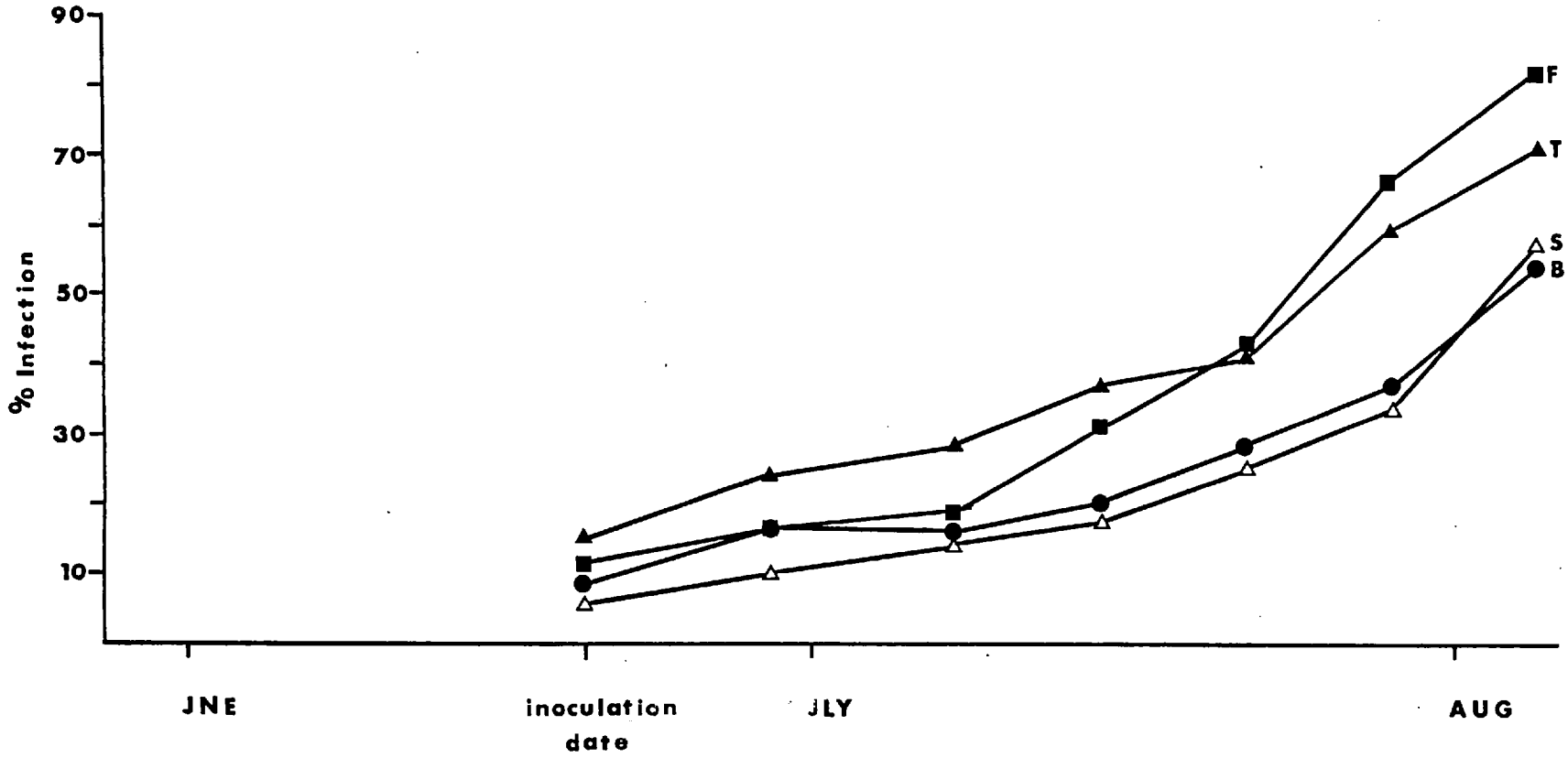


Figure 23:- Mean weekly % infection; whole plants. Treatment 3, spring wheat 1974

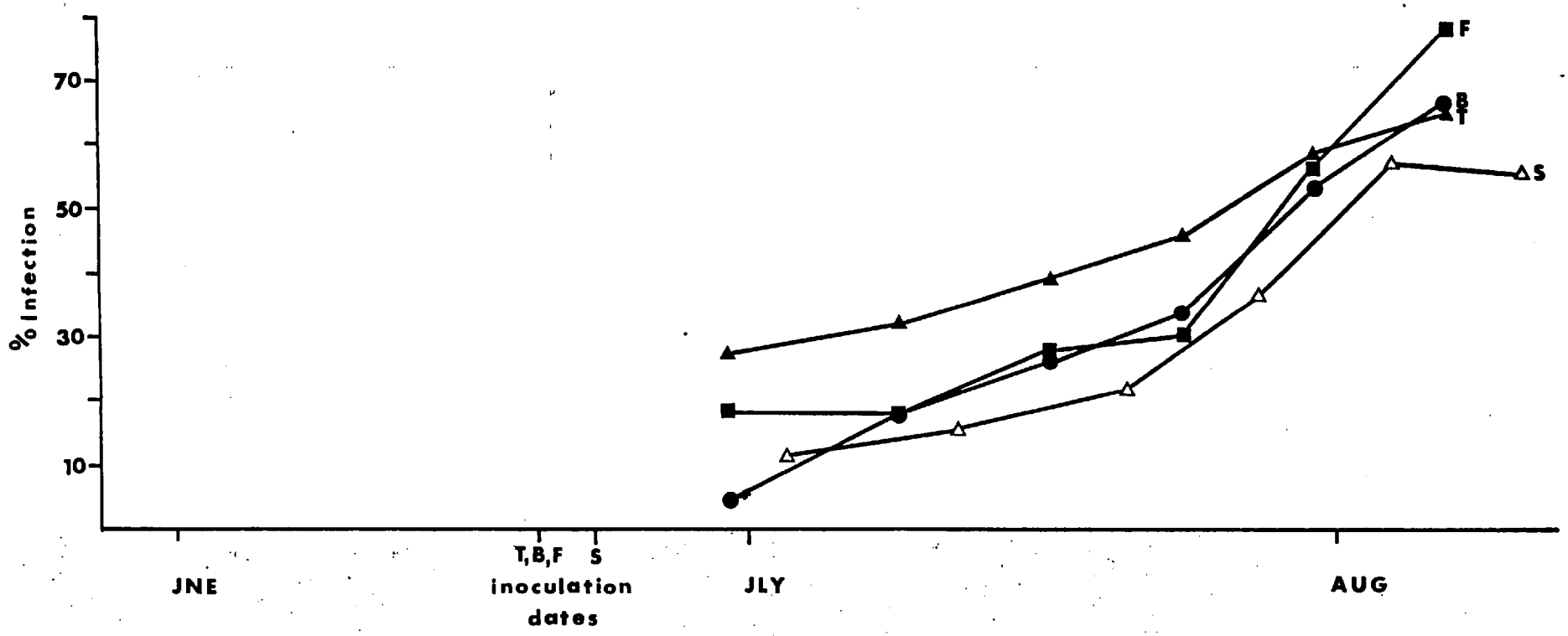


Figure 24:- Mean weekly % infection; whole plants. Treatment 4, spring wheat 1974

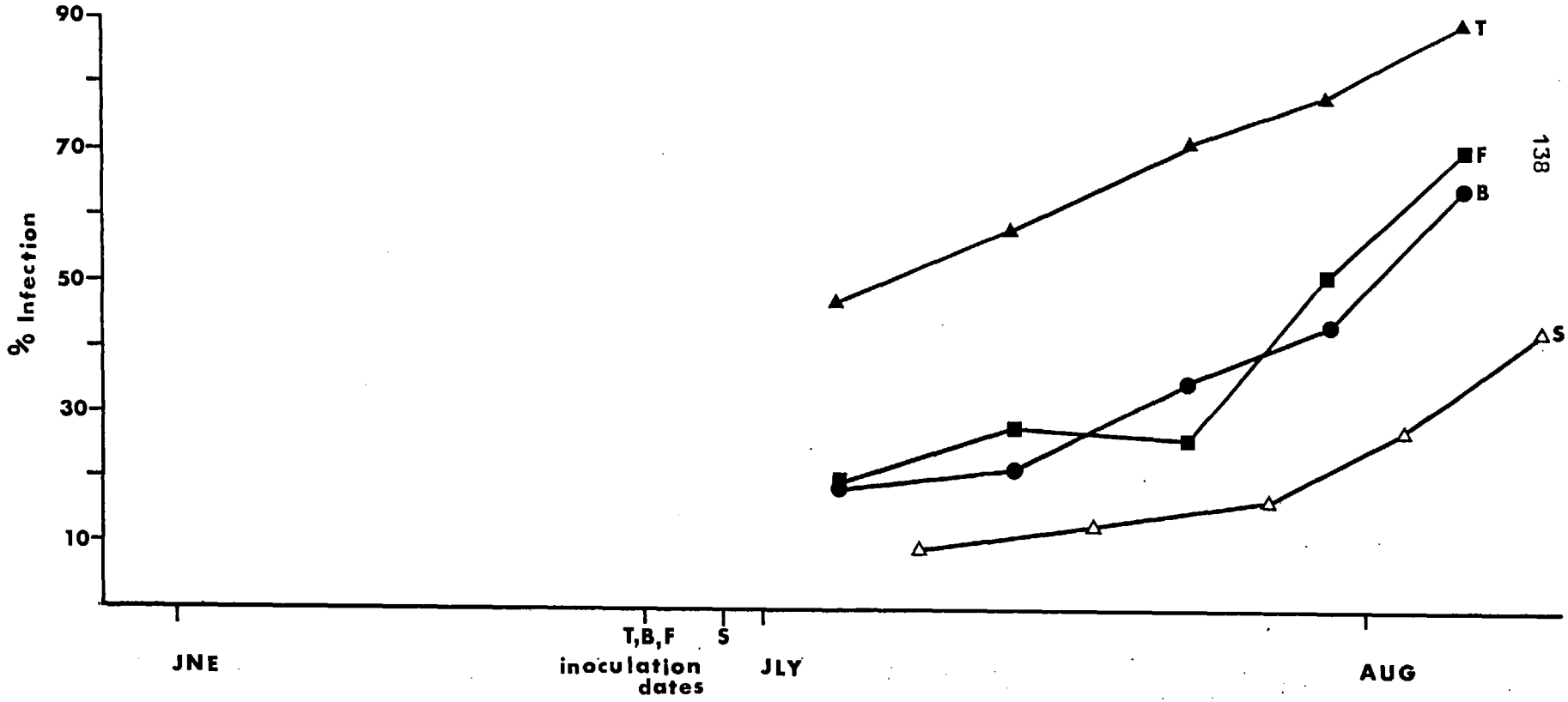


Figure 25:- Mean weekly % infection; whole plants. Uninoculated Control, spring wheat, 1974

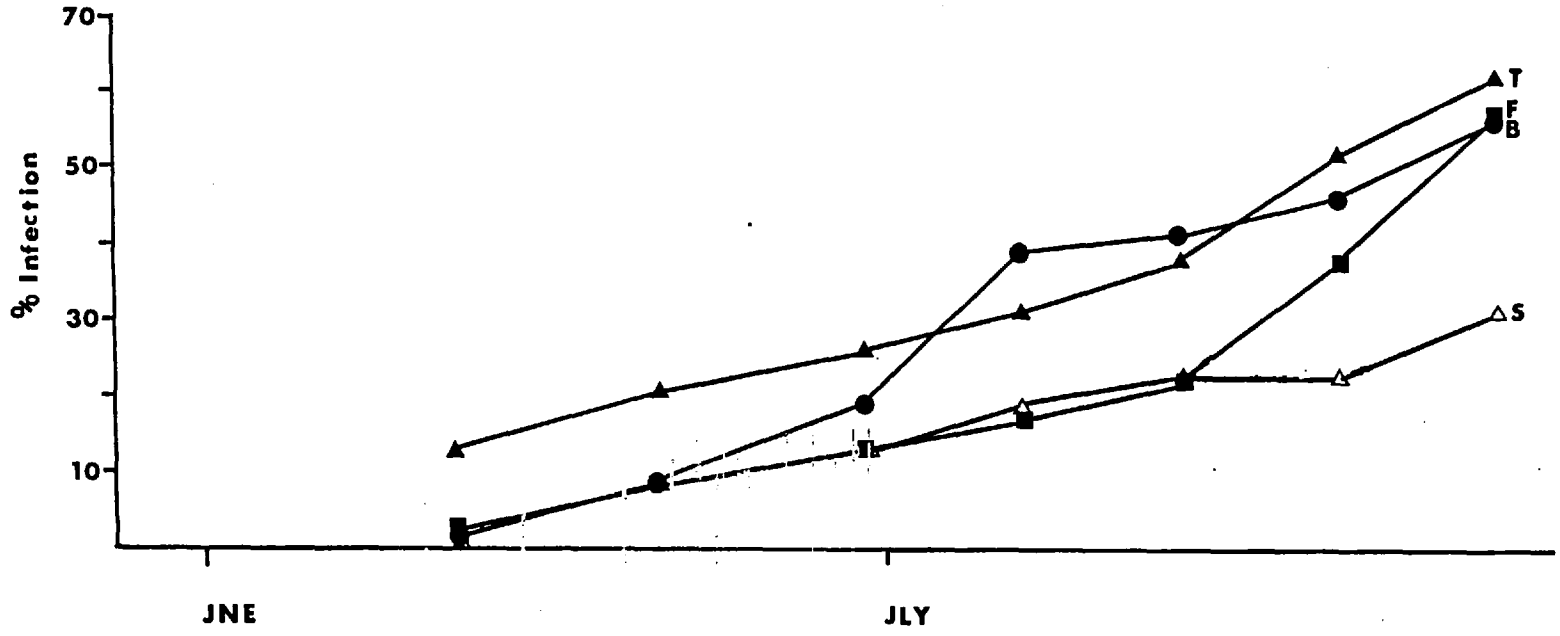


TABLE 18 Field Experiment 2A

The effects and interactions of inoculation date, variety and leaf on development of S. nodorum infection in spring wheat

Analysis of variance (whole plants)

F values + level of significance

Days after inoculation	Variables		
	Treatment	Cultivar	Leaf
10	13.28** (All trts.)	40.77**	71.90**
19	5.40*	25.66**	87.35**
28	10.32*	19.56**	119.11**
35	10.72*	20.73**	156.58**
42	28.34**	16.00**	238.64**
49	17.08** (Trt.1-3+c)	56.2	301.56**
56	13.42* (Trt 1+2+c)	20.89**	248.81**

Interactions

Days after inoculation	Variables		
	Treatment & Cultivar	Treatment & Leaf	Cultivar & leaf
10	3.17*	3.31**	9.05**
19	2.28*	1.64	2.92**
28	2.11	1.50	4.28**
35	2.92*	0.57	5.44**
42	2.93*	2.10*	4.18**
49	4.74**	6.10**	8.05**
56	5.30**	4.49**	5.35**

* sig. at 5% level
** " " 1% "

In treatment 1 (Fig. 21) and the control (Fig. 25) there is a relatively low linear rise in infection with a slight increase at the end of the season for the more resistant cultivars, B519 and Svenno. Bounty 208 shows a consistently higher level for most of the time. In treatments 2 and 3 (Figs. 22 and 23) there is a relatively steep rise in % infection, towards the end of the season, which occurs in all cultivars. Bounty 208 is again the more susceptible and Svenno the more resistant cultivar.

In treatment 4 (Fig. 24) there is a higher initial level of infection particularly in Bounty 208 indicating its greater susceptibility to infection at this growth stage. Svenno stays at a much lower level of infection while Fortuna and B519 have similar, but intermediate values.

The analysis of variance (Table 18) shows that these differences in infection between treatments were significant throughout the season.

It is also evident from Table 18 that there are significant differences between cultivars for all times following inoculation. Bounty 208 and Svenno represent the two extremes in leaf susceptibility and resistance respectively, and for all treatments and times this relationship generally held true.

The difference between Fortuna and B 519 which was marked in detached leaf tests (Section I) was less apparent in the field. While Fortuna had a similar or lower level of infection than B 519 in the early part of the season for treatments 2-4, it tended to show a steep rise in % infection towards the end, resulting in higher levels than B 519.

This apparent reversal in % infection for Fortuna and B 519 was not consistent for all treatments and is probably the cause of the significant treatment/cultivar interaction in Table 18.

At 10 days after inoculation the ranking order for all treatments except 3 is Bounty 208 (T) > Fortuna (F) > B 519 (B) > Svenno (S), while in treatment 3 (inoculation at G.S.10) the order in T > F > S > B.

At 35 and 42 days for the control B 519 becomes the most susceptible cultivar, the order being B > T > F ≥ S.
In contrast at 42 days treatment 2, 49 days treatment 2 and 3 and 56 days treatment 2, Fortuna assumes the position of most susceptible while the others vary: T ≥ B > S, T > S > B. Thus throughout, Bounty 208 is more heavily infected than Svenno, but B 519 and Fortuna vary in comparison. On the whole, B 519 is more resistant at the end of the season and Fortuna more susceptible.

The prevailing meteorological conditions

In relating disease development to the meteorological data (Figs. 8 and 9) treatments 1, 3 and 4 (except Svenno) had near optimum leaf surface wetness conditions following removal of inoculation bags. The level of infection on Svenno, treatment 4, is very much lower than the other cultivars and may be a result of the sub-optimum conditions following bag removal.

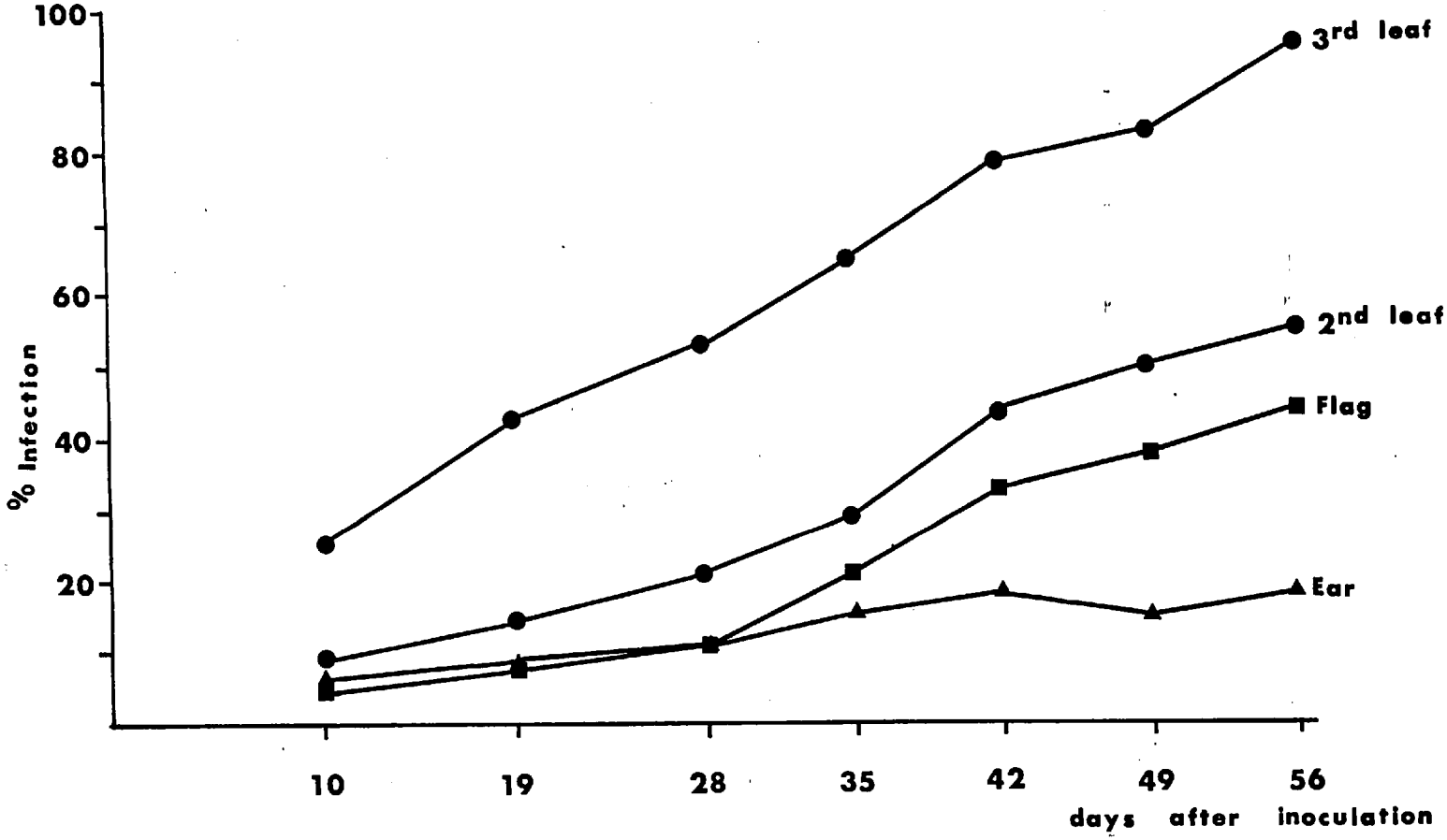
The steep increases in infection at the end of July - beginning of August follow an eight day period of leaf surface wetness in the third week of the month and coincide with higher minimum temperatures.

Temperatures within the crop canopy were not measured because of lack of equipment.

Differences between part of plant (averaged over cultivars and treatments)

The progress of infection on different leaves and the ear following inoculation is given in Fig. 26. The three leaves follow a similar pattern of a gradual rise in infection up to 35 days and then an increase after this time. The thid leaf has very much higher levels of infection throughout. The flag leaf and ear have the same level of infection up to 28 days after inoculation. Infection on the flag then rises more steeply than that on the ear - which remains relatively low. The differences between leaves are significant throughout the season (Table 18).

Figure 26:- Mean weekly % infection of different parts of the culm; averaged over all treatments & varieties; spring wheat, 1974



Apparent infection rates for individual leavee

The apparent infection rates for parte of plant/variety treatment combinations are given in Table 19.

The ranking order of 'r' for leaves across all treatments is given below ('1' indicates highest value of 'r')

Leaf	Cultivar			
	Bounty 208	B519	Fortuna	Svenno
3	3	1	3	2
2	2	3	2	2
Flag	1	2	1	1

Ths ranking order for leaves of the cultivars across all treatments is the same, there being a decreasing gradient of 'r' down ths plant, except in the case of B519. In this cultivar the highest rate of 'r' is on the third leaf and lowest on the second leaf; the flag leaf having an intermediate value of 'r'

The high value of 'r' for the third leaf of B519 compared to Fortuna may explain the apparent greater susceptibility of the former cultivar at the beginning of the season.

As discussed in experiment 1A, the infection rates for ears are based on late observations only and will, therefore, be considered separately.

The high loss cultivars, Bounty 208 and Svenno, tend on the whole to have rather higher 'r' values on the ears than leaves compared with the low loss cultivars.

The overall ranking order for varieties is Bounty 208 $>$ B519 = Svenno $\geqslant$ Fortuna.

TABLE 19

Field Experiment 2A

Spring wheat 1974

Apparent infection rates 'r' of spring wheat (units. day^{-1})

(calculated as the linear regression coefficients of $\log_e(\frac{x}{1-x})$ on time

Treatment	Leaf	Cultivar			
		Bounty 208	B519	Fortuna	Svenno
1	3	0.1055	0.1435	0.0328	0.0998
	2	0.0567	0.0968	0.0693	0.0760
	Flag	0.1256	0.0652	0.0890	0.0912
	Ear	0.0458	0.0664	0.0500	0.1004
2	3	0.0814	0.0658	0.1077	0.0702
	2	0.0508	0.0591	0.1215	0.0737
	Flag	0.1091	0.0722	0.1312	0.0807
	Ear	0.0970	0.0896	0.0440	0.1145
3	3	0.0335	0.1698	0.0376	0.0413
	2	0.0372	0.1587	0.1208	0.0829
	Flag	0.0632	0.0837	0.1209	0.1203
	Ear	0.0815	0.1221	0.0456	0.0840
4	3	0.0943	0.2219	0.0734	0.0658
	2	0.1211	0.1426	0.1468	0.0844
	Flag	0.1436	0.0544	0.1539	0.0937
	Ear	0.0276	0.0599	0.0205	0.0823
Control	3	0.0547	0.1503	0.0717	0.0940
	2	0.0631	0.0885	0.1084	0.0898
	Flag	0.1226	0.0980	0.0994	0.0901
	Ear	0.0964	0.0364	0.0526	0.1027

The former cultivar stands out as having high values of 'r' throughout, while the other three are rather similar.

The ranking order for treatments across cultivars is given below.

Treatment	Leaf			
	3	2	Flag	Ear
1	1	3	5	3
2	4	5	2	1
3	4	2	3	1
4	3	1	1	5
Control	2	3	3	3

A definite pattern is difficult to distinguish here, but generally in the later inoculations, treatments 3 and 4, the infection rates for leaves tend to be greater indicating a greater susceptibility of the plant at those times.

Summary of infection data

The overall seasonal means for treatments and varieties are presented in Table 20.

As in the winter wheat experiment the later treatments result in higher average % infections than the earlier ones.

The ranking order for varieties is Bounty 208 > Fortuna ≫ B 519 > Svenno, which is somewhat different from the ranking order for infection rates.

Svenno tended to have similar values of 'r' to B519 and Fortuna. Thus the low mean % infection represents a lower initial level of infection at the start of the season.

The levels of infection obtained did not correlate entirely with experiments carried out in the laboratory with detached leaves (Section I), in which Fortuna was very susceptible, Bounty 208 and Svenno similar but less than Fortuna, and B519 the more resistant..

TABLE 20

Field Experiment 2A

Spring wheat 1974

Mean % S. nodorum infection for the season

Treatment	Cultivar				Mean
	Bounty 208	B519	Fortuna	Svenno	
1	35.5	25.7	20.0	11.1	23.1
2	39.5	26.3	38.5	23.7	32.0
3	45.0	33.9	38.1	33.1	37.5
4	69.1	36.5	38.9	21.7	41.5
Control	35.0	30.5	22.7	17.0	26.3
	-	-	-	-	-
Mean	44.8	30.6	31.6	21.3	

Field Experiment 2B

As in the winter wheat trial (1B) a more detailed study of infection development in naturally infected and artificially inoculated plants was made, in order to assess in particular the relative importance of increase of lesion number and spread of existing lesions.

The data was collected and analysed as described in Field Experiment 1B p.110 Figures 27-32 represent the increase in lesion number and % infection for naturally infected and artificially inoculated flag leaves, second leaves and ears on the four spring wheat cultivars. The % of lesions exceeding 3 mm is tabulated in Table 21.

The data from which the figures are prepared is given in Appendix 2B Tables 47-58.

Field Experiment 2B

Spring wheat 1974

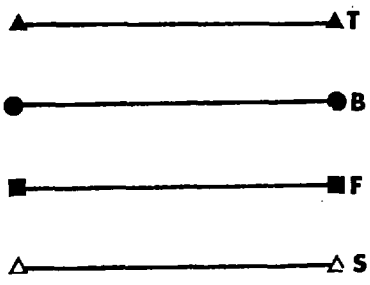
Detailed Study of Infection Development

Key to Figs. 27-32

Scale: Vertical axis 2 cm represents 10% infection.

2 cm " 1.0 lesions/cm^2

Horizontal axis 3 cm " 1 day



Bounty 208

B519

Fortuna

Svenno

Each point on the graph is an average of up to five leaves (or ears) on five plants.

Figure 27:- Increase in % infection and lesion number for naturally infected flag leaves of spring wheat, 1974

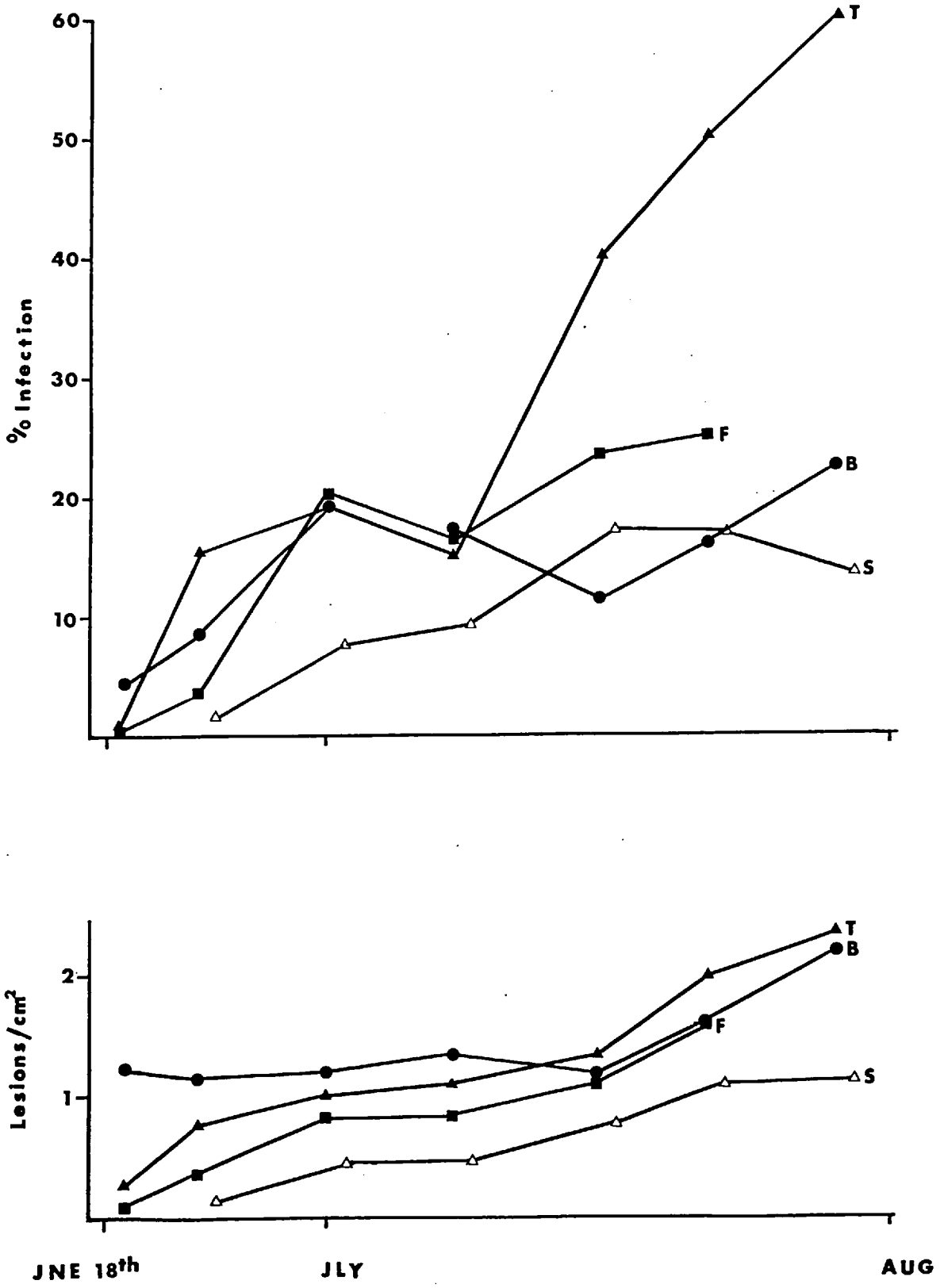


Figure 28:- Increase in % infection and lesion number for artificially inoculated flag leaves of spring wheat, 1974

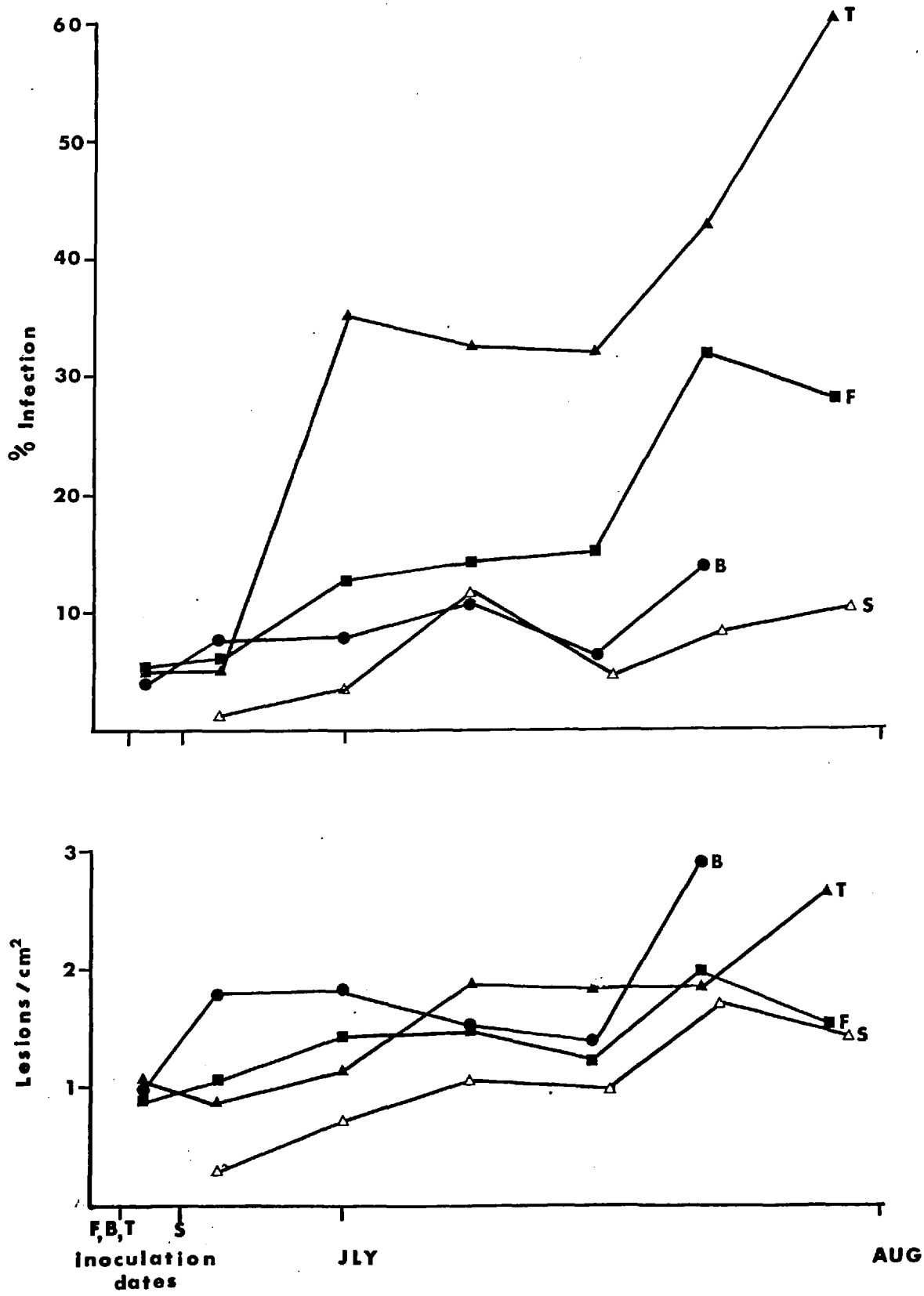


Figure 29:- Increase in % infection and lesion number for naturally infected 2nd leaves of spring wheat, 1974

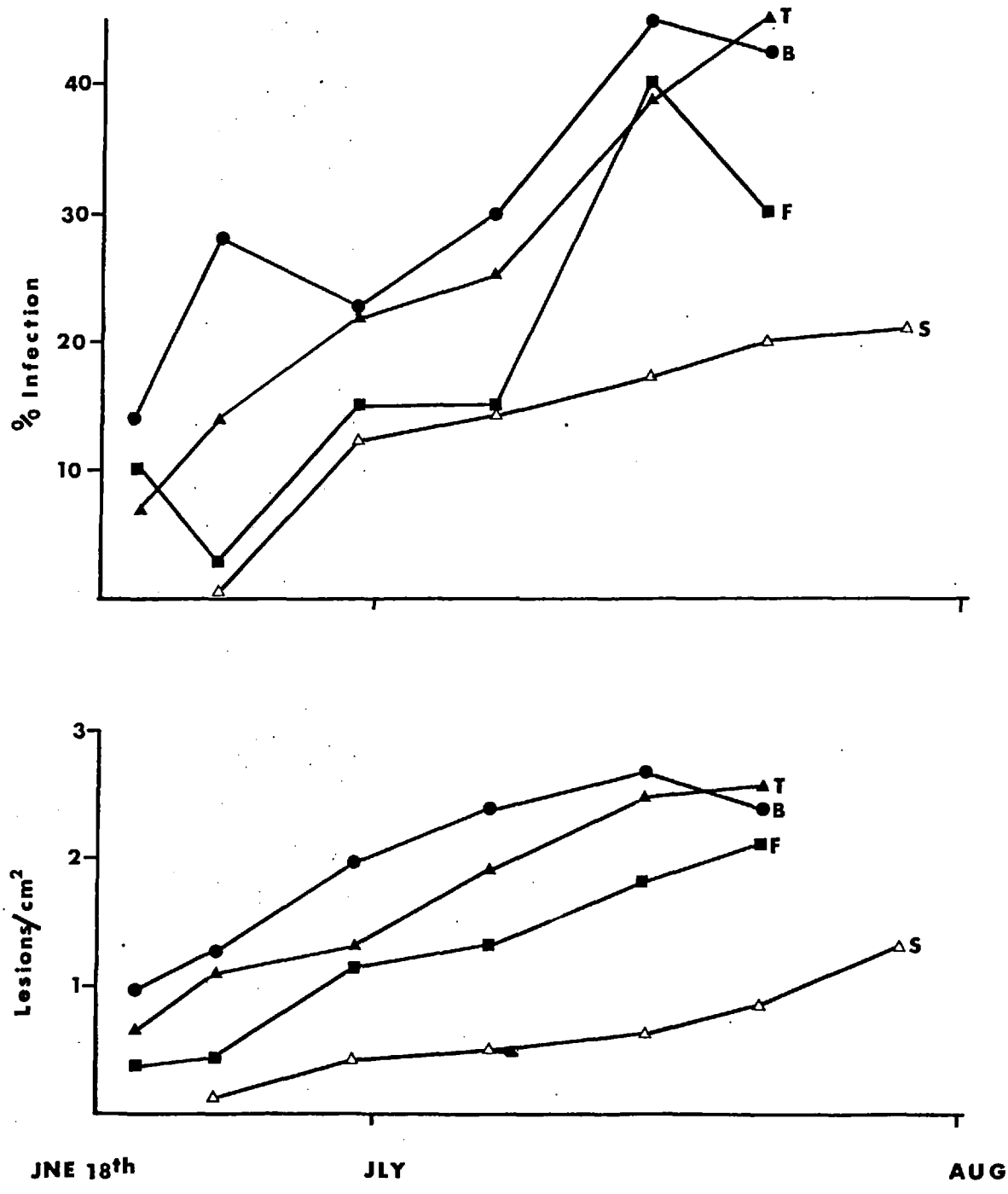


Figure 30:- Increase in % infection and lesion number for artificially inoculated 2nd leaves of spring wheat, 1974

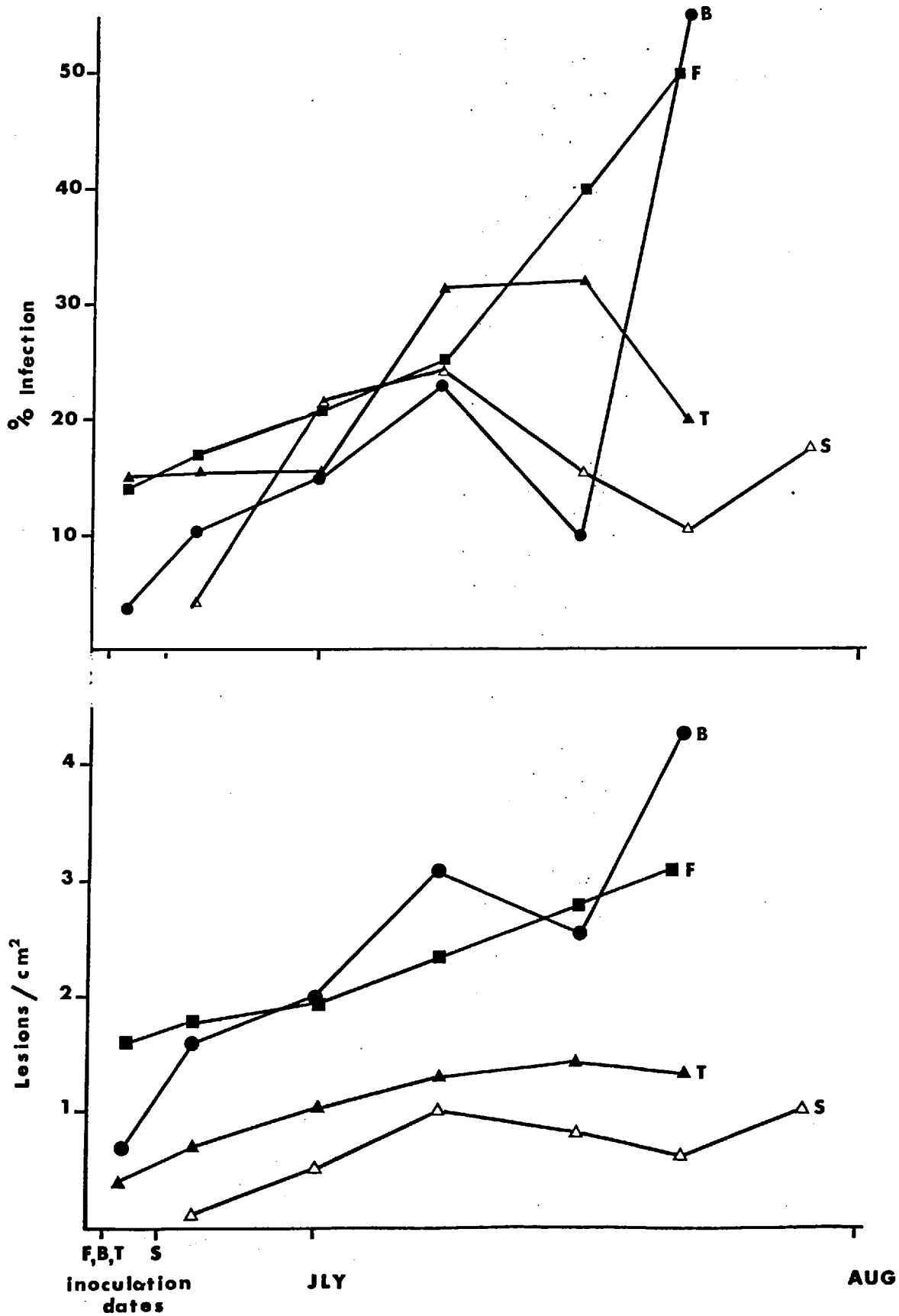


Figure 31:- Increase in % infection and lesion number for naturally infected ears of spring wheat, 1974

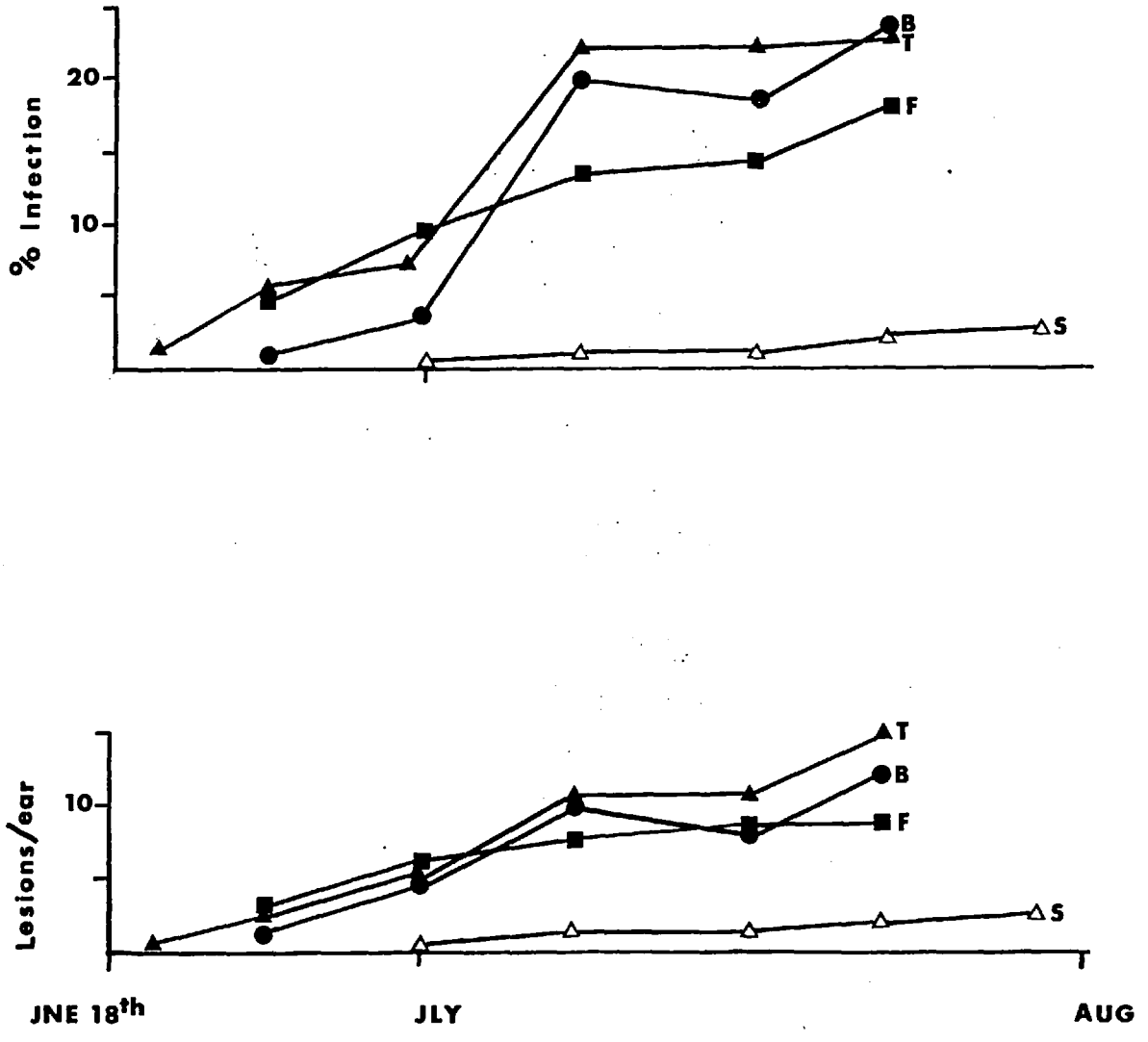


Figure 32:- Increase in % infection and lesion number for artificially inoculated ears of spring wheat, 1974

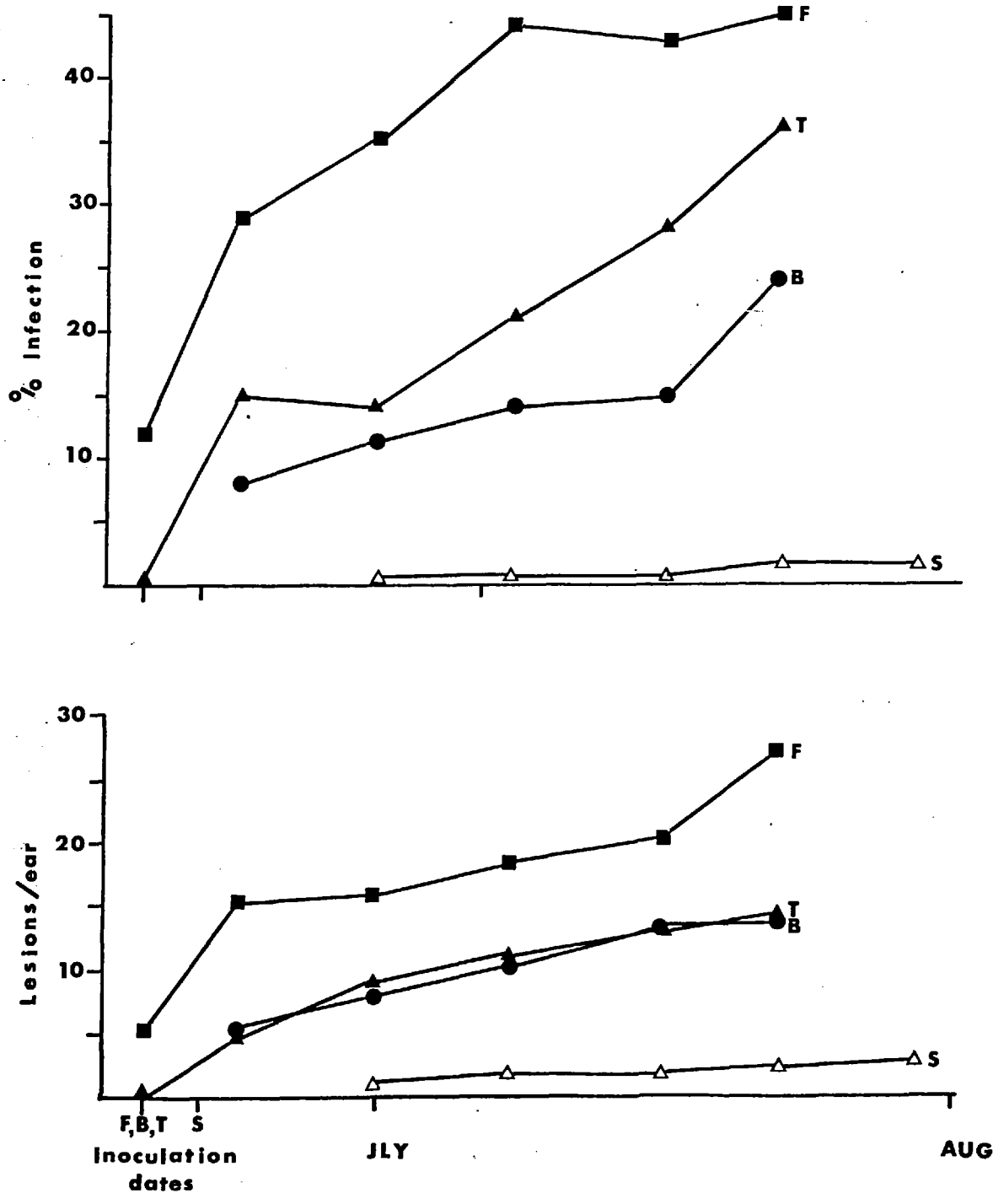


TABLE 21

Field Experiment 28

Spring wheat 1974

% lesions exceeding 3 mm

*Time	Part	Cultivar			
Days	Inoculation type	Bounty 208	8519	Fortuna	Svenno
1		28.0	24.5	5	-
5(2)	Flag	55.4	36.4	20	14.0
12(9)	Nat. Inf.	42.8	23.0	46.6	51.4
19(16)		45.7	17.8	44.6	40.7
27(24)		49.3	11.2	43.6	40.9
33(30)		52.5	17.8	73.1	28.6
40(37)		53.3	17.0	-	20.7
	Flag Art. Inoc.				
1		44.2	12.1	56.8	-
5(2)		47.1	8.2	41.7	0
12(a)		54.8	8.7	44.2	24.5
17(16)		36.0	9.1	34.8	19.0
27(24)		56.9	15.3	45.2	11.3
33(30)		57.2	12.2	42.3	33.0
40(37)		60.8	-	77.1	35.0
	2nd leaf Nat. Inf.				
1		37.6	34.7	10.0	-
5		37.6	51.4	23.1	14.3
12		41.6	34.0	50.3	44.1
19		43.7	41.3	45.0	42.9
27		59.0	66.7	65.0	50.3
33		54.5	65.5	48.0	45.8
40		-	-	-	47.3

TABLE 21 Cont'd. Field Experiment 28

Spring wheat 1974

% lesions exceeding 3 mm

* Time	Part	Cultivar			
Days	Inoculation type	Bounty 208	B519	Fortuna	Svenno
	2nd leaf Art. Inoc.				
1		49.2	32.4	59.0	-
5		50.2	34.5	52.0	30.0
12		42.7	38.6	53.0	70.0
19		60.0	33.0	51.8	44.7
27		81.2	21.0	49.0	58.3
33		75.0	47.0	64.0	68.9
40		-	-	-	59.5
	Ear Nat. Inf.				
1		0	-	-	-
5 (2)		57.6	0	44.2	-
12 (9)		67.0	4	34.8	0
19(16)		57.4	39.5	57.2	50.0
27(24)		68.2	61.7	54.0	50.0
33(30)		66.2	35.0	57.2	45.8
-(37)		-	-	-	39.8
	Ear Art. Inoc.				
1		0	-	57.6	-
5		97.2	37.5	53.0	-
12		67.2	29.0	78.4	0
19		59.8	29.6	81.8	0
27		65.6	32.2	74.4	0
33		58.8	42.6	79.6	19.8
-		-	-	-	15.0

* Figures in brackets are time for Svenno

Infection on flag leaves

The flag leaves show a relatively slow rate of increase in lesion number throughout the season (Figs. 27 and 28) with a steeper rise at the end. Bounty 208 and B519 have higher numbers of lesions than Fortuna and Svenno, the latter cultivar having the lowest levels.

Increase in % infection in Fortuna, Svenno and B519 is similar. However, Bounty 208 shows a steep increase in infection from 19 days and 5 days after inoculation in naturally infected and artificially inoculated plants respectively, which can be explained by an increase in the size of existing lesions. From Table 21 it can be seen that about 17% lesions exceed 3 mm in B519 while in Fortuna the figure is about 77%. This is reflected in the fact that while B519 has more lesions , it has a lower % infection since the lesionsare restricted.

Infection on second leaves

In naturally infected second leaves (Fig. 29) varieties show parallel increases in lesion number with B519 > Bounty 208 > Fortuna > Svenno. In artificially inoculated leaves however, (Fig. 30) the order is changed. Svenno and Bounty 208 have parallel low rates of increase while the other two cultivars show similar but steeper and larger increases, with B519 exhibiting a sharp increase at the end.

Increase in % infection for both types of infection parallels that of increase in lesion numbers. But, while in artificially inoculated leaves the% infection values are similar for all cultivars up to 19 days after inoculation, B519 and Fortuna subsequently show steep increases in infection, while Bounty 208 and Svenno level out. Up to 19 days, therefore, lesions are smaller on B519 and Fortuna and then size increases at a greater rate than for the other cultivars.

Infection on ears

The number of lesions in both naturally infected and artificially inoculated ears of Svenno remains relatively low throughout the season (Figs. 31 and 32). Bounty 208 and B519 have similar numbers of lesions for both types of infection. Fortuna, on the other hand, shows a large difference in lesion numbers on the ear, there being many more lesions on the artificially inoculated compared to the naturally infected (Figs. 31 and 32).

% infection increases are analogous to the increases in total lesion numbers for all varieties, except that there is a large difference between infection % on Bounty 208 and B519, while they have almost identical lesion numbers on artificially inoculated ears. This may be explained by the fact that about 60-90% of lesions spread on Bounty 208 ears compared to only 30-40% on B519. (Table 21).

The prevailing meteorological conditions

Excepting a few days, the leaf surface wetness conditions (Fig. 8) throughout the duration of the experiment were generally favourable to the pathogen. This was also true for temperature (Fig. 9): minima around 10-11°C and maxima 17.5-21°C.

Statistical analysis

The data for flag leaves was subjected to a multiple linear regression analysis with % infected leaf area the dependent variable and number lesion/cm^2, % lesions >3 mm, internode length, and time the independent variables as described in experiment 18 p.123

The partial regression coefficients for lesion number/cm^2, % lesions >3 mm and internode length on % infection are given in Table 22. The levels of significance are indicated.

As in the winter wheat experiment, the regression coefficient for lesion number/cm^2 assumes greatest significance in all cultivars with the exception of Bounty 208. In this cultivar internode length is significant. The regression coefficient in this case is negative indicating that less infection is associated with longer internodes.

Increase in size of lesions is significant for B519 and for Svenno (naturally infected only). This is difficult to explain in the case of the former cultivar since on the whole the % of lesions exceeding 3 mm was rather low.

TABLE 22

Field Experiment 28

Spring wheat 1974

Detailed study of infection

Partial regression coefficients

(Dependent variable: % infection)

Independent Variable	Leaf	Cultivar			
	Flag Nat. Inf.	Bounty 208	B519	Fortuna	Svenno
No. lesions/cm^2		7.571	5.263**	25.106***	18.030***
% lesions > 3mm		0.027	0.175*	0.012	0.154**
Internode length		-2.542	-3.835	-0.329	-2.322*
	Art.Inoc. Flag				
No. lesions/cm^2		3.666	2.637**	10.717***	5.562*
% lesions > 3mm		0.218	0.343***	0.118	0.121
Internode length		-5.232*	-0.423	-0.199	-1.106

Summary of infection data

A summary of data from the detailed study of infection for each cultivar and variable is given in Table 23.

Bounty 208 and Svenno show high and low levels of % infection respectively, with B519 and Fortuna intermediate.

Although B519 has a greater number of lesions/cm^2 than Fortuna, the increase in size of lesions in the former is rather less, and thus the total % infection is less also.

In contrast with the other varieties, Fortuna shows a relatively greater increase in lesion number/cm^2 (2-fold) and size when inoculated compared to natural infection. However, the rate of infection in artificially inoculated leaves of Fortuna is less than in naturally infected ones. This may reflect the success of the initial inoculation, which results in a high level of infection that does not then increase at a high rate.

B519 and Svenno have slightly less infection in the artificially inoculated leaves compared to naturally infected ones. More lesions are developing in the former case but less of these increase in size.

Fortuna had longer internodes than the other three cultivars which were all similar.

The lowest infection rates occur on B519, the low disease, low loss cultivar, and the highest rates on Bounty 208, the high disease, high loss cultivar.

TABLE 23

Field Experiment 2B

Spring wheat 1974

Detailed study of infection

Summary table: (means for all variables on Flag leaves)

Variable	Leaf	Cultivar			
	Nat.Inf. Flag	Bounty 208	B519	Fortuna	Svenno
% infection ('r')		24.72 (0.1006)	14.28 (0.0323)	12.00 (0.1135)	10.28 (0.0509)
lesion No./cm^2		1.13	1.40	0.68	0.69
% lesions > 3mm		47.60	21.63	32.67	33.13
Internode length		16.24	16.77	19.29	16.03
	Art.Inoc. Flag				
% infection ('r')		27.77 (0.0799)	8.32 (0.0200)	15.48 (0.0532)	6.38 (0.0512)
lesion No./cm^2		1.49	1.68	1.38	0.95
% lesion > 3mm		50.43	10.58	47.15	20.43
Internode length		16.57	17.20	24.98	16.44

DISCUSSION

Varietal effect on lesion development

The levels of infection obtained for Fortuna are rather lower than expected from previous work (Sharpe et al, 1972) however the other three cultivars emerge in the foreseen order; Bounty having overall higher levels and infection rates and Svenno the converse.

Compared with the other cultivars, Fortuna tends to show a greater increase in % infection at the end of the season, which may reflect a relatively greater increase in plant susceptibility at this time. The prevailing weather conditions, which were favourable at the end of the season, may be another factor in this apparent increase in susceptibility.

In the detailed study of infection it was evident that the lower level of infected tissue in B519 compared to Fortuna, was due, not to fewer lesions, but to a limitation in the size of those lesions. Thus B519, although not able to resist infection initiation, is able to a certain extent to restrict spread of the fungus within the leaf.

It should be noted that growth of the spring wheat was rather poor, owing to a late and then very dry spring. The resulting lack of plant vigour may affect the varietal reaction. In the winter wheat the vigorous crop of the second season tended to exhibit higher levels of resistance to infection.

The total amount of infection resulting from different inoculation dates paralleled that of the winter wheat; the early inoculation and control resulting in less infected tissue than the later ones. However, the pattern of infection development was slightly different in that there was an increase in % infection throughout the season with a sharper rise at the end.

The differential response of cultivar to time of inoculation, may be due to an interaction of the speed of maturing of varieties with the time of infection. (Pirson, 1960). An interaction of this kind was reported by Williams and Jones (1972) for the spring wheat cultivars Opal, Kolibri and Sterling. The effect here was largely due to differential response of infection of B519 and Fortuna to inoculation date. Bounty 208 was always more susceptible than Svenno. In general, for the early inoculation and control B519 ranks more susceptible than Fortuna; while in the later inoculations, particularly from 42 days after inoculation onwards, Fortuna becomes the most susceptible cultivar overall. The apparent greater susceptibility of B519 early in the season may be due to the relatively high 'r' values on the third leaves. In Fortuna relatively higher 'r' values occur on the flag leaf which would tend to cause higher % infection towards the end of the season.

In Fortuna the peduncle is relatively long and thus the ear is held high above the flag leaf. Under conditions of natural infection little inoculum passes up to the ear and the level of infection remains low. However, on direct inoculation of the ear the increase in lesion number and spread, compared to natural infection, is very marked. This may explain the interaction discussed above.

A direct correlation of the infection data with meteorological conditions is difficult to establish. The rise in % infection occurs at the end of the season in favourable weather conditions, but also when the state of plant resistance may be altered anyway. Of note is the low level of disease on Svenno inoculated at heading, which is considered a relatively susceptible stage; this may be a reflection of the dry conditions following removal of the inoculation bag.

The differential infection rates on various parts of the culm for the varieties may explain in part the differential yield effects of the pathogen. Thus in the high loss varieties the infection rate on the flag leaf and ear, which contribute mainly to filling the kernels, are higher than on the rest of the plant. The converse is true for the low loss cultivars. Thus yield loss may be related to the value of 'r' for the flag leaf and ear, rather than the % infection *per se*.

SECTION 3:

STUDIES ON PRE- AND POST-FORMED ANTIFUNGAL COMPOUNDS IN WINTER WHEAT

I. Studies on Pre and Post-formed Antifungal Compounds in Winter Wheat Resistant and Susceptible to S. nodorum

Research into the role of antifungal compounds in disease resistance of wheat has so far been confined largely to seedlings from 7-14 days old, (Elnaghy and Linko, 1962; Knott and Kumar, 1972; Morgan, 1974). In addition these authors were concerned almost exclusively with the part played by the benzoxazinone derivatives. That these compounds are active against S. nodorum has been demonstrated by Morgan (1974); however their presence in adult plants was difficult to establish, (Chigrin and Rozum, 1959) and their role in resistance remains an unanswered question.

The presence of pre-and post formed inhibitors of S. nodorum in crude and partially purified extracts of winter wheat tissues of varying ages.

In the following experiments plants from the glass-house and field were extracted by two methods at different stages of growth. Firstly tissues were plunged into boiling ethanol so inactivating enzymes and thus retaining the chemical regime of the intact plant with respect to enzyme activity and in particular hydrolysis. Alternatively tissues were macerated and left to stand for 3 hours at room temperature, so allowing maximum enzyme activity and in particular hydrolysis. The crude and partially purified extracts were then assayed against spores of S. nodorum in order to determine the presence of any active substances. The times of extraction were chosen arbitrarily but cover a number of stages of plant growth.

1. Healthy week old seedlings

7 day old seedlings of the cultivar Maris Huntsman, germinated on blotting paper in trays, were extracted by the method of maximal hydrolysis described in Materials and Methods H2. The alcoholic extract, adjusted to 60% ethanol was partitioned three times against petroleum ether

(40-60°C boiling range), and then used in the Standard Droplet bioassay with S. nodorum spores. (Materials and Methods K.6).

The mean % germination and mean germ tube length of germinated spores are given in Table 24.

TABLE 24 Growth of S. nodorum in nutrient solution containing alcohol soluble substances from maximally hydrolysed extracts of Maris Huntsman wheat seedlings

Dilution	Mean % germination	Mean germ tube Length µm + S.E.
Tissue conc. (1g f.w t./ml)	0	0
x ½	80	17 ± 0.8
x ¼	90	26 ± 1.3
x 1/8	100	34 ± 1.7
x 1/16	100	36 ± 1.7
x 1/32	100	91 ± 4.8
Control (Nutrient medium) only	100	89 ± 3.7

Thus healthy seedlings of the resistant cultivar contain substances which, upon hydrolysis, are completely inhibitory to S. nodorum at tissue concentration and below.

f.wt. = fresh weight

S.E. = Standard error of the mean

2. Healthy and inoculated 30 day old glass-house grown plants

Leaves of 24 day old plants of Maris Huntsman and Maris Ranger were detached, set up in plastic trays and inoculated with droplets of a 10^6 spores/ml suspension of S. nodorum as described in Materials and Methods, D. There were also uninoculated controls. Six days after inoculation the infected and control leaves of both cultivars were extracted by the method of maximal hydrolysis.

The total crude extract, ether soluble and water soluble substances (following partition, Materials and Methods J.1) were assayed using the Standard Droplet bioassay.

The results are given in Table 25 and 26.

TABLE 25 Growth of S. nodorum in nutrient solution containing total crude extract of maximally hydrolysed 30 day old plants, inoculated and control of Maris Huntsman and Maris Ranger

Dilution	Mean % germination				Mean germ tube length µm			
	MRI	MRC	MHI	MHC	MRI	MRC	MHI	MHC
Tissue conc. (1g f.w. t./ml)	63	57	0	40	25 ± 1.7	25 ± 2.2	0	15 ± 1.3
x ½	97	100	77	97	58 ± 4.4	82 ± 4.1	22 ± 2.1	37 ± 3.8
x ¼	100	100	-	100	122 ± 5.1	130 ± 6.0	-	73 ± 4.5
x 1/8	100	100	73	100	152 ± 6.9	164 ± 4.6	36 ± 3.2	123 ± 4.7
Control (nutrient medium only	100	100	100	100	173 ± 3.3	177 ± 4.3	115 ± 8.3	156 ± 6.8

Key MR - Maris Ranger
MH - Maris Huntsman
I - inoculated
C - uninoculated control.

(a) Total crude extract

(i) Healthy

From Table 25 it can be seen that healthy 30 day old tissues of both Maris Ranger and Maris Huntsman contain substances at tissue concentration, which upon hydrolysis cause about 50% inhibition to germination and 70-90% inhibition of germ tube growth. This inhibition is maintained at x $\frac{1}{4}$ tissue concentration in Maris Huntsman and x $\frac{1}{2}$ tissue concentration in Maris Ranger.

(ii) Infected

The infected tissue, on the other hand, was totally inhibitory at tissue concentration for the resistant cultivar, but the same as for the control in the susceptible variety (Table 25). Throughout the dilutions, for both cultivars, the infected tissue was more inhibitory upon hydrolysis than the control tissue similarly hydrolysed. This was more pronounced for the resistant cultivar in which at x $\frac{1}{8}$ tissue concentration of the infected tissue extract, germ tube length was still only 33% of the control.

TABLE 25 Growth of S. nodorum in nutrient solution containing ether soluble substances of maximally hydrolysed 30 day old plants, inoculated and control, of Maris Ranger and Maris Huntsman

Dilution	Mean % germination				Mean germ tube length µm			
	MRI	MRC	MHI	MHC	MRI	MRC	MHI	MHC
Tissue conc. (1g f.w.t. /ml)	27	40	0	57	16 ± 1.6	18 ± 1.8	0	16 ± 1.2
x ½	47	93	37	97	24 ± 2.0	51 ± 4.4	9 ± 1.0	62 ± 4.7
x ¼	90	100	90	100	65 ± 5.6	71 ± 5.2	30 ± 3.2	85 ± 5.1
x 1/8	100	97	90	100	88 ± 4.1	79 ± 4.3	72 ± 6.8	80 ± 6.8
Control (nutrient medium only	95	97	97	100	104 ± 3.6	108 ± 5.8	85 ± 5.5	90 ± 5.4

Key MR - Maris Ranger I - inoculated
MH - Maris Huntsman C - uninoculated control.

(b) Ether solubles

(i) Healthy

For uninoculated 30 day old leaves, the ether soluble substances of maximally hydrolysed tissue of both cultivars gave about 50% inhibition of germination and 84% inhibition of germ tube growth at tissue concentration, and to a lesser extent at half this value(Table 26). This was similar to or slightly less than the activity of the total crude extract, thus indicating some loss of activity on partitioning.

(ii) Infected

The inhibition of infected tissues was enhanced by ether partition at tissue concentration and $\times \frac{1}{2}$, but the effect was not apparent at dilutions above this, particularly for the susceptible cultivar (Table 26)

(c) Water soluble

The water soluble partition of the 30 day old, maximally hydrolysed extracts of inoculated and control tissue of both cultivars was not inhibitory to S. nodorum growth. The active substances are therefore all going into the ether fraction during partition.

3. Healthy and inoculated 7 week old glasshouse grown plants

The youngest fully expanded leaves of 7 weeks old plants for both Marie Huntsman and Maris Ranger were set up and inoculated as described in Materials and Methods, D. Extraction by both minimal and maximal hydrolysis was carried out six days after inoculation. Uninoculated controls were also extracted. Standard droplet bio-assays were set up using the total crude, alcohol, ether and water soluble substances for each variety control and the total crude, ether and water solubles for the infecteds.

The results are presented in Tables 27-29, except those for the minimally hydroloysed inoculated tissue extracts which were lost during experimentation.

TABLE 27 Growth of S. nodorum in distilled water containing the total crude, alcohol, ether and water soluble substances of minimally hydrolysed, 7 week old, healthy plants

	Cultivar	Maris Ranger					Maris Huntsman				
Partition	Dilution	x 2 Tissue conc.	Tissue conc.	x ½	x ¼	Control	x 2 Tissue conc.	Tissue conc.	x ½	x ¼	Control
Total crude	% Germination	83	93	100	97	100	100	100	100	100	100
	Germ tube length µm	63 ± 5.8	125 ± 14.1	112 ± 9.3	122 ± 11.9	69 ± 3.3	107 ± 8.6	101 ± 8.1	121 ± 9.6	158 ± 8.6	65 ± 2.9
Alcohol solubles	% Germination	90	93	100	100	90	0	0	70	90	91
	Germ tube length µm	105 ± 11.6	137 ± 12.0	171 ± 10.7	177 ± 9.3	84 ± 7.7	0	0	98 ± 5.1	170 ± 10.9	78 ± 6.9
Ether solubles	% Germination	80	100	100	100	90	83	87	90	100	100
	Germ tube length µm	31 ± 3.1	102 ± 6.4	105 ± 6.7	143 ± 8.6	76 ± 6.5	55 ± 5.5	103 ± 9.6	127 ± 8.3	133 ± 8.7	87 ± 5.4
Water solubles	% Germination	NO INHIBITION					NO INHIBITION				
	Germ tube length µm										

N.B. Control in distilled water

TABLE 28 Growth of S. nodorum in distilled water containing the total crude, alcohol ether and water soluble substances of maximally hydrolysed, 7 week old, healthy leaves

Cultivar		Maris Ranger					Maris Huntsman				
Partition	Dilution	x 2 Tissue conc.	Tissue conc.	x ½	x ¼	Control	x 2 Tissue conc.	Tissue conc.	x ½	x ¼	Control
Total crude	% Germination	43	83	86	90	90	100	80	90	100	100
	Germ tube length µm	12 ± 1.2	34 ± 2.5	105 ± 9.5	131 ± 10.9	92 ± 5.9	103 ± 9.6	68 ± 5.5	109 ± 7.7	158 ± 9.7	43 ± 3.7
Alcohol solubles	% Germination	0	63	80	90	90	0	33	63	90	90
	Germ tube length µm	0	48 ± 4.2	81 ± 7.5	133 ± 8.1	111 ± 6.6	0	22 ± 1.8	58 ± 5.2	139 ± 11.7	90 ± 6.8
Ether solubles	% Germination	0	20	90	100	100	40	90	90	-	100
	Germ tube length µm	0	28 ± 2.7	97 ± 5.4	108 ± 7.9	83 ± 5.3	58 ± 3.1	81 ± 8.1	97 ± 5.2	-	76 ± 4.6
Water Solubles		NO I N H I B I T I O N					NO I N H I B I T I O N				

N.B. Control in distilled water

TABLE 29 Growth of S. nodorum in nutrient medium containing the total crude, ether and water soluble substances of maximally hydroylsed 7 week old, inoculated and control plants

Cultivar		Maris Ranger					Maris Huntsman				
Partition		Tissue conc.	× ½	× ¼	× 1/8	Control	Tissue conc.	× ½	× ¼	× 1/8	Control
Infected Total crude	% Germination	63	97	100	100	100	0	77	-	73	100
	Germ tube length µm	26 ± 1.6	57 ± 1.9	122 ± 5.0	154 ± 4.7	173 ± 3.3	0	21 ± 1.9	-	36 ± 3.1	117 ± 7.4
Infected Ether solublee	% Germination	27	47	90	100	95	0	37	90	90	97
	Germ tube length µm	15 ± 1.2	24 ± 2.4	65 ± 4.0	88 ± 3.8	104 ± 5.4	0	8.3 ± 0.8	30 ± 3.1	72 ± 6.6	85 ± 5.4
Infected water solubles	% Germination	NO I N H I B I T I O N					NO I N H I B I T I O N				
	Germ tube length µm										

N.B. Control in nutrient medium

Cont'd .....

TABLE 29 (Continued) Growth of S. nodorum in nutrient medium containing the total crude, ether and water soluble substances of maximally hydroylsed 7 week old, inoculated and control plants

Infection	Cultivar	Maris Ranger					Maris Huntsman				
Partition		Tissue conc.	× ½	× ¼	× 1/8	Control	Tissue conc.	× ½	× ¼	× 1/8	Control
Control Total crude	% Germination	57	100	100	100	100	40	97	100	100	100
	Germ tube length µm	25 ± 2.1	82 ± 4.0	124 ± 5.8	163 ± 5.2	177 ± 5.1	16 ± 3.8	37 ± 3.7	73 ± 4.5	123 ± 4.5	157 ± 6.4
Control Ether solubles	% Germination	40	93	100	97	97	58	97	100	100	100
	Germ tube length µm	17 ± 1.3	51 ± 2.8	71 ± 4.2	79 ± 4.1	107 ± 3.9	16 ± 1.1	62 ± 4.7	85 ± 5.0	86 ± 5.6	90 ± 5.3
Control water solubles	% Germination	NO I N H I B I T I O N					NO I N H I B I T I O N				
	Germ tube length µm										

N.B. Control in nutrient medium.

Effect of extraction method on inhibition

The method of extraction appears to have little effect on inhibitory substances of Maris Huntsman, while in Maris Ranger, maximal hydrolysis gives more activity than minimal. (Tables 25 and 26).

Solubility of inhibitory material

In all cases the inhibitory material goes into the ether and not the water partition. The inhibitory affect is enhanced in most cases after petroleum ether partition, except for the minimal hydrolysis, Maris Ranger extract, where all inhibition is lost.

Effect of infection on inhibition

The infected tissue extracts, particularly for Maris Huntsman show higher levels of inhibition at the x $\frac{1}{4}$ and x 1/8 dilutions over the uninoculated control tissue extracts.

The rather low values for the controls, arising from the fact that distilled water and not nutrient medium was used, tends to diminish the apparent effect of the inhibitor. At the high dilutions of the extracts the stimulatory effect of nutrients present overcomes possible inhibition. Thus when estimating % inhibition it is better to compare the low dilutions with the highest dilution, rather than the control.

Effect of variety on inhibition

Overall Maris Huntsman tissue extracts are more inhibitory than the corresponding Maris Ranger ones.

4. Healthy, Naturally Infected and Inoculated field grown material of 6 month old wheat

The youngest fully expanded second leaves, and naturally infected lower leaves of the cultivars Maris Ranger and Maris Huntsman were excised from 6 month old field grown plants (in April) and extracted by the method of minimal hydrolysis. Further, youngest fully expanded leaves of both cultivars were excised and set up in plastic trays for inoculation with S. nodorum as described in Materials and Methods, D. Five days later the leaves, inoculated and uninoculated controls were extracted by the method of minimal hydrolysis. Standard droplet bioassays were set up using the total extract in 60% alcohol following petroleum ether partition. The results of these bioassays are summarised in Tables 30-33.

TABLE 30 Growth of S. nodorum in nutrient medium containing the 60% alcohol soluble substances of minimally hydrolysed, healthy, youngest fully expanded leaves of 6 month old field grown plants.

Dilution	Mean % germination		Mean germ tube length µm	
	M. Ranger	M. Hunteman	M. Ranger	M. Hunteman
x 2 Tissue conc.	53	13	14 ± 1.5	45 ± 3.8
Tissue conc. (1g f.w. t./ml)	90	60	96 ± 4.7	57 ± 3.9
x ½	97	73	178 ± 4.4	90 ± 4.3
x ¼	100	87	185 ± 5.3	112 ± 5.0
Control in nutrient medium	100	97	171 ± 8.2	87 ± 7.0

Table 30 shows that both cultivars contain substances at tissue concentration active against S. nodorum. The resistant cultivar has a markedly higher inhibitory effect on germination compared to the susceptible one.

TABLE 31 Growth of S. nodorum in nutrient medium containing the 60% alcohol soluble substances of minimally hydrolysed healthy 2nd leaves of 6 month old field grown plants

Dilution	Mean % germination		Mean germ tube length µm	
	M. Ranger	M. Huntsman	M. Ranger	M. Huntsman
x Tissue conc.	77	77	55 ± 4.3	71 ± 5.0
Tissue conc 1g f.w t./ml	90	97	86 ± 5.8	116 ± 6.0
x ½	97	97	132 ± 6.5	162 ± 7.7
x ¼	93	93	155 ± 8.7	169 ± 7.8
Control in nutrient medium	97	97	111 ± 6.2	124 ± 8.9

From Table 31 it is apparent that in second leaves the susceptible cultivar, Maris Ranger, appears to contain slightly more activity against S. nodorum than does Maris Huntsman. However, in both cultivars activity is less than for the younger leaves (see Table 30).

TABLE 32 Growth of S. nodorum in nutrient medium containing the 60% aocohol soluble substances of minimally hydrolysed, naturally infected, 4th and 5th leaves of 6 month old field grown plants

Dilution	Mean % germination		Mean germ tube length µm	
	M. Ranger	M. Huntsman	M. Ranger	M. Huntsman
x 2 Tissue conc^{n}	67	30	23 ± 1.7	28 ± 4.1
Tissue conc^{n} lg f.w. t./ml	83	60	37 ± 2.0	44 ± 5.3
x ½	90	80	48 ± 3.6	49 ± 5.1
x ¼	97	87	54 ± 4.2	66 ± 5.6
Control nutrient medium	90	90	70 ± 6.5	58 ± 4.5

It is evident in Table 32 that Maris Huntsman is causing a greater inhibitory effect on germination, while Maris Ranger has a slightly greater effect on germ tube length. In both cultivars the effect is slightly greater than for the uninoculated 2nd leaves (Tablee 30 and 31) excepting Maris Ranger, youngest fully expanded leaves (Table 30) which has the highest level of inhibition of germ tube length.

TABLE 33. Growth of S. nodorum in nutrient medium containing the 60% aocohol soluble substances of minimally hydrolysed youngest leaves of 6 month old field grown plants 5 days after inoculation

Dilution	Mean % germination				Mean germ tube length µm			
	MRI	MRC	MHI	MHC	MRI	MRC	MHI	MHC
Tissue conc^{n} lg f.w t./ml	93	67	77	73	83 ± 6.9	91 ± 5.8	39 ± 3.7	39 ± 3.8
x ½	93	100	90	90	75 ± 4.9	92 ± 5.9	86 ± 7.9	88 ± 7.7
x ¼	93	100	100	93	110 ± 5.4	108 ± 6.0	105 ± 6.3	73 ± 5.2
Control nutrient medium	97	100	93	97	96 ± 6.6	101 ± 4.9	105 ± 6.2	101 ± 6.8

Key MR - Maris Ranger I - inoculated

MH - Maris Huntsman C - uninoculated control

In comparing Tables 32 and 33 it can be seen that the artificially inoculated tissue is slightly more inhibitory than the naturally infected. Activity of the inoculated tissue against S. nodorum is similar to that of the uninoculated control and activity is greater for Maris Huntsman.

A summary of Tables 24-33 - S. nodorum inhibition by crude and partially purified extracts of healthy and infected, maximally and minimally hydrolysed wheat tissue of varying ages, is given in Table 34. The relative activities of the various extracts are represented in order to give a simple visual comparison.

TABLE 34

Summary of S. nodorum inhibition by crude or partially purified extracts of healthy and infected maximally and minimally hydrolysed wheat tissue at varying ages

Age	Infection	Hydrolysis	Partition	Cultivar inhibition	
				Maris Huntsman	Maris Ranger
7 Days	H	M	AS	++++	-
30 "	E	A	TC	+++	+++
	A	X	ES	+++	+++
7 week	L	I	TC	++	++
	T	M	1) AS	+++	++
	H	U	ES	+	++
	Y	M	2) TC	+++	+++
			ES	+++	+++
	═══				
30 days	1		TC	++++	+++
	N		ES	++++	+++
7 week	F		TC	++++	+++
	E		ES	++++	+++
	C				
	T				
	E				
	D				

Cont'd.....

TABLE 34 (Continued)

Summary of S. nodorum inhibition by crude or partially purified extracts of healthy and infected maximally and minimally hydrolysed wheat tissue of varying ages

Age	Infection	Hydrolysis	Partition	Cultivar inhibition	
				Maris Huntsman	Maris Ranger
7 week	H	M	TC	+	0
	E	I	AS	++++	+
	A	N	ES	+	+
6 month young leaves	L	I	AS	+	+
	T	M			
Older leaves	H	U	AS	+	+
	Y	M			
Young leaves (detached 5 days)			AS	++	+
6 month	Naturally Infected		AS	+	+
	Artificially Inoculated		AS	++	+

Key ++++ 100% inhibition of germ tube length
+++ 75-00% " " " " "
++ 50-74 % " " " " "
+ < 50% " " " " "

TC - Total crude extract
AS - Alcohol soluble substances
ES - Ether soluble substances.

Summary

Effect of extraction method on activity of extracts

Overall the tissue extracts which were allowed to hydrolyse contain the highest levels of inhibition - generally over 75% inhibition of germ tube length at tissue concentration. One exception is the minimally hydrolysed, alcohol solubles extract of healthy 7 week old Maris Huntsman leaves, which is totally inhibitory. The total crude and ether soluble fractions of this extract were however only slightly inhibitory.

Effect of infection

For the maximally hydrolysed extracts, infection of tissue leads to slightly higher levels of inhibition. In minimally hydrolysed extracts, however, there is only slightly greater activity in infected tissue compared to the uninoculated healthy tissue.

Effect of variety and age of tissue

Maris Huntsman, the resistant cultivar has slightly higher levels of inhibition compared to Maris Ranger. Age of tissue appears to have little effect on the activity of extracts.

II. Chromatographic separation and assay of pre-and post-formed antifungal compounds in wheat

In the preceeding pages the presence of antifungal compounds in wheat tissue has been established. The next step was to attempt to purify and characterise the compounds by thin-layer chromatography and UV spectral analysis.

In locating antifungal activity on chromatograms an assay technique is required that should be relatively fast to minimise poasible decomposition of compounds. One method of location is the direct growth of the fungus on the developed chromatogram. However, the lack of pigment in S. nodorum which makes visualisation difficult and moreover, the relatively slow growth of the fungus, render it unsuitable in direct assay of chromatograms. The agar imprint bioassay of Morgan (1974) is open to errors from differential diffusion of compounds and in addition the slow growth of S. nodorum maximises possible chemical breakdown.

It was thus decided to utilise the sensitive non-pathogen, Cladosporium sp, whose fast growth and dark green pigmentation makes visualisation of assayed chromatograms relatively quick and simple. Strips of developed chromatograms can be assayed with Cladosporium while the remainders are stored in the deep freeze to minimise chemical decomposition. These can then be eluted for spectral analyses and Standard droplet bioassay with S. nodorum.

1. Preformed inhibitors present in healthy intact wheat plants

In using the extraction method in boiling alcohol the chemical regime with respect to enzyme activity in the intact leaf will be maintained, and it should then be possible to determine the presence of compounds which are immediately antifungal.

The boiling of tissue used in the extraction method may inactivate inhibitory substances which are not thermostable, although, the inhibitory substances obtained from the method of maximal hydrolysis are stable after boiling for 5 mins. However, it cannot be assumed that the possible inhibitory compounds in the tissue are thermostable and extraction at temperatures below which enzyme activity is nil would be necessary to confirm this.

Healthy 1-3 week old field grown plants of Maris Ranger and Maris Huntsman were extracted by the method of minimal hydrolysis. The ether and water soluble substances after partition against petroleum ether were chromatogrammed at 1g f.w t./cm, and run in 5% methanol in chloroform. Following development, strips were cut from chromatograms and Cladosporium assays set up. (Materials and Methods, H, J and K).

In all cases the water soluble substances were rather gummy and did not run well if at all. Changing the solvent system to a more polar mixture did not help in the separation of those water soluble substances.

The Cladosporium assays are summarised in Table 35.

TABLE 35 Inhibition of Cladosporium on chromatograms of the ether and water soluble substances of minimally hydrolysed healthy plant extracts

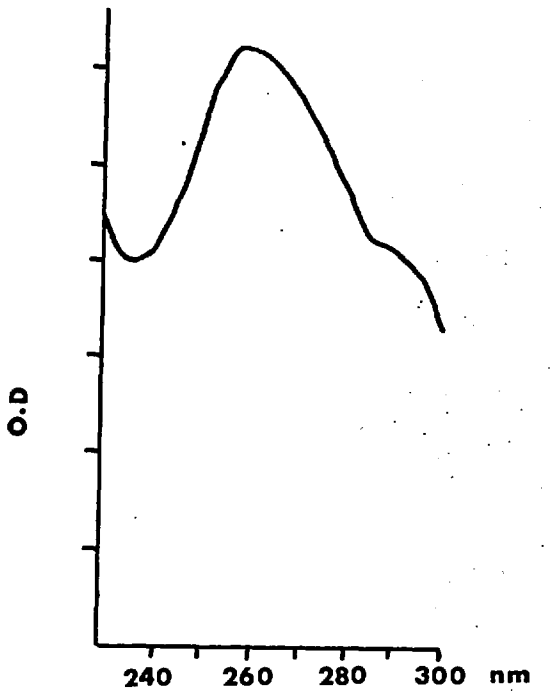
Age	Water Soluble		Ether soluble	
(Weeks)	MR	MH	MR	MH
1	None	+	None	None
2	+	+	None	None
3	+	++	None	None

+ represents rel.amount of inhibition

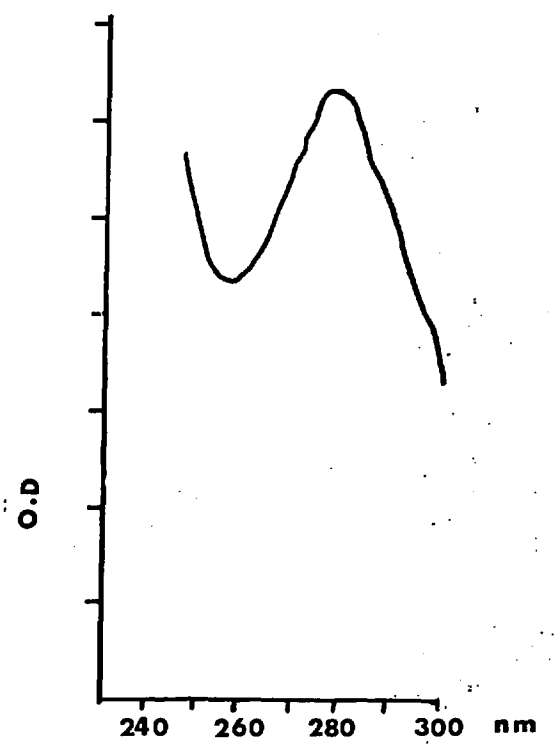
In all cases the inhibition occurred at the origin and it constituted an area of less dense growth, rather than complete inhibition. There was no inhibitory material in the ether soluble partition. The presence of the benzoxazinone glucoside in both cultivars was confirmed for week 1 by a blue reaction with $F_e Cl_3$ spray at the water soluble origin. There was no positive colour reaction in the subsequent weeks.

Figure 33:- UV spectra in ethanol of antifungal substances from 1 week old wheat leaves

a - $FeCl_3$ + band R_F 0·63



b - R_F 0·8 - 1·0



2. The Potential antifungal compounds present in healthy wheat plants

The extraction of plants by maximal hydrolysis gives some estimate of the potential of antifungal compounds within the healthy intact plant, which might be released upon infection.

Glasshouse grown plants of the cultivars Maris Huntsman and Maris Ranger were extracted by the method of maximal hydrolysis each week for 5 weeks, after planting. Both the ether and water soluble substances were chromatogrammed, run in 5% methanol in chloroform and Cladosporium assays set up as described in Marerials and Methods, HJ and K.

As in the previous experiment the water soluble substances were almost impossible to chromatogram and develop owing to their gummy nature. In all cases they proved almost totally inhibitory at the origin, but stimulated growth to a large extent around this area. (See Plate 11). This inhibition increased with time and was generally greater for the resistant cultivar.

For the ether soluble substances (also shown in Plate 11) there was an inhibitory zone behind the solvent front, which occurred at all times. In addition, for the week 1 extraction there was a narrow pink pigmented band at R_F 0.63, which possessed some antifungal activity. On spraying with ferric chloride spray this band turned violet-blue indicating the presence of a benzoxazinone derivative.

Both the ether soluble inhibitory zones for week 1 were eluted, their UV spectra obtained (Fig. 33) and Standard droplet assays set up with S. nodorum spores (Table 36).

The positive ferric chloride reaction and the characteristic UV spectrum of (a) Fig. 33 confirm that it is the benzoxazolinone aglucone.

Plate 11:- Cladosporium assay of ether and water soluble substances from 5 week old glasshouse grown plants of wheat



TABLE 36 Growth of S. nodorum in nutrient solution containing the two ether soluble Cladosporium inhibitors from week old wheat plants

Dilution	Mean % germination		Mean germ tube length µm	
Inhibitor R_F	0.63	0.8-1.0	0.63	0.8 - 1.0
x 2 Tissue conc^n	43	0	20 ± 1.9	0
Tissue conc^n 1g f. wt. /ml	57	1	16 ± 1.7	9 ± 1.8
x ½	93	0	56 ± 3.4	0
x ¼	90	60	72 ± 3.8	24 ± 2.9
x 1/8	87	-	85 ± 4.7	-
Control nutrient medium	83	90	79 ± 3.6	83 ± 5.7

Thus, both zones of inhibition from the week 1 extract, that of the benzoxazinone aglucone and the compound(s) behind the solvent front, are considerably active at tissue concentration against S. nodorum.

In the extracts from 2-5 week old plants there was no positive blue colour reaction with $FeCl_3$ at R_F 0.63, which indicated the absence of the benzoxazolinone derivative. However the R_F corresponding to the benzoxazinone aglucone was eluted. The UV spectrum had become a single peak, and the antifungal activity was lost. The activity behind the solvent front persisted for the duration of the experiment. In order to determine if this band was more than one compound the ether soluble substances were run in different solvents. The R_F values obtained are listed in Table 37.

TABLE 37 R_F Values of ether soluble inhibitors from 2 and 3 week old maximally hydrolysed wheat plants

Solvent system	R_F
5% methanol in chloroform	0.80 - 1.00
10% " "	0.50 - 0.85
10% Diethyl ether in petroleum ether	origin
50% chloroform-acetone	0.55 - 0.85
50% cyclohexane-ethyl acetate	0.40 - 0.60

The chloroform-acetone (50:50) system gave the better separation; although the zone was continuous it appeared to be composed of 3-4 bands. (Plate 12) R_F 0.59, 0.69 and 0.76 approximately. These bands will be referred to as A, B, C respectively.

A, B and C from the ether solubles were eluted, as was the water soluble inhibitory zone, UV spectra obtained and S. nodorum assays set up.

The UV spectra in ethanol are presented in Fig. 34.

The spore droplet assay results of the eluates A, B and C are given in Table 38.

Plate 12:- Antifungal zones on Cladosporium assay of ether soluble substances from 2-3 week old plants of wheat cultivars Maris Ranger and Maris Huntsman, grown in the glasshouse

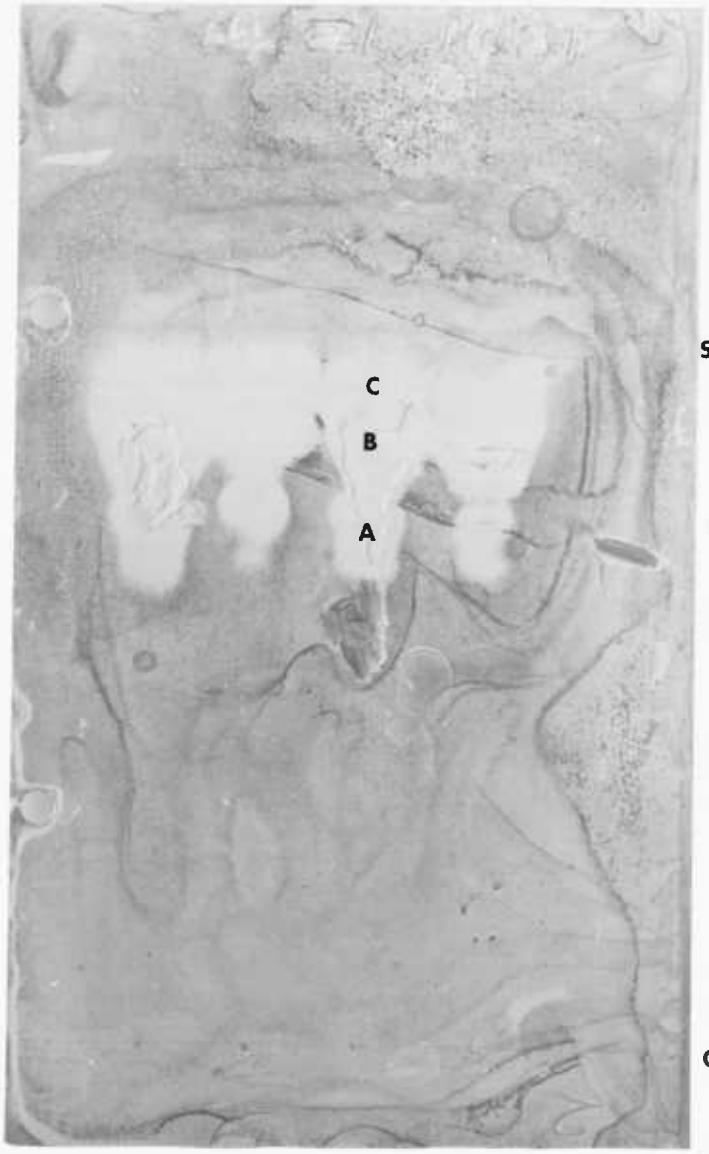
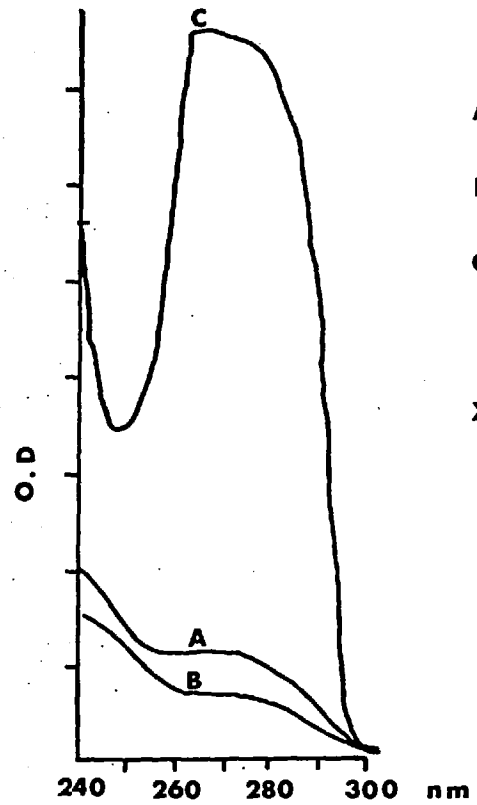


Figure 34:- UV spectra in ethanol of inhibitory areas from ether & water solubles of maximally hydrolysed, healthy, 2-3 week old plants

a) ETHER SOLUBLES



R_F VALUES (50:50 clfm:actn)

A = 0·59

B = 0·69

C = 0·76

X = ORIGIN

b) WATER SOLUBLES

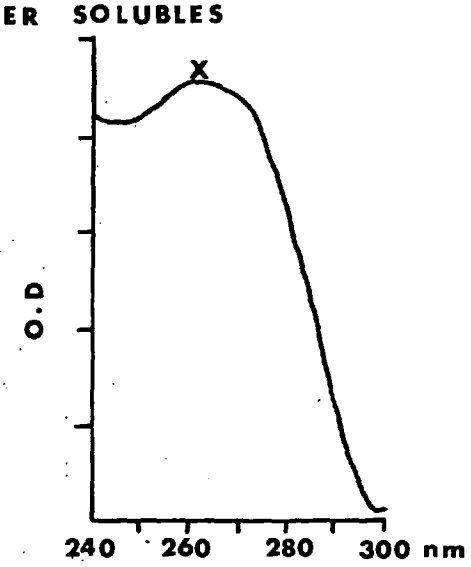


TABLE 38 Growth of S. nodorum in nutrient medium containing ether soluble inhibitors from maximally hydrolysed 2-3 week old, healthy wheat plants

	Mean % germination			Mean germ tube length µm		
Dilution	A	B	C	A	B	C
Tissue conc^{n} lg f.w.t./ml	90	90	0	45 ± 4.3	66 ± 5.1	0
x ½	90	90	0	66 ± 5.2	93 ± 8.3	0
x ¼	-	-	80	-	-	29 ± 5.1
x 1/8	-	-	80	-	-	48 ± 3.5
Control nutrient medium	100	90	93	79 ± 7.6	88 ±12.5	88 ± 5.8

There was no inhibition of S. nodorum by the Cladosporium inhibitory substance(s) eluted from the water solubles origin. The UV spectrum of this substance(s) is given in Fig. 34 (b) - it has only a broad peak around 260nm.

It was noticeable in the S. nodorum assay that for zone C many of the spores had produced two germ tubes, while for zone A there was comparatively more branching of the germ tubes.

From Table 38 it is evident that the band with the highest R_F, C, is most inhibitory to S. nodorum growth. This may be a reflection of the higher concentration evident from the UV spectrum (Fig. 34).

3. Antifungal compounds in artificially inoculated wheat plants resistant and susceptible to S. nodorum

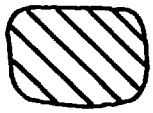
The youngest fully expanded leaves of field grown plants of Maris Ranger and Maris Huntsman, 22, 29 and 43 days old, were detached and inoculated as described in Materials and Methods D. Uninoculated controls were also set up. The leaf tissue was extracted 7 days after inoculation by minimal and maximal hydrolysis. The former method enabled the amount of hydrolysis due to infection to be estimated, while the latter gave an estimate of the pool of potentially antifungal compounds present. The diethyl ether soluble fractions for the different times were bulked, loaded at 1g f.w.t./ml and run in 10% chloroform in methanol and 50% cyclohexane in ethylacetate. Cladosporium assays were set up on the developed chromatograms. In view of the difficulty of application and development and the lack of activity of the water soluble substances it was decided not to chromatogram them.

The results of the Cladosporium assays are represented diagrammatically in Fig. 35. Fig. 35(a) shows that under minimal hydrolysis, for both varieties, inoculated and controls, there was an inhabitory zone occurring at R_F 0.42 - 0.55 in 10% chloroform in methanol. This band will be referred to as A. In 50% cyclohexane/ethyl acetate the band split apparently into 2 for the Maris Ranger infected extract, R_F 0.09 and 0.18. The Maris Huntsman control had one band at 0.18, while the inoculated and Maris Ranger control had one band at 0.09.

For the maximal hydrolyses the Maris Ranger infected extract gave inhibitory zones corresponding to those for minimal above (Fig. 36). The remaining 3 extracts, however gave inhibitory zones at R_F 0.75 - 0.91 in 10% chloroform in methanol and R_F 0.41 - 0.65 in 50% cyclohexane/ethyl acetate. These bands will be referred to as C.

KEY TO FIGURE 35

POSITIONS OF INHIBITORY AREAS IN

 = 10:90 METHANOL : CHLOROFORM

 = 50:50 CYCLOHEXANE : ETHYL ACETATE

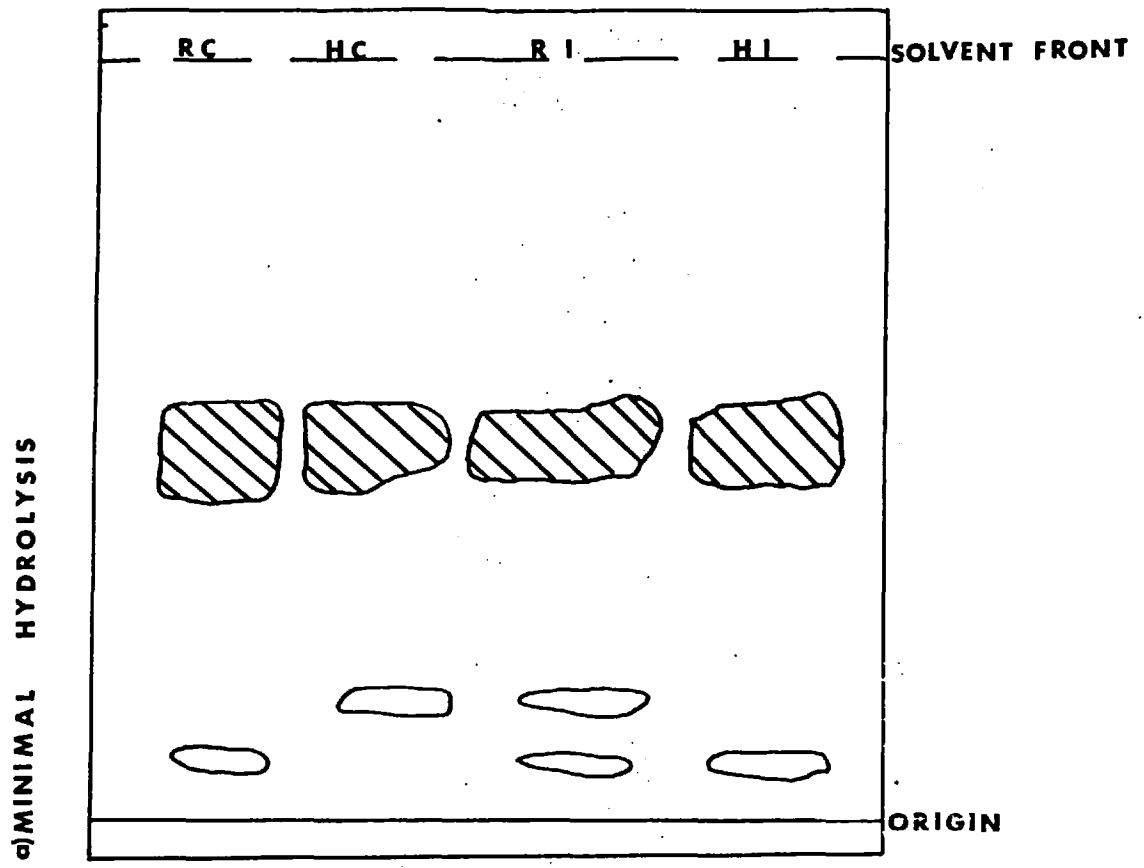
R = MARIS RANGER

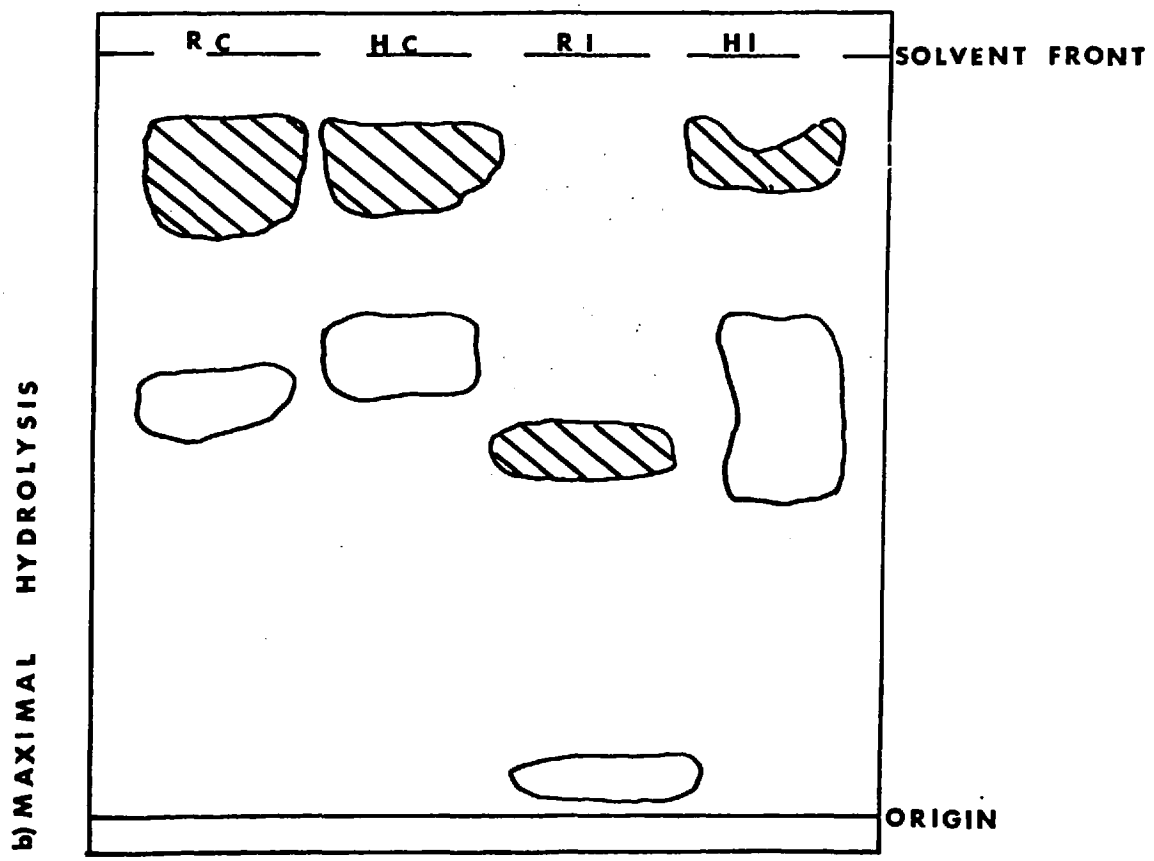
H = MARIS HUNTSMAN

C = CONTROL

I = INOCULATED

Figure 35:- Diagram of Cladosporium assay on chromatograms of ether soluble substances from inoculated & uninfected leaves, 4-7 weeks old



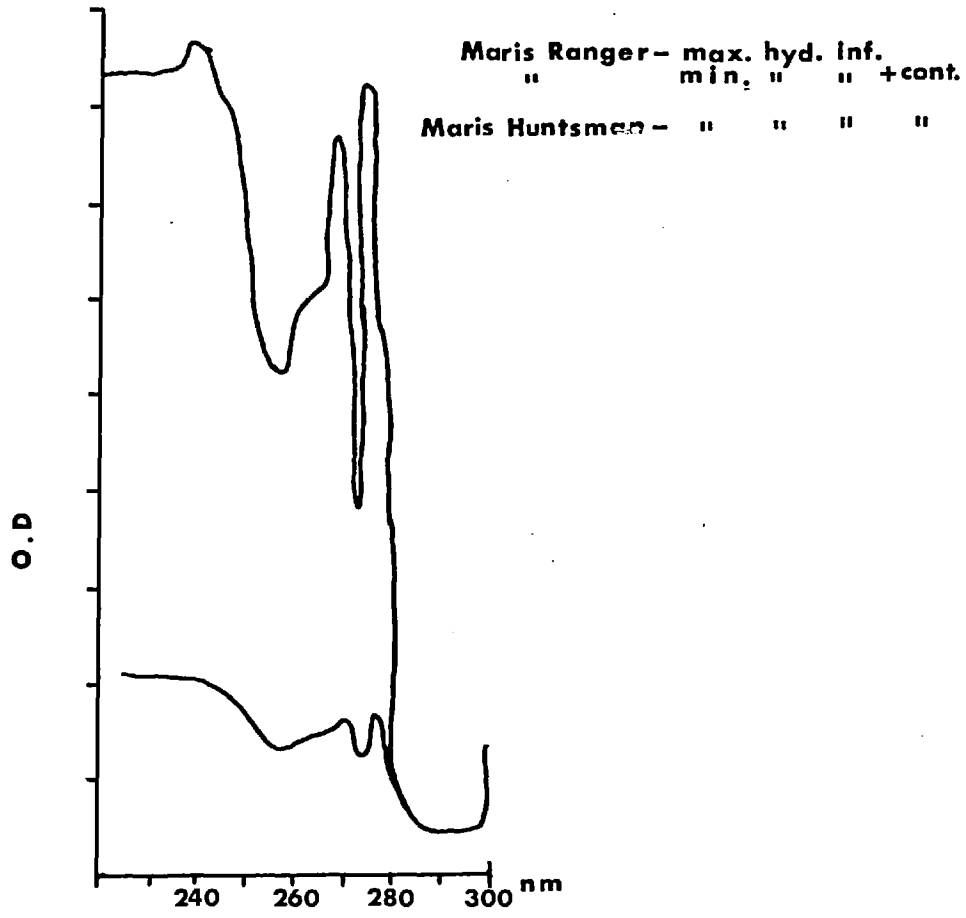


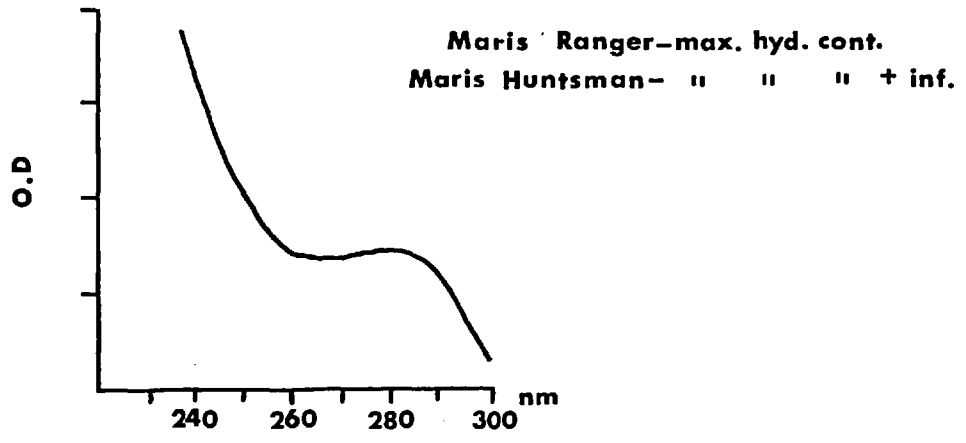
Thus in the intact plant, when the chemical regime is maintained by eliminating enzymic activity,there is no apparent difference between cultivars whether inoculated or not; they all contain the inhibitory zone A. However on maximal hydrolysis Maris Ranger infected tissue still contains zone A, while for the other extracts the inhibitory areas correspond to C. This anomaly of the maximally hydrolysed Maris Ranger extract cannot readily be explained.

The inhibitory areas A and C were eluted and the UV spectra obtained are shown in Fig. 36.

The R_F values for these substances correspond to zones A and C respectively from the preceeding experiment, although the UV spectrum for A is different.

Figure 36:- UV spectra of inhibitory zones in inoculated & control leaves of field grown wheat





The inhibitory zone A giving the well defined double peak was bulked and part re-run in ethyl acetate: cyclohexane. Under UV light there was a violet band corresponding to the origin where the compound occurred. The origin was eluted and a further UV spectrum obtained. This gave the same double peak showing relative stability of the compound(s).

Other portions of the bulked inhibitory zone were run in the following solvents.

(i) Butanol:acetic acid:water (BAW) 4:1:1

(ii) Chloroform:acetone (CA) 50:50

(iii) Chloroform:methanol (CM) 90:10 (check)

The chromatograms were observed under UV light and the violet UV absorbing area marked with pencil. The developed chromatograms were then sprayed with alkaline potassium permanganate in order to possibly characterise the compound.

In

(i) BAW a yellow spot appeared ahead of the UV band at R_F 0.75

(ii) CA " " " behind at R_F 0.20

(iii) CM the " and UV band coincided at R_F 0.50

A further chromatogram was run in (i) BAW and the UV band and 'yellow spot' area eluted and UV spectra obtained. The former still the characteristic double peak while the latter gave only a gradual rise with no obvious peaks.

TABLE 39

Summary of chromatographic separation of pre- and post-formed antifungal compounds in winter wheat (G.S. 1-4)

Growth stage	Infection	Hydrolysis	Part	Cultivar + Zone inhibition	
				Maris Huntsman	Maris Ranger
1-2	HEALTHY	Min	seedling just forming tillers	None	None
1-3	HEALTHY	Max	" and 3 tillers	ABC	ABC
2-4	AHEAD INFECTION + NOCHY	Min	" up to 4 strong tillers	A	A
2-4	AHEAD INFECTION + NOCHY	Max	"	C	C(A)

Zone A - R_F 0.59)
" C - R_F 0.76) in chloroform :acetone 50:50

From the experiments so far, summarised in Table 39 above, there do not appear to be any immediately available antifungal compounds in healthy plants up to 3 weeks old. However there are substances, which upon infection, or under maximal enzyme activity, become active.

4. Antifungal compounds at various growth stages in healthy and naturally infected field grown winter wheat plants

This experiment was set up to determine the presence and relative levels of antifungal compounds throughout the season in field grown plants resistant and susceptible to S. nodorum, and if possible to evaluate their importance in adult plant resistance in the field.

Plant material was collected from the 1975 winter wheat trial planted in October, and details of which are given in Materials and Methods L.

Material of Maris Huntsman and Maris Ranger, at the following growth stages was extracted by the methods of minimal and maximal hydrolysis:-

Growth stage	Parts extracted	Infection
6-7 Stem extension	(i) youngest fully expanded leaf (2nd)	uninfected
	(ii) next youngest fully expanded leaf (3rd)	"
	(iii) basal leaves (4th x 5th)	infected
10 in boot	(i) flag leaves & sheath	infected & uninfected
	(ii) unemerged ear	uninfected
11 ripening	(i) flag leaves	infected
	(ii) 2nd leaves	"
	(iii) ears	"

The diethyl ether soluble substances in all cases were chromatogrammed at 1g f.wt/cm and run in 10% methanol in chloroform. Cladosporium assays were set up on strips while the remainders of chromatograms were stored in the deep freeze. As soon as any inhibition was visible on the assay strips, usually after 2-3 days, the corresponding areas on the stored chromatograms were eluted, UV spectra recorded and Standard droplet assays set up using S. nodorum spores. (Materials and Methods, H, J, and K.)

The Cladosporium assay results are summarised in Table 40. Although it may be a gross over-simplification, the inhibitory zones have been divided into 3 bands occurring at R_F 0.50 - 0.60 = A 0.62 - 0.73 = B and 0.74 - 0.83 = C. Thus the bands B and C were continuous, while A was very definitely separate and behind. Plate 13 shows the zones A - C for infected flag leaves of Maris Huntsman at ripening.

Plate 13:- Antifungal zones on Cladosporium assay of ether soluble substances from flag leaves of field grown wheat at G.S. 10.5

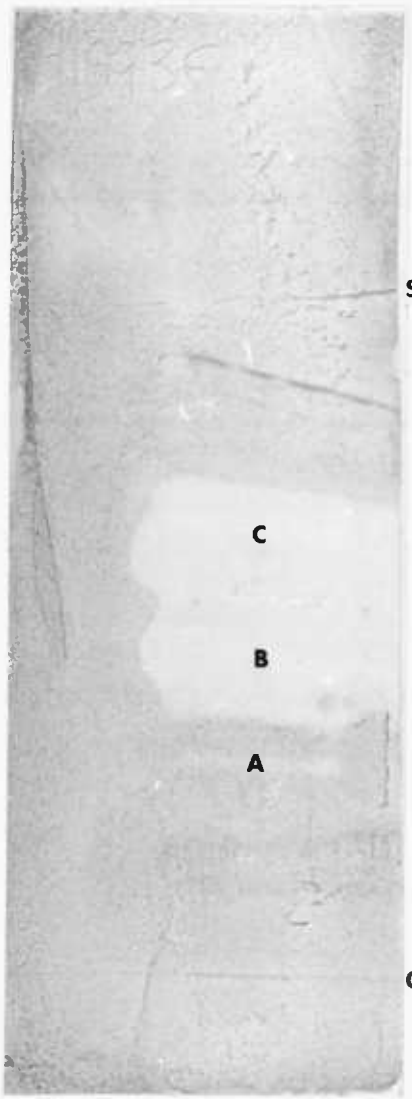


TABLE 40 Cladosporium inhibition by ether soluble substances from Minimally and maximally hydrolysed, healthy and infected field grown winter wheat at various growth stages

G.S.	Infection	Hydrolysis	Part of culm	Cultivar and Inhibitors	
				Maris Huntsman	Maris Ranger
6-7	NO	M	2nd leaf	(A)	None
10	INFECTION	I N I M U M	Flag	None	None
10			Ear unemerged	None	None
10			Flag proximal portion of infected leaf	None	-
11			Flag	None	None
11			Ear	B	None
6-7	70%	M	4th/5th leaves	None	None
10	30%	I	Flag	(C)	(A)
11	50%	N	Flag	(A)	(A)
11	50%	I M U M	Ear	(B)	(B)(C)

Key Inhibitory R_F in 10% methanol in chloroform

A 0.58 - 0.61

B 0.62 - 0.73

C 0.74 - 0.83

Letters in parenthesis indicate only trace of inhibition on chromatogram.

Cont'd.....

TABLE 40 (Continued)

Cladosporium inhibition by ether soluble substances from minimally and maximally hydrolysed, healthy and infected field grown winter wheat at various growth stages

G.S.	Infection	Hydrolysis	Part of culm	Cultivar & Inhibitors	
				Maris Huntsman	Maris Ranger
10	NO	M	Flag	A B	A B
10	I	A	Flag	B (c)	-
	N	X	proximal		
	F	I	portion		
	E	M	of infected		
	C	U	leaf		
10	T	M	Ear unemerged	(C)	(C)
11	I		Flag	B C	B C
11	O		Ear	A B C	A B C
	N				
6-7	70%	M	4th and 5th leaves	C	C
10	30%	A	Flag	(A) B C	(A) B C
11	50%	X	Flag	A B C	A B C
11	50%	I	Ear	A B C	A B C
		M			
		U			
		M			

Key

Inhibitory R_F in 10% methanol in chloroform

A 0.58 - 0.61

B 0.62 - 0.73

C 0.74 - 0.83

Letters in parenthesis indicate only trace of inhibition on chromatogram

From Table 40 it can be seen that in general the inhibitory zones are the same for both cultivare, there being no obvious qualitative difference except for healthy minimally hydrolysed ears of Huntsman which contain an inhibitor(s) which is not present on the corresponding Maris Ranger chromatogram.

On the whole, healthy minimally hydrolysed leaves contain no inhibitory compounds, while the corresponding infected tissues have some activity corresponding to zones A and C. This suggests that A and C are hydrolysed upon infection of leaves. The minimally hydrolysed, infected ears contain B in addition to some C.

As expected from previous experiments the maximally hydrolyeed healthy tissues contain relatively more inhibition corresponding to zones B and C, with some A. The infected maximally hydrolysed tissues contain all three compounds A, B and C.

Thus compound(s) A does not occur free in intact tissues but is a product of hydrolysis, released when leaves become infected. The relatively lower levels of A for healthy maximally hydrolysed leaves tends to indicate that A is hydrolysed by fungal, or host-induced enzymes rather than by plant enzymes in macerated tissues.

B appears in maximally hydrolysed leaf extracts only and is thus a product of host enzymic hydrolysis and not due to the fungal enzymes. B does occur in minimally hydrolysed infected ears suggesting that the fungus can <u>per se</u>, or by host induction, hydrolyse the precurser in this tissue.

C, like A, is a product of hydrolysis released upon infection and tissue maceration of both ears and leaves. In maximum hydrolysed unemerged ears only traces of C are present, while after emergence all three bands are present. For the flag leaves,A and B are present at G.S.10 and then B and C at G.S. 11. Thus there are qualitative differences in the compounds at varying ages.

The Standard droplet bioassay results for the eluted Cladosporium inhibitors with activity are summarised in Table 41. Eluates which were not active are omitted. The corresponding band (Table 40) is included in brackets.

The area of inhibition on the chromatogram is directly proportional to the inhibition of S. nodorum germ tube growth (except for maximally hydrolysed, infected Maris Huntsman flag leaves at G.S. 11 and minimally hydrolysed, infected Maris Ranger ears at G.S.11.) Thus maximally hydrolysed and infected tissue extracts are more inhibitory than minimally hydrolysed and uninfected tissues. Activity is slightly greater for Maris Huntsman except for maximally hydrolysed infected flags and minimally hydrolysed infected ears, both at flowering.

The three zones A - C all exhibit some activity. Their effects are not additive and the relatively large amount of inhibition when all three are assayed together suggests a synergistic effect.

Germination was not on the whole greatly affected; inhibition being less than 30% for the resistant cultivar. The % germination and actual germ tube lengths for all assays are presented in the Appendix 3. Tables 59 and 60.

The inhibitory zone A - C was eluted from maximally hydrolysed uninfected ears at flowering for both cultivars and re-run in cyclohexane/ethylacetate 50:50, and a Cladosporium assay set up, in order to determine if there was any separation of inhibitors. The separation

TABLE 41 % Inhibition of S. nodorum in the eluted ether soluble Cladosporium inhibitors in nutrient medium, from minimally and maximally hydrolysed field plants at various growth stages

G.S.	Infection	Hydrolysis	Part of	Cultivar &	% inhibition of germ tube growth
			Culm	Maris Huntsman	Maris Ranger
6-7	HEALTHY	MINIMUM	2nd leaf	0*(A)	-
11			Ear	0 (B)	-
10	30%	MINIMUM	Flag	28 (C)	28 (A)
11	50%		Flag	36 (A)	28 (A)
11	50%		Ear	-	39 (B C)
10	HEALTHY	MAXIMUM	Flag	68 (A B)	0 (A B)
10			Flag:proximal portion of infected leaf	52 (B C)	-
10			Ear unemerged	10 (C)	6 (C)
11			Flag	5 (B C)	5 (B C)
11			Ear	49 (A B C)	28 (A B C)
10	30%		Flag	68 (A B C)	-
11	50%		Flag	18 + 51 (A+BC)	100 (A B C)
11	50%		Ear	44 (A B C)	48 (A B C)

* % inhibition of germ tube length at tissue concentration 1g f.wt/ml

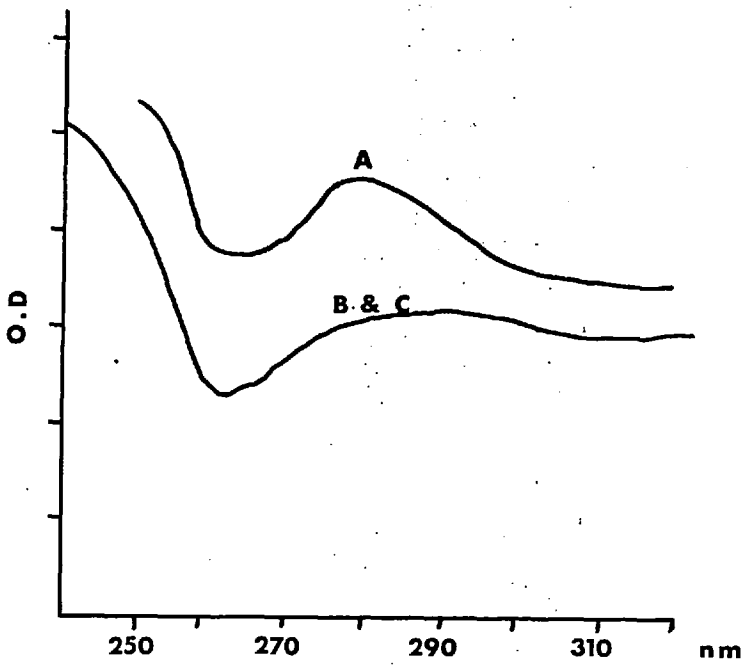
Letters A - C correspond to antifungal zones on Cladosporium assays, Table 40.

was not greatly improved. 'A' remaining as a narrow band and B and C continuous and much less active.

In an attempt to characterise A, B and C the UV spectra were recorded for all eluates before S. nodorum assay. The spectrum of A (in Fig. 37) peaks at around 280nm, while that of B and C is a very broad barely detectable peak from 270-300nm.

None of the zones reacted with ferric chloride. Unfortunately time did not permit further elucidation of these inhibitors.

Figure 37:- UV spectra of antifungal zones A, B & C of ether soluble extracts of maximally & minimally hydrolysed healthy & infected field grown wheat



III. The Disappearance of the Benzoxazinone compounds in winter wheat

During the preceeding experiments in part II, it has become increasingly clear that the benzoxazinone compounds are absent from wheat tissue over about 2-3 weeks old. This is evident since after this time the R_Fs corresponding to the benzoxazinone compounds no longer react with ferric chloride and the UV spectrum obtained does not have the characteristic double peak. Their role in adult plant resistance is therefore questioned.

The following time course experiments were set up to determine the presence or absence of the benzoxazinone compounds in plants grown under varying conditions.

A. 1. Glasshouse grown plants

At weekly intervals from planting, the shoot tissue of glasshouse grown plants of Maris Ranger and Maris Huntsman were excised, and extracted by the method of maximal hydrolysis. The ether soluble substances were chromatogrammed at 1g f.wt./cm and run in 10% methanol in chloroform. A strip was cut from the chromatogram and sprayed with a 5% ethanolic solution of ferric chloride. The presence of the benzoxazinone aglucone was confirmed by a violet-blue colour reaction.

A positive reaction was obtained only from seven day old plants of both varieties. The two and three week old plants gave negative reactions thus indicating the absence of the benzoxazinone compounds.

To ensure that the inability to recover the aglucone from the glucosidic precursor was not due to a lack of β-glucosidase enzyme activity, plants (4 weeks old) were extracted by the method of minimal hydrolysis. The butanol soluble substances, and the butanol partition from the water soluble substances of maximally hydrolysed 2 and 3 week old plants were chromatogrammed (1 g f.wt./cm) and run in 50% methanol in chloroform.

But no positive reaction with ferricchloride wae obtained indicating the abeence of the glucosidic precureor in wheat plante more than 2 weeks old.

2. Cooled cabinet grown plante

Elnaghy and Shaw (1966) showed that plante carrying the temperature seneitive gene for reeistance, Sr6, to stem rust, had a high concentration of the glucoside when grown at moderate temperaturee but a lower concentration when grown at higher temperaturee.

It was therefore decided to repeat the serial extraction using plants grown at the lower temperature of 15°C. Seede of the cultivare Maris Huntsman and Marie Ranger were planted in pots, as described in Materials and Methode, and these were placed in a cooled 15°C cabinet with bands of Philipe white fluorescent lights set to a 16h day. Plante were extracted by the method of maximal hydrolysis, ae described, daily from 9-15 daye inclusive and at 3 and 4 weeks old. The ether eoluble substances were chromatogrammed, run in 10% methanol in chloroform and a strip of the resulting developed chromatogram sprayed with ferric chloride.

For both cultivars the ether eoluble eubstances gave a positive reaction for 9-14 days old. The 15 day old plants gave a purple-brown reaction while the 3 and 4 week old extractions were completely negative in their colour reaction for the aglucone.

3. Field grown plants

Plants in the field are subject to relatively large fluctuations in temperature in comparison to those in the glasshouse. To ehow a role of the benzoxazinone compounde in resistance it is necessary to demonstrate their presence in field grown plante.

Field grown plants were extracted by the method of minimal hydrolysis at 7, 11, 15 and 22 days after planting. The alcohol soluble substances, following petroleum ether partition, were separated on an LH-20 sephadex column. The resulting fractions were run on the DB spectrophotometer, similar fractions bulked, and then run on chromatograms in 50% methanol in chloroform. The presence of a benzoxazinone glucoside was determined by spraying with ferric chloride, and the appearance of a blue reaction. Seven day old shoots of both cultivars were positive in their colour reaction, but the 11, 15 and 22 day old shoots were negative. Thus indicating the absence of the glucoside at an earlier stage than in plants grown under controlled conditions.

B. Means of quantifying the benzoxazinone compounds

1. Assessment of ferric chloride colour reaction by optical density measurements

In order to quantify the levels of benzoxazinone compounds in wheat it was first decided to estimate the accuracy of visual assessment of colour development following application of the spray reagent ferric chloride to developed chromatograms.

The ether soluble substances from maximal hydrolysis of week old plants of Maris Ranger and Maris Huntsman were chromatogrammed at 0.4, 0.8 and 1.6g fresh weight/cm and run in 10% methanol in chloroform.

The blue areas were eluted with 95% methanol and their optical denisty at 560nm measured on the Beckman DB spectrophotometer (Knott and Kumar, 1972). Blanks were prepared from areas of the chromatogram which were sprayed, but free from any extract. See Table 42.

2. An analagous chromatogram was run but not sprayed and the corresponding areas eluted for UV spectral analysis. The results are given in Table 43.

TABLE 42 O.D. Measurement of the colour reaction to assess levels of benzoxazinone aglucone

Loading gm f. wt/cm.	Cultivar	
	Maris Huntsman	Maris Ranger
0.4	0.14	0.02
0.8	0.85	0.33
1.6	0.95	0.74

It is evident from Table 42 that the O.D. measurements do not reflect the loadings and thus do not represent the relative levels of aglucone in the 2 cultivars used. M. Ranger has more aglucone at 1.6g f.wt/cm but not below this loading, compared to Maris Huntsman.

TABLE 43 UV Spectral analysis to assess levels of the aglucone

(O.D. at 264/288 nm)

Loading g.f.wt/cm	Cultivar	
	Maris Huntsman	Maris Ranger
0.4	0.110/0.070	0.149/0.105
0.8	0.269/0.190	0.300/0.222
1.6	0.450/0.320	1.05/0.70

This is apparently more accurate than measuring the OD of the coloured $FeCl_3$ complex.

It is of interest that the cultivar more susceptible to S. nodorum has a higher level of the inhibitory aglucone. However this is following hydolysis and does not represent inhibitor in the intact plant.

C. The levels of benzoxazinone glucoside and aglucone in healthy wheat plants resistant and susceptible to S. nodorum

This time course experiment was set up to follow the levels of both the aglucone and the glucoside in healthy plants of Maris Huntsman and Maris Ranger.

Two replicates (20g) each of glasshouse grown Huntsman and Ranger were extracted at regular intervals after germination by the methods of minimal and maximal hydrolysis to obtain the glucoside and aglucone respectively.

The 60% alcohol soluble substances, following petroleum ether partitio (40-60°C boiling range) of the total crude minimal hydrolysis extract, were chromatogrammed at 0.5g fresh wt/cm and run in 50:30:5:10 ethyl acetate : methyl ethyl ketone: formic acid:water.

The ether soluble substances of the maximal hydrolysis extract were chromatogrammed at 1g. fwt/cm and run in 10% methanol in chloroform.

In both cases the benzoxazinone compounds were located by spraying a strip of the chromatogram with ferric chloride.

The glucoside was eluted from the chromatogram with water and UV absorption at 272 and 286nm measured.

The aglucone was eluted with ethyl alcohol and the UV absorption at 264 and 288nm measured.

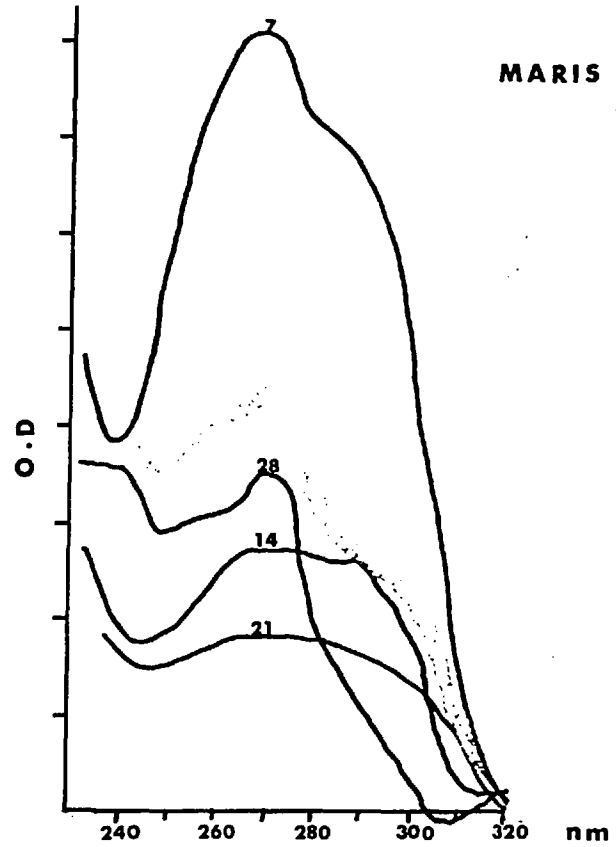
Blanks were prepared from areas of the chromatogram which were sprayed but free from any extract.

In the later extractions where no blue reaction occurred the area was eluted where the benzoxazinone would have been expected to run. The spectra are given in Figures 38 and 39.

From Figs. 38 and 39 it is evident in both cultivars that the levels of benzoxazinone compounds fall off with time, being undetectable after 3 weeks. In the early stages the UV spectra have sharp peaks and shoulders, while with increasing age of plant the peak disappears almost completely with only background absorbing.

For the glucoside (Fig. 38), Maris Huntsman levels are roughly similar for the first 3 weeks and then at 28 days there is no obvious UV spectrum. In Maris Ranger, the initial level at 7 days is relatively high and then fall off is rapid in the following 2 weeks.

Figure 38:- UV spectrum of benzoxazinone glucoside in water (age in days)



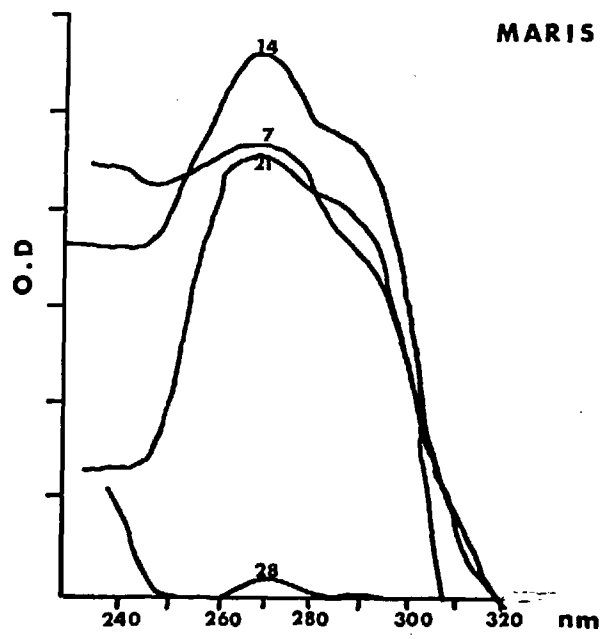
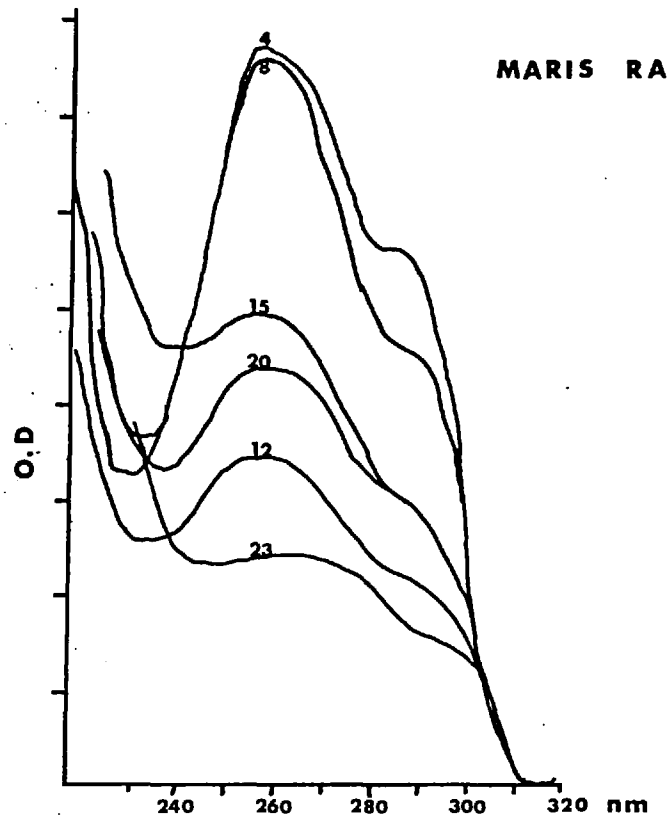
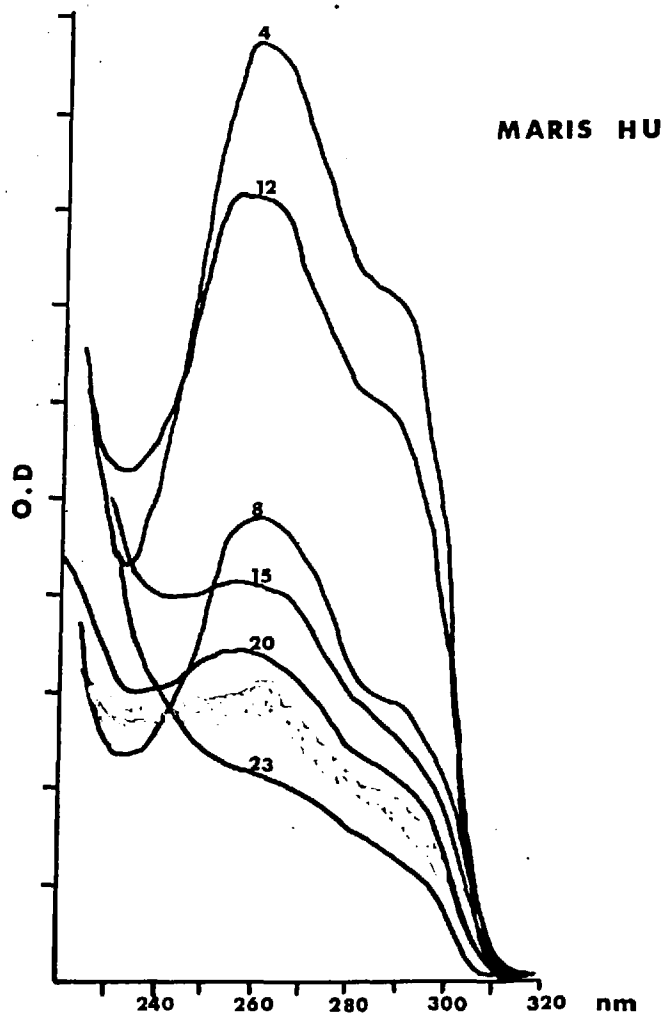


Figure 39:- UV spectrum of benzoxazinone aglucone in ethanol (age in days)





days 4, 8, 12, 15, 20 only, +ve ferric chloride reaction

The pattern for the aglucone is somewhat different (Fig. 39). In Huntsman there is a high initial level and again at 12 days. The 8 day extract has a lower level similar to the 15, 20 and 23 day extracts. In Maris Ranger there were relatively high levels in the 4 and 8 day old plants. The levels in later extracts are similar and slightly higher than for Maris Huntsman. The high background absorbance is probably due to other substances of the same R_F. The zone C compounds (Part II) correspond to this R_F and may be present simultaneously. Thus not only do the benzoxazinones disappear, but the levels are similar for both cultivars and so their role in resistance per se is questionable for seedlings and non-existent for adult plants in the field.

DISCUSSION

That there are compounds in healthy intact wheat tissue, which upon the appropriate extraction method are antifungal is evident. However the question remains - can these compounds be invoked in the resistance of wheat to S. nodorum? Also are there further antifungal substances which are not detected by these extraction methods of minimal and maximal hydrolysis? In the former case substances which are not thermostable would be inactivated and in the second case labile substances may be denatured by hydrolytic and other enzymes.

In considering the role of antifungal compounds in resistance it is necessary to compare the qualitative and quantitative differences in activity for the resistant and susceptible cultivars both healthy and infected at various growth stages.

Cultivar differences

No qualitative differences were apparent between cultivars in crude extracts, or partially purified, except for the minimal hydrolysed uninfected ears at flowering where an inhibitory zone was found in Maris Huntsman only. However, on S. nodorum assay this zone was found to be inactive.

On a quantitative basis, activity of extracts, crude and purified, against S. nodorum was generally greater for the resistant cultivar than for the susceptible one. The inhibitory effect of infected resistant tissue may be an under-estimate since in this case there are small limited lesions surrounded by relatively large areas of symptomless tissue, which tends to have a diluting effect. Thus when the proximal portions (usually symptomless) of infected resistant flag leaves were extracted the levels of inhibition obtained were less

than for the infected distal portions. Morgan (1974) showed that antifungal activity of lesions was greater than for the surrounding symptomless areas and that activity was related to degree of pathogen damage. This resulted in relatively high levels of inhibition for the susceptible cultivar. There are obvious difficulties with obtaining large areas of infected resistant leaf tissue.

Differences between infected and uninfected tissues

In most cases where healthy intact tissues were extracted by minimal hydrolysis which maintained the chemical regime of the cells there was no activity. Thus it appears there are no immediately antifungal compounds in wheat tissue. However, upon infection, tissues extracted in the same way did exhibit some activity which suggests a production of compounds. An increase of 10-20% in the activity against S. nodorum from maximally hydrolysed infected tissue over that of the corresponding control supports the concept of production of antifungal compounds.

The question now arises - are these substances a result of de novo synthesis, or enzymic activity on existing compounds? The qualitative studies from chromatograms suggest the compounds are a result of enzyme action, possibly hydrolysis of existing chemicals. Using minimal hydrolysis in the intact plant, upon infection one or two inhibitory zones became apparent, A and sometimes C. Healthy, maximal hydrolysed tissue contained C and to a lesser extent A, but in addition a further inhibitory band, B, while infected maximal hydrolysed tissue contained all 3 zones of inhibition. It seems likely, therefore, that the plant contains enzymes which act on the precursors of B and C and to a lesser

extent A. The fungus per se or by host induction increases the level of enzymes which produces A and C. The fungus does not appear, except in ears, to produce or induce the necessary enzyme(s) to form B. The zones A - C appear to act synergistically against S. nodorum in spore germination assays.

Differences with increasing age

The apparent disappearance of the benzoxazinone compounds from wheat plants over two weeks old, and their absence from adult plants in the field, tends to negate their role in resistance of adult plants. Further, even when present, there are no quantitative differences between healthy resistant and susceptible cultivars which could account for the differential reaction of wheat varieties. However a differential release of the active aglucone, on infection, may provide an explanation. Morgan (1974) has shown S. nodorum to be capable of hydrolysing the innocuous glucoside the active aglucone. In addition workers have shown (Chigrin and Rozum, 1969; Couture et al, 1971) that the β-glucosidase enzyme activity of some wheat cultivars is variable. No investigations as to the relative levels of this enzyme were made in Maris Ranger and Maris Huntsman, but in staining reactions (Materials and Methods) a large background activity of β-glucosidase enzyme was evident. The crucial investigation into the role of benzoxazinone compounds in resistance is their relative levels in infected tissue. Using 1st and 2nd leaves, Morgan (1974) demonstrated the presence of glucoside around lesions in the resistant cultivar, but its absence within the lesion. He was unable, however, to detect glucoside in or around lesions of the susceptible cultivar.

The fact that the compounds are present and at concentrations which totally inhibit S. nodorum remains, and clearly further investigation

of infected seedlings is required. However the difficulties associated with applying spore suspensions to coleoptiles makes obtaining an extensive area of infection for analysis very difficult. The use of suitable wetting agents may be profitable in this respect.

In addition to the disappearance of the benzoxazinone compounds there is another qualitative difference with age. Thus healthy ears, in boot, appear to contain only the precursor of zone C while after emergence the precursors of B and A are apparent as well.

FINAL DISCUSSION

The basic question this research attempts to answer is: What is the nature of the interaction which occurs between S. nodorum and cultivars such as Maris Huntsman, which does not occur in cultivars such as Maris Ranger? Why do lesions remain limited necrotic flecks with little or no chlorosis in the former cases, but spread and coalesce with extensive chlorosis in the latter cultivars?

Clearly no single mechanism of resistance emerges. This is corroborated by the genetic studies on this host/pathogen combination (Brönnimann, 1970). He demonstrated a linear and additive gene effect with respect to 1000 grain weight. This polygenic inheritance and the consistent ranking order of cultivars with respect to susceptibility are evidence of horizontal resistance defined by Robinson (1969). No major resistance genes have been reported.

The continuous variation in symptoms through different degrees of lesion extent, necrosis and chlorosis suggests that the "resistance factors" are varying quantitatively rather than qualitatively. In light microscopy investigations the presence of structural barriers was not detected, in accordance with the studies of Morgan, (1974). Ride (1975) demonstrated a possible role for lignin in restricting non-pathogens, and suggested that the ability of S. nodorum to counter host defenses could be due in part to the breakdown of lignified cell walls by specific enzymes produced only by the pathogen. The differential rapidity of lignin deposition might account for the variation in lesion extent in certain cultivars infected by S. nodorum. However the present author was unable to demonstrate the presence of lignin in the cultivars Maris Ranger and Maris Huntsman.

The ranking order of cultivars in detached leaf tests was maintained in the field, although symptoms were slightly different. In the resistant cultivar in the field there was often some chlorosis and lesions at the tip of the leaf usually spread. Spreading of lesions was not observed in laboratory tests with the resistant cultivar. This differential reaction may be associated with the physiological state of the leaf. In the laboratory healthy leaves were used and observed for only 5-10 days after inoculation, while in the field leaves were observed for much longer periods. The spread of lesions at the distal end of resistant leaves may be associated with a senescence of that tissue.

At inoculum levels normally used in laboratory tests lesions always developed on the resistant cultivar. However at inoculum concentrations below 10^3 spores/ml lesions developed very slowly or not at all. This probably accounts for the phenomenon brought out by the detailed study of infection in the field, that fewer and smaller lesions developed on the resistant cultivar, since inoculum levels might often be low in this location.

Some cultivars sustain relatively high yields despite high infections and vice versa. This anomaly is explained to some extent in the detailed study of infection of spring wheats in the field, considering that the flag leaf and ear are the most important contributors to grain filling (Thorne, 1965). The high yielding cultivars had a lower % infection and lower infection rates on the flag leaf and ear compared to the lower yielding cultivars, although in the former case a high % infection of the lower leaves resulted in an apparently greater overall infection.

Morgan (1974) demonstrated the ability of S. nodorum to colonise healthy leaves which had been autoclaved. The apparent inability of the fungus to grow extensively and sporulate in the dead lesion tissue of resistant cultivars indicates the presence of inhibitory substances

in the lesion. The present research has shown that, using the appropriate extraction method, there are compounds in healthy and infected wheat plants which are inhibitory to S. nodorum.

The benzoxazolinone derivatives, demonstrated by Morgan (1974) to be highly active against S. nodorum, have been shown to be absent in plants older than 2-3 weeks. Therefore it is unlikely that they contribute to adult plant resistance in the field.

Time did not permit further elucidation of the other inhibitory substances and clearly much work remains to be done. Plants could, for example, be extracted in liquid nitrogen, which would minimise the loss of inhibitors labile to boiling and enzyme activity. Although the presence of antifungal compounds is evident, no qualitative difference between the cultivars was established and quantitative differences in activity were not pronounced. This may be due to a loss of activity during preparation of extracts for bioassays. Alternatively the relatively large area of symptomless tissue which surrounds lesions in the resistant cultivar may cause an artefactual dilution of inhibitory substances if they are only produced at infection sites. The possible role of these compounds in resistance to S. nodorum is difficult to assess. Their biological significance depends on correlation of the presence of these compounds with morphological and physiological events during infection. The biology of the infection process remains to be defined. Electron microscopy or adaptation of its preparation techniques to light microscopy observations may resolve the exact relationship of the fungal hyphae with the host cells, and so provide a meaningful model to which the biochemical changes can be related.

BIBLIOGRAPHY

ASHFORD, Anne E. (1970). Histochemical localisation of β-glucosidases in roots of Zea mays 1. A simultaneous coupling azo-dye technique for the localisation of β-glucosidase and β-galactosidase. Protoplasma 71, 281-293.

BAILEY, J.A. and BURDEN, R.S. (1973). Biochemical changes and phytoalexin accumulation in Phaseolus vulgaris following cellular browning caused by tobacco necrosis virus. Physiological Plant Pathology 3, 171-178.

BAKER, C.J. (1969). Studies on Leptosphaeria nodorum Muller and Septoria tritici Desm. on wheat. Ph.D. Thesis, University of Exeter.

BAKER, C.J. (1970). Influence of environmental factors on development of symptoms on wheat seedlings infected with Leptosphaeria nodorum. Transactions of the British Mycological Society 55, 443-447.

BECKER, G.J.F. (1963). Glume blotch of wheat caused by Leptosphaeria nodorum Muller. Technische Bericht Nederlands Graan-Centrum 11, 1-36.

BENIDICT, W.G. (1973). Effect of light and of peroxidase on pathogenicity and sporulation of Septoria apiicola on celery. Physiological Plant Pathology 3, 69-78.

BERKELY, M.J. (1845). Diseases in the wheat crop. Gardeners Chronicle 5, 601.

BOTTALICO, A. (1969). Quoted in Phytotoxins in Plant Disease. Edited by Wood, R.K.S., Ballio, A., Graniti, A., Academic Press, 530pp. 4. Production and bioassay of phytotoxins. S.Neaf-Roth.

BOUSQUET, J.F. and SKAJENNIKOFF, M. (1974). Isolement et mode d'action d'une phytotoxine produite en culture par Septoria nodorum Berk. Phytopathologische Zeitschrift 80, 355-360.

BRIAN, P.W., DAWKINS, A.W., GROVE, J.F., HEMMING, H.G., LOWE, D., and NORRIS, G.L.F. (1961). Phytotoxic compounds produced by Fusarium equiseti. Journal of Experimental Botany 12, 1-12.

BRÖNNIMANN, A. (1968a). Zur kenntnis von Septoria nodorum Berk., dem erreger der Spelzenbräune und einer Blattdüre des Weizens. Phytopathologische Zeitschrift 61, 101-146.

————, (1968b). Zur Toleranz des Weizens gegenüber Septoria nodorum Berk. Phytopathologische Zeitschrift 62, 365-370.

BRÖNNIMANN, A. (1969a). Ursachen der unterschiedlichen Verträglichkeit des Weizens gegenüber Befall durch Septoria nodorum Berk. Phytopathologische Zeitschrift 66, 353-364.

————, (1969b). Befall und Schadigung von Weizen durch S.nodorum Berk., in Abhangigkeit von der Stickstoffdüngung. Schweizerische landwirtschaftliche Forschung.VII 2, 185-193.

————, (1970). Zur Vererbung der Toleranz des Weizens gegenüber Befall durch Septoria nodorum Berk. Zeitschrift fur Pflanzenzüchtung 63, 333-340.

BRÖNNIMANN, A., KÜNZLI, W., and HANI, F. (1972). Reaktion einiger Weizensorten auf Septoria befall nach CCC-Behandlung. Mitterlungen für die Schweizerische Landwirtschaft 8, 20.

BRÖNNIMANN, A., SALLY, B.K., and SHARP, E.L. (1972). Investigations on Septoria nodorum in spring wheat in Montana. Plant Disease Reporter 56, 188-191.

BURHARDT, Z. (1954). Septoria nodorum Berk., on tillering spring wheat. Abst. in Review of Applied Mycology 37, 33.

CHIGRIN, V.V., and ROZUM, L.V. (1969). Changes in phenolic metabolism of spring wheat infected with stem rust. Fiziologiya Rastenii 6, 330-335.

CHKANIKOV, D.I., TARABRIN, G.A., SHABANOVA, A.M., and KONSTANTINOV, P.T. (1969). Localisation of β-glucosidase in the cells of higher plants. Fiziologiya Rastenii 16, 322-325.

COHEN, R.B., RUTENBURG, S.H., KWAN-CHUNG TSOU, WOODBURY, M.A., and SELIGMAN, A.N. (1951). The colorimetric estimation of β-D-glucosidase. Journal of Biological Chemistry 195, 607-614.

COOKE, B.M., and JONES, D.G. (1970a). Epidemiology of S.tritici and S.nodorum II. Comparative studies of head infection by Septoria tritici and Septoria nodorum on spring wheat. Transactions of the British Mycological Society 54, 395-404.

————, (1970b). A field inoculation method of Septoria tritici and Septoria nodorum. Plant Pathology 19, 72-74.

————, (1971). Epidemiology of S.tritici and S.nodorum III. The reaction of spring and winter wheat varieties to infection by Septoria tritici and Septoria nodorum. Transactions of the British Mycological Society 56, 121-135.

COUTURE, R.M., ROUTLEY, D.G., and DUNN, G.M. (1971). Role of cyclic hydroxamic acids in monogenic resistance of maize to Helminthosporium turcicum. Physiological Plant Pathology 1, 515-521.

COVEY, R.P., (1962). Mode of action of Septoria linicola in flax. Phytopathology 52, 1229-1230.

CRUICKSHANK, I.A.M. (1966). Defense mechanisms in plants. World Review Pest Control 5, 161-175.

DEREVEYANKIN, A.I. (1969). Specialisation of causal organisms of wheat septoriosis. Mikologiya fitopatologiya 3, 256-258.

DEVERALL, B.J. (1967). Biochemical changes in infection droplets containing spores of Botrysis spp. incubated in the seed cavities of pods of bean (Vicia faba L.). Annals of Applied Biology 59, 375-387.

ELNAGHY, M.A., and LINKO, P. (1962). The role of 4-o-glucosyl-2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one in resistance of wheat to stem rust. Physiologia Plantarum 15, 764-771.

ELNAGHY, M.A., and SHAW, M. (1966). Correlation between resistance to stem rust and the concentration of glucoside in wheat. Nature, London 210, 417-478.

FINK, K., and FINK, R.M. (1949). in Thin Layer Chromatography. A Laboratory Handbook. Edited by E.Stahl. 2nd edition. George Allen and Unwin 1041 pp. p.875.

FINNEY, M.E. (1973). Growth analysis of barley (cv. Zephyr) infected with E.graminis DC. Ph.D. Thesis, University of London.

FRANCIS, C.M., MILLINGTON, A.J., and BAILEY, E.T. (1967). Distribution of oestrogenic isoflavones in the genus Trifolium. Australian Journal Agricultural Research 18, 47-54.

GAHAGAN, H.E., and MUMMA, R.O. (1967). The isolation of 2-(2-hydroxy-7-methoxy-1,4-benzoxazin-3-one)β-D-glucopyranoside from Zea mays. Phytochemistry 6, 1441-1448.

HIETALA, P.K., and VIRTANEN, A.I. (1960). Precursors of benzoxazolinone in rye plants. II. Precursor I. The Glucoside. Acta chemica scandinavica 14, 502-504.

HIRAI, T. (1956). Quoted in Physiology and biochemistry of disease resistance in plants. K.Tomiyama. Annual Review of Phytopathology (1963), 1, 295-324.

HOFMAN, J. and HOFMANOVÁ, O. (1969). 1,4-benzoxazine derivatives in plants- Sephadex fractionation and identification of a new glucoside. European Journal of Biochemistry 8, 109-112.

————, (1971). 1,4-benzoxazine derivatives in plants- Absence of 2,4 dihydroxy-7-methoxy,2 hydroxy,1,4-benzoxazin-3-one from uninjured Zea mays plants. Phytochemistry 10, 1441-1444.

HOLMES, S.J.I., and COLHOUN, J. (1974). Infection of wheat by Septoria nodorum and Septoria tritici in relation to plant age, air temperature and relative humidity. Transactions of the British Mycological Society 63, 329-338.

JENKINS, J.E.E., and MORGAN, W. (1969). The effect of Septoria diseases on the yield of winter wheat. Plant Pathology 18, 152-156.

JOHNSTON, C.O., and HUFFMAN, M.D. (1958). Evidence of local antagonism between two cereal rust fungi. Phytopathology 48, 69-70.

JONES, D.G., and ODEBUNMI, K. (1971). The epidemiology of Septoria tritici and Septoria nodorum. IV. The effect of inoculation at different growth stages and on different plant parts. Transactions of the British Mycological Society 56, 281-288.

KEITREIBER, M. (1961). Die Erkennung des Septoria - Befalles von Weizenkörnen bei der Saatgutprüfung. Pflanzenschutz-Berichte 26, 129-157

KERN, H., NAEF-ROTH, S., and DEFAGO, G. (1971). In Phytotoxins in Plant Disease. Edited by Wood, R.K.S., Ballio, A., and Graniti,A. Academic Press, 530pp. 4. Production and bioassay of phytotoxins. S. Naef-Roth.

KLUN, J.A., and ROBINSON, J.F. (1969). Concentration of two 1,4-benzoxazinones in dent corn at various stages of development of the plant and its relation to resistance of the host plant to the European corn borer. Journal of Economic Entomology 62, 214-220.

KNOTT, D.R., and KUMAR, J. (1972). Tests of the relationship between a specific phenolic glucoside and stem rust resistance in wheat. Physiological Plant Pathology 2, 393-399.

KRUPINSKY, J.M., SCHAREN, A.L., and SCHILLINGER, J.A. (1973). Pathogenic variation in Septoria nodorum Berk., in relation to organ specificity, apparent photosnythetic rate and yield of wheat. Physiological Plant Pathology 3, 187-194.

LARGE, E.C. (1954). Growth stages in cereals. Illustrations of the Feekes scale. Plant Pathology 3, 128-129.

LAUBSCHER, F.X., von WECHMAR, B., and van SCHALKWYK, D. (1966). Heritable resistance of wheat varieties to glume blotch (Septoria nodorum Berk.). Phytopathologische Zeitschrift 56, 260-264.

LEBEDEVA, L.N. (1960). Septoria infection of spring wheat in Novosibirsk Oblast. Biological Abstracts 45, 52960.

LOOMIS, R.S., BECK, S.D., and STAUFFER, J.F. (1957). The European corn borer, Pyrausta nubilalis Hubn., and its principle host plant. V. A chemical study of host plant resistance. Plant Physiology 32, 379.

LUKE, H.H., and WHEELER, H.E. (1955). Toxin production by Helminthosporium victorae. P hytopathology 45, 453-458.

LUPTON, F.G.H. (1971). Report of cereals department. P.B.I. Cambridge Annual Report 1971, pp 68-69.

MAXIMAV, O.B., and PANTHINKHINA, L.S. (1965). In Thin Layer Chromatography. A Laboratory Handbook. Edited by E. Stahl. 2nd edition. George Allen and Unwin 1041pp. p 893.

MORGAN, W.M. (1974). Physiological studies of diseases of wheat caused by Septoria spp and Fusarium culmorum. Ph.D. Thesis. University of London.

OBST, A. (1971). Infectionsquellen fur Septoria nodorum. Nachrichten der Deutschen Pflanzenschutzdienst 23, 177-178.

OKU, H., OUCHI, S., and TANI, T. (1974). Role of phytoalexins in obligate parasitism. Abstracts of papers: 2nd International Congress of Plant Pathology. Minneapolis, 1973. Abstract no. 1012.

PIRSON, H. (1960). Prüfung verschiedener Winterweizensorten auf Auffälligkeit gegen Septoria nodorum Berk., mit Hilfe von künstlichen Infektionen. Phytopathologische Zeitschrift 37, 330-342.

P.B.I. Cambridge. Annual Report 1968.

POOLE, D.D. and MURPHY, J.C. (1952). Reactions of oat varieties to black stem and to a toxic substance produced by the causal organism. Phytopathology 42, 16.

PURKAYASTA, R.P. and DEVERALL, B.J. (1965). The detection of antifungal substances before and after infection of broad beans (Vicia faba) by Botrytis spp. Annals of Applied Biology 56, 269-277.

RENFRO, B.L., and YOUNG, M.C. (1956). Techniques for studying varietal response to Septoria leaf blotch of wheat. Phytopathology 46, 23-24.

RIDE, J.P. (1975). Lignification in wounded leaves in response to fungi and its possible role in resistance. Physiological Plant Pathology 5, 125-134.

ROBINSON, R.A. (1969). Disease resistance terminology. Review of Applied Mycology 48, 593-606.

SCHAREN, A.L. (1963). Effect of age of wheat tissues on susceptibility to Septoria nodorum. Plant Disease Reporter 47, 952-954.

————, (1964). Environmental influences on development of glume blotch in wheat. Phytopathology 54, 300-303.

————, (1966). Cyclic production of pycnidia and spores in dead wheat tissues by Septoria nodorum. Phytopathology 56, 580-581.

SCHAREN, A.L., and KRUPINSKY, J.M. (197Da). Cultural and inoculation studies of Septoria nodorum cause of glume blotch of wheat. Phytopathology 60, 1480-1485.

————, (197Db). Effect of Septoria nodorum infection on CO_2 absorption and wheat yield. Phytopathology 59, 1298-1301.

SCHAREN, A.L.,SCHAEFFER, G.W., KRUPINSKY, J.M., and SHARPE, F.T.Jr. (1975). Effects of flag leaf axial lesions caused by Septoria nodorum on ^{14}C translocation and yield of wheat. Physiological Plant Pathology 6, 193-198.

SCHAREN, A.L. and TAYLOR, J.M. (1968). CO_2 assimilation and yield of Little Club wheat infected by Septoria nodorum. Phytopathology 58, 447-451.

SCHMIEDEKNECHT, M. (1967). Die Braunfleckigkeit des Weizens. Nachrichten der Deutschen Pflanzenschutzdienst 21, 54-60.

SCOTT, P.R. (1973). Incidence and effects of Septoria nodorum on wheat cultivars. Annals of Applied Biology 75, 321-329.

SEEVERS, P.M., and DALY, J.M. (1970). Studies on wheat stem rust resistance controlled at the Sr.6 locus. I. The role of phenolic compounds. Phytopathology 60, 1322-1328.

SHARP, E.L., BRÖNNIMANN, A., and McNEAL, F.H. (1972). Reaction of selected spring wheat varieties to infection by Septoria nodorum. Plant Disease Reporter 56, 761-764.

SHEARER, B.L., and ZADOKS, J.C. (1972). The latent period of Septoria nodorum in wheat. I. The effect of temperature and moisture treatments under controlled conditions. Netherlands Journal of Plant Pathology 78, 231-241.

————, (1974). The latent period of Septoria nodorum in wheat. II. The effect of temperature and moisture under field conditions. Netherlands Journal Plant Pathology 80, 48-60.

SHIPTON, W.A., BOYD, W.R.J., ROSIELLE, A.A., and SHEARER, B.L. (1971). The common Septoria diseases of wheat. Botanical Review 37, 231-262.

SHIPTON, W.A., and BROWN, J.F. (1962). A whole leaf clearing and staining technique to demonstrate host-pathogen relationships of wheat stem rust. Phytopathology 52, 1313.

SUTTON, G.L. (1920). Take-all, Septoria, Rust and Wheat Mildew. Western Australia dept. Agriculture Bull. 69, 27pp. 10 figs.

THOMAS, M.J. (1962). Factors affecting glume blotch development on wheat and variation in the causal organism, Septoria nodorum. Dissertation Abstracts 23, 789.

THORNE, G.N. (1965). Photosynthesis of ears and flag leaves of wheat and barley. Annals of Botany.(N.S.) 29, 317-329.

VAN DER PLANK, J.E. (1963). Plant Diseases: Epidemics and Control. Academic Press Inc., New York and London. 349pp.

VIRTANEN, A.I. (1958). Antimikrobiologische substanzen in unseren Kulturpflanzen und ihre Bedeutung für die Pflanzen und für die Ernährung des Menschen und der Tiere. Schweizerische Zeitschrift für allgemeine Pathologie und Bakteriologie 21, 970.

————, (1961). Über einen neuen Typ von Glucosiden in jungen Roggen-, Weizen und Mais- Pflanzen. Brauwissenschaft 14, 98-101.

VIRTANEN, A.I., and HIETALA, P.K. (1955). 2(3)-benzoxazolinone, an anti-Fusarium factor from rye seedlings. Acta chemica scandinavica 1543-1544.

————, (1960). Precursors of benzoxazolinone in rye plants. I. Precursor II, the Aglucone. Acta chemica scandinavica 14, 499-502.

VIRTANEN, A.I., HIETALA, P.K., and WAHLROOS, Ö. (1957). A ntimicrobial substances in cereals and fodder plants. Archives of Biochemistry and Biophysics 69, 486-500.

WAHLROOS, Ö. and VIRTANEN, A.I. (1958). On the antifungal effect of benzoxazolinone and 6-methoxybenzoxazolinone, respectively, on Fusarium nivale. Acta chemica scandinavica 12, 124-128.

————, (1959). The precursors of 6-methoxybenzoxazolinone in maize and wheat plants, their isolation and some of their properties. Acta chemica scandinavica 13, 1906-1908.

————, (1964). Free 2.4,dihydroxy-7-methoxy-1,4-benzoxazin-3-one in maize. Journal Pharm. Science 53, 844.

WEBER, G.F. (1922). Septoria diseases of cereals. II. Septoria diseases of wheat. Phytopathology 12, 537-585.

von WECHMAR, M.B. (1966). Investigations on the survival of Septoria nodorum on crop residues. South African Journal Agricultural Science 9, 93-100.

WILLIAMS, J.R., and JONES, D.G. (1972). Epidemiology of Septoria tritici and Septoria nodorum. VI. Effect of time of initial infection on disease development and grain yield in spring wheats. Transactions of the British Mycological Society 59, 273-283.

WOOD, R.K.S. (1967). Physiological Plant Pathology. Blackwell Scientific Publications Ltd, Oxford and Edinburgh. 570pp.

ACKNOWLEDGEMENTS

I wish to express my sincere gratitude to my supervisor, Dr. Ian Smith, for his helpful guidance, useful discussions and criticism throughout the duration of the experimental work and in the preparation of this manuscript.

I would like to thank Professor R.K.S. Wood in whose department this research was carried out.

Further thanks go to Ted Green, Cheryl Warren and Pam Tyler for technical assistance, particularly with the field work.

Additional thanks to all my friends, in particular David, who have made life at Silwood very happy.

A special thankyou to my parents for their kindness and generosity.

I am grateful to Sheila Douglas for typing the manuscript.

Finally I should like to acknowledge the Science Research Council whose financial support during the academic years 1972-1975 made this research possible.

APPENDICES

Appendix No.	Contents	Table No.
1A	Field Experiment 1A	
	Weekly Mean % *S. nodorum* infection	
	Winter wheat 1974.	1-18
1B	Field Experiment 1B	
	Detailed study of infection	
	Winter wheat 1975.	19-24
	Summary of data for 1974	25
	Summary of data for 1975	26
2A	Field Experiment 2A	
	Weekly Mean % *S. nodorum* infection	
	Spring wheat 1974.	27-46
2B	Field Experiment 2B	
	Detailed study of infection	
	Spring wheat 1974	47-58
3	Spore droplet bioassays	
	Growth of *S. nodorum* in ether-soluble antifungal eluates (from field grown plants).	59-60

APPENDIX 1A

TABLES 1-18

Weekly Mean % Septoria nodorum infection

Winter wheat 1974

Values are means of up to 10 culms in a clump

Key R 1, R 2 } Replicates

DAI - Days after inoculation

Treatments	1	inoculation at		G.S.	6.7
	2	"	"	G.S.	8.9
	3	"	"	G.S.	10
	4	"	"	G.S.	10.5
	5	Ears only	"	G.S.	10.5

TABLE 1 — Field Experiment 1A

Maris Ranger — Treatment 1

Mean % Septoria nodorum infection (of 10 plants)

R1

DAI	Leaf				
	4	3	2	Flag	Ear
19	15.0	7.5	2.5	0	0
26	19.0	10.2	5.5	1.4	0
33	25.1	5.7	4.0	1.2	0
40	56.5	12.5	4.7	3.0	0
47		28.0	5.7	1.6	2.5
55		48.5	7.2	5.9	6.3
63		79.4	16.5	23.6	20.5
70			47.8	23.0	41.0

R2

19	59.0	38.0	5.1	0	0
26	41.5	17.1	3.9	0.7	0
33	31.6	24.1	9.7	1.0	0
40	19.6	24.5	14.0	10.2	0
47		34.0	30.5	24.0	3.5
55		35.0	22.5	26.3	4.3
63		51.4	30.5	31.6	11.0
70			35.0	31.6	21.5

Overall cultivar/treatment mean 17.78%

TABLE 2 — Field Experiment 1A

Maris Ranger — Treatment 2

Mean % Septoria nodorum infection (of 10 plants)

R1

DAI	Leaf				
	4	3	2	Flag	Ear
10	32.5	11.7	1.3	0	0
19	22.5	19.6	8.3	2.6	0
26	29.0	11.0	7.3	3.5	0
33	45.5	15.1	13.5	9.3	0
40	41.0	15.6	17.7	6.5	3.0
47		30.1	24.6	22.8	6.7
55		39.0	25.0	24.0	19.5
63		61.0	46.0	30.0	18.5
70				48.1	30.0

R2

10	36.6	15.5	2.6	0	0
19	13.5	55.5	1.3	0.4	0
26	14.5	7.8	2.7	0.9	0
33	29.0	6.2	6.2	1.8	0
40	32.0	15.1	15.0	9.6	1.6
47		11.6	16.0	6.6	3.5
55		20.0	22.5	20.0	12.1
63		30.0	13.0	8.6	11.0
70				32.5	11.0

Overall mean: 17.95 cultivar/treatment

TABLE 3 — Field Experiment 1A

Maris Ranger — Treatment 3

Mean % Septoria nodorum infection (mean of 10)

R1

DAI	Leaf				
	4	3	2	Flag	Ear
10	16.0	33.5	19.0	5.1	0
19	12.7	28.1	15.5	10.0	0
26	33.4	35.5	17.5	2.2	12.7
33	43.0	31.5	19.2	3.4	15.1
40	85.0	46.0	25.5	11.5	31.1
47		65.0	43.0	34.5	46.0
55		99.0	85.0	49.5	55.0

R2

10	33.0	55.5	44.5	10.0	0
19	29.5	51.0	39.0	4.8	0
26	62.0	61.5	45.0	30.5	1.5
33	73.5	63.5	37.5	26.1	4.4
40	81.0	66.0	49.0	44.5	7.1
47		99.0	25.9	39.5	10.0
55		94.0	86.0	50.7	34.0

Overall mean 42.85

TABLE 4 — Field Experiment 1A

cv Maris Ranger — Treatment 4

% Septoria nodorum infection (means of 10)

R1

DAI	Leaf				
	4	3	2	Flag	Ear
10	49.5	53.6	25.5	22.0	7.3
19	72.0	60.5	43.5	13.0	12.3
26	88.0	73.5	37.5	17.2	9.7
33	94.0	62.5	51.5	38.0	47.5
40	99.9	75.5	30.0	18.0	48.0
47		87.0	60.0	46.0	56.0

R2

10	14.0	20.0	19.6	8.5	11.1
19	33.0	38.5	25.0	14.5	40.0
26	53.5	45.5	25.6	8.4	23.5
33	70.0	44.5	25.0	13.6	33.5
40	78.0	70.1	36.1	33.0	33.0
47		95.0	52.0	43.0	34.0

Overall cv/treatment mean 41.51

TABLE 5 Field Experiment 1A

cv Maris Ranger Treatment 5

% Septoria nodorum infection (means of 10)

R1

DAI	Leaf				
	4	3	2	Flag	Ear
10	59.0	32.0	12.6	9.2	2.0
19	80.0	44.5	38.0	16.3	23.6
26	84.0	40.0	23.0	19.1	41.0
33	84.0	58.0	50.5	22.8	59.0
40	99.9	90.0	83.5	55.4	65.0
47		99.9	97.0	91.0	90.0

R2

10	36.5	22.5	6.7	2.2	20.1
19	77.5	26.0	9.3	5.1	17.2
26	99.9	97.0	93.2	81.9	15.0
33	92.5	32.0	12.0	10.1	24.5
40	99.9	54.5	11.5	33.1	40.5
47		99.9	62.0	36.2	15.5

Overall cv/treatment mean 47.48

TABLE 6 — Field Experiment 1A

Maris Ranger Uninoculated control

% Septoria nodorum infection (means of 10)

R1

DAI	Leaf				
	4	3	2	Flag	Ear
1	8.0	7.2	3.5	0.9	0
9	10.6	5.4	4.1	0.6	0
16	38.0	11.6	6.8	1.0	0
23	57.5	19.6	5.1	2.7	1.2
30		21.5	6.0	14.5	8.2
37		31.1	12.7	33.0	18.0
45		48.5	12.5	11.2	16.5
53			33.0	34.5	17.5

R2

1	14.1	9.3	1.8	1.8	0
9	41.5	12.7	1.8	1.9	0
16	80.5	25.0	8.1	5.3	0
23	92.5	36.0	14.6	14.4	0.9
30		31.5	12.1	18.5	3.0
37		45.0	23.0	21.6	6.5
45		43.5	22.0	13.2	8.0
53			47.8	38.0	8.5

Overall cv/treatment mean 16.49

TABLE 7 — Field Experiment 1A

Cappelle Desprez — Treatment 1

% Septoria nodorum infection (means of 10)

R1

DAI	Leaf				
	4	3	2	Flag	Ear
19	2.3	0	0	0	0
26	11.2	2.6	2.2	2.2	0
33	2.7	2.7	1.0	1.0	0
40	3.6	2.6	2.2	3.9	0
47		4.6	1.8	9.2	0.4
55		3.9	2.6	1.3	1.8
63		8.7	3.4	9.5	3.8
70			9.0	2.6	5.5

R2

19	26.0	6.5	0	0	0
26	31.5	11.0	3.9	0.5	0
33	49.0	9.5	5.2	1.0	0
40	58.5	13.0	6.1	3.9	0
47		14.5	9.0	7.3	1.8
55		28.5	10.1	9.9	1.3
63		56.0	24.5	22.7	3.4
70			43.5	26.0	6.7

Overall cv/treatment mean 8.3

TABLE 8 — Field Experiment 1A

Cappelle Desprez — Treatment 2

% Septoria nodorum infection (means of 10)

R1

DAI	Leaf				
	4	3	2	Leaf	Ear
10	7.2	4.3	5.1	0	0
19	32.5	8.4	3.8	2.5	0
26	25.0	12.0	5.0	2.4	0
33	42.5	14.5	6.0	8.0	0
40	57.5	11.0	4.6	3.4	0.5
47		18.0	9.1	7.1	2.0
55		30.5	23.5	9.1	6.6
63		67.0	54.0	15.0	15.0
70				40.0	17.2

R2

10	9.0	3.6	1.0	0	0
19	12.0	4.4	5.3	1.3	0
26	4.3	2.3	4.2	1.8	0
33	6.6	2.6	8.6	2.3	0
40	20.5	4.8	14.6	2.6	0.3
47		10.5	11.0	2.6	1.4
55		33.0	27.5	3.4	3.0
63		82.5	45.0	6.4	4.3
70				36.0	14.0

Overall cv/treatment mean 15.77

TABLE 9 — Field Experiment 1A

Cappelle Desprez — Treatment 3

% Septoria nodorum infection (means of 10)

R1

DAI	Leaf				
	4	3	2	Flag	Ear
10	35.1	8.3	3.4	3.9	0
19	68.5	25.5	12.6	16.0	0
26	76.5	39.5	14.5	14.5	1.9
33	87.0	36.0	11.0	9.6	2.6
40	94.0	42.0	23.0	25.0	4.8
47		56.5	30.0	27.0	6.6
55		99.9	78.0	39.0	6.1
63					

R2

10	10.6	3.6	2.2	7.6	0
19	21.0	5.1	4.7	11.6	0
26	31.0	14.0	7.5	11.5	1.8
33	72.0	11.5	9.7	15.0	1.8
40	81.0	28.0	16.0	29.0	2.6
47		57.5	26.0	18.6	4.7
55		99.9	79.0	66.0	6.6
63					

Overall cv/treatment mean 31.80

TABLE 10 Field Experiment 1A

cv Cappelle Desprez Treatment 4

% Septoria nodorum infection (mean of 10)

R1

DAI	Leaf				
	4	3	2	Leaf	Ear
10	65.5	29.0	13.0	19.0	4.3
19	77.5	23.0	13.0	16.5	5.8
26	90.5	39.5	15.5	9.5	4.2
33	95.5	64.5	23.0	21.5	9.0
40	99.9	99.9	50.0	21.5	13.5
47		99.9	82.0	64.0	31.5

R2

10	49.0	6.2	5.2	7.6	4.9
19	91.5	20.2	4.7	6.6	5.7
26	99.9	48.5	21.0	9.5	5.0
33	99.9	87.0	59.0	19.5	15.0
40	99.9	99.9	75.0	57.0	21.0
47		99.9	99.9	96.0	61.5

Overall treatment mean 44.10

TABLE 11 — Field Experiment 1A

cv Cappelle Desprez. Treatment 5

% Septoria nodorum infection (mean of 10)

R1

DAI	Leaf				
	4	3	2	Leaf	Ear
10	58.5	25.1	5.3	3.1	1.6
19	70.0	27.5	5.3	3.5	1.8
26	88.0	48.0	15.1	7.5	3.0
33	99.9	67.5	45.5	22.5	5.7
40	99.9	99.9	88.0	28.5	8.5
47		99.9	99.9	9.5	25.5

R2

10	66.0	16.0	10.5	5.4	4.8
19	64.0	22.5	12.0	4.8	6.3
26	67.0	31.5	19.0	11.8	5.4
33	79.0	37.5	12.6	14.5	7.6
40	99.9	68.0	46.0	23.0	5.6
47		99.9	84.0	31.5	18.0

Overall treatment mean 35.75

TABLE 12 — Field Experiment 1A

Cappelle Oesprez Uninoculated control

% Septoria nodorum infection (means of 10)

R1

DAI	Leaf				
	4	3	2	Flag	Ear
0	40.5	16.1	1.8	0.8	0
9	51.5	36.0	3.4	0.9	0
16	59.4	34.0	8.1	5.4	0
23	84.5	46.0	20.5	6.0	0.4
30		54.0	21.0	11.0	1.4
37		73.5	33.0	8.1	2.2
45		88.5	43.0	3.5	1.8
53			30.0	24.9	2.6

R2

0	16.0	6.3	1.9	0.9	0
9	12.2	8.7	4.0	2.2	0
16	30.7	11.8	5.1	1.8	0
23	11.0	3.0	2.2	2.7	1.4
30		8.3	3.4	2.2	1.8
37		6.1	3.5	2.6	3.4
45		12.0	4.4	1.4	3.5
53			22.5	13.2	4.0

Overall cv/treatment mean 15.45

TABLE 13 Field Experiment 1A

Maris Huntsman Treatment 1

Mean % Septoria nodorum infection (of 10 plants)

R1

DAI	Leaf				
	4	3	2	Leaf	Ear
19	28.5	1.4	1.0	0	0
26	45.0	20.5	9.2	2.2	0
33	52.9	48.5	20.6	1.8	0
40	61.0	56.5	47.5	7.8	0
47		59.0	49.0	29.0	7.1
55		58.0	47.0	30.9	3.9
63		63.9	53.0	28.1	20.0
70			55.0	17.9	15.0

R2

19	60.5	13.2	1.2	0.	0
26	77.5	62.5	22.6	0.8	0
33	73.0	60.5	33.2	4.8	0
40	85.5	71.0	54.0	27.5	0
47		75.0	64.0	53.5	7.6
55		74.0	55.5	53.6	3.9
63		78.0	57.0	57.1	3.0
70			58.0	51.7	9.1

Overall cv/treatment mean 32.29

TABLE 14 Field Experiment 1A

Maris Huntsman Treatment 2

% Septoria nodorum infection (means of 10)

R1

DAI	Leaf				
	4	3	2	Leaf	Ear
10	28.5	14.0	3.0	0	0
19	62.5	22.1	5.7	0.3	0
26	21.0	5.3	1.8	0.6	0
33	54.0	13.0	6.0	1.4	0
40	65.0	13.1	6.8	1.8	2.9
47		26.0	11.3	2.7	6.3
55		25.0	13.6	3.9	9.0
63		11.0	4.4	1.8	4.8
70			57.0	5.1	3.0

R2

10	49.4	10.1	0.3	0	0
19	58.5	21.5	6.4	0.5	0
26	44.0	17.6	1.6	0.5	0
33	54.5	18.6	12.7	0.8	0
40	75.0	3.7	3.8	5.0	16.5
47		35.0	4.7	4.5	3.9
55		41.0	34.1	3.0	3.9
63		77.5	5.9	3.6	6.6
70			88.0	6.2	3.9

Overall cv/treatment mean 13.73

TABLE 15 — Field Experiment 1A

Maris Huntsman — Treatment 3

% Septoria nodorum infection (means of 10)

R1

DAI	Leaf				
	4	3	2	Flag	Ear
10	56.5	33.5	20.8	1.4	0
19	74.5	48.5	18.3	1.8	0
26	96.5	67.0	39.0	14.5	5.9
33	99.0	68.0	23.0	6.6	4.9
40	89.9	77.5	32.0	9.3	10.5
47		86.0	33.0	11.6	10.0
55		97.0	48.0	3.7	4.8

R2

10	42.0	18.5	6.4	1.8	0
19	51.0	22.0	17.3	1.4	0
26	66.5	42.0	31.5	12.2	11.1
33	80.5	43.5	29.5	11.3	11.1
40	89.0	51.0	37.0	9.6	8.6
47		64.5	29.0	5.0	7.7
55		93.0	61.0	5.7	6.1

Overall cv/treatment mean 34.65

TABLE 16

Maris Huntsman Treatment 4

% Septoria nodorum infection (means of 10)

R1

DAI	Leaf				
	4	3	2	Flag	Ear
10	20.5	14.0	9.0	7.1	40.5
19	38.5	13.0	9.0	7.7	21.5
26	86.0	24.0	17.5	18.0	25.5
33	86.0	36.5	12.3	7.7	35.0
40	99.9	80.0	35.0	11.6	20.0
47		99.9	99.0	88.0	20.0

R2

10	81.5	49.5	40.5	34.1	1.2
19	73.5	51.5	49.5	2.7	6.8
26	82.0	57.5	53.5	48.0	68.0
33	91.2	57.5	48.0	38.8	4.9
40	99.9	99.0	6.7	4.7	87.0
47		76.0	86.0	65.0	90.0

Overall cv/treatment 38.19

TABLE 17

Maris Huntsman Treatment 5

% Septoria nodorum infection (means of 10)

R1

DAI	Leaf				
	4	3	2	Flag	Ear
10	66.0	33.5	20.5	13.7	24.5
19	73.5	27.0	15.0	6.8	13.6
26	82.0	33.0	26.0	8.2	18.5
33	85.0	40.5	20.0	9.2	2.4
40	99.9	57.0	29.0	4.0	7.1
47		99.0	84.0	42.0	14.6

R2 abandoned

Overall cv/treatment mean 33.21

TABLE 18 Field Experiment 1A

Maris Huntsman Uninoculated Control

% Septoria nodorum infection

R1

DAI	Leaf				
	4	3	2	Flag	Ear
0	28.5	16.4	2.8	0.8	0
9	43.5	26.2	5.6	0.2	0
16	45.5	25.8	4.1	1.9	0
23	54.5	17.4	9.2	1.0	2.5
30		32.0	13.5	9.6	3.1
37		36.5	16.6	3.0	10.0
45		8.0	10.2	2.1	4.7
53			23.0	3.0	7.0

R2

0	13.6	1.9	1.4	1.0	0
9	9.9	2.7	0.5	0.2	0
16	6.3	3.7	0.9	0.7	0
23	13.6	4.1	1.8	1.4	1.2
30		1.9	1.0	1.0	5.0
37		9.0	1.0	1.0	4.0
45		11.4	1.4	1.4	8.6
53			26.0	3.1	6.3

Overall cv/treatment mean 9.21

APPENDIX 1B

Tables 19 - 24 (Winter wheat 1975)

Tables 25 - 26 (Winter wheat 1974 and 1975)

Detailed study of infection

Values are means of up to 5 leaves.

Key

TI - Type of Infection

LA - Leaf area cm^2

%I - % Leaf area affected by *S. nodorum*

TLN - Total lesion number

TLN >3 - Total lesion number exceeding 3 mm

% >3 - % " " " " 3 mm

L/cm^2 - Lesions per cm^2

IL - Internode length

T - Time in days (after inoculation)

Tables 25 and 26 are summary tables for the same experiment performed in 1974 and 1975.

TABLE 19 — Field Experiment 1B

Maris Ranger — Flag Leaf

TI	LA	%1	TLN	TLN >3	L/cm^2	IL	T	
	82.5	0	0	0	0.00	8.8	0	(23.5.75)
	84.8	0.4	1.0	0	0.00	18.4	6	
	84.7	0.4	0.8	0	0.00	22.6	13	
Natural		2.6	3.2	0.8	0.04	23.6	20	
		3.4	3.8	2.0	0.04	23.8	27	
		5.2	3.4	2.2	0.04	24.0	34	
		7	3.8	2.0	0.04	24.0	41	
		7	4.4	2.8	0.05	24.0	48	
		33	22.7	16	0.27	24.0	55	
	77.3	1	3	0	0.04	23	0	(6.6.75)
		6	8.4	3	0.11	25.6	7	
		9	9.8	3	0.12	26.2	14	
Aritificial Inoculation		9	11.6	5	0.15	26.4	21	
		20	13.4	8	0.17	26.4	35	
		47	18.0	15	0.25	25.5	42	

TABLE 20

Maris Ranger 2nd Leaf

TI	LA	%1	TLN	TLN >3	L/cm^2	IL	T	
Natural	73.6	0.8	1.6	0.6	0.02	116.2	0	(15.5.75)
		4.2	2.4	1.0	0.03	116.2	8	
		5.0	2.8	1.0	0.04		14	
		5.0	3.4	1.0	0.05		21	
		5.0	3.0	1.0	0.04		28	
		9.0	6.0	5.0	0.08		35	
		61.0	29.5	9.8	0.41		49	
Artificial Inoculation	62.5	0.6	2.2	0.2	0.03	112.8	0	(6.6.75)
		10.0	17.0	7.8	0.28	112.8	6	
		10.0	19.8	9.0	0.42		13	
		16.0	30.8	17.4	0.50		20	
		28.0	32.6	25.2	0.53		34	
		86.0					48	

TABLE 21

Cappelle Desprez Flag Leaf

TI	LA	%I	TLN	TLN>3	L/cm^2	IL	T	
	63.4	0.4	1.4	0	0.01	10.2	0	(29.5.75)
	63.4	0.8	2.4	0	0.04	16.6	7	
	63.2	0.8	2.2	0	0.03	28.4	14	
Natural		2.6	3.6	1.2	0.06	28.4	21	
		3.4	3.6	1.2	0.06	27.8	28	
		4.2	3.4	1.2	0.06	27.8	35	
		4.2	3.2	1.2	0.05	27.8	42	
		18	20.4	9.8	0.32	27.8	49	
	63.1	0.2	0.4	0	0.01	28.4	0	(12.6.75)
		1.0	3.0	0.6	0.05	28.8	7	
		2.6	5.0	2.2	0.08	28.8	14	
Artificial Inoculation		4.2	5.4	2.4	0.08	28.8	21	
		6.0	8.2	2.6	0.13	28.8	28	
		38.8	29.5	14.5	0.40	29.5	35	

TABLE 22

Cappelle Desprez 2nd Leaf

TI	LA	%I	TLN	TLN>3	L/cm^2	IL	T	
	59.4	0	0	0	0	87.4	0	(15.5.75)
		0	0	0	0	97.2	8	
		1.0	3.4	0	0.06	106.8	14	
		1.0	4.0	0	0.06	112.6	21	
Natural		1.8	3.8	0.8	0.06	123.0	28	
		5.2	5.0	1.0	0.08	123.2	35	
		6.0	5.8	1.0	0.10	123.2	42	
		7.0	5.2	1.0	0.09	123.2	49	
		8.0	6.8	4.2	0.11	123.2	56	
		62.0	22.5	12.0	0.36	124.5	70	
	59.2	1.2	1.6	0.4	0.03	118.6	0	(12.6.75)
		2.2	2.6	1.6	0.07	116.3	7	
		2.6	4.2	2.2	0.08	116.4	14	
Artificial Inoculation		5.3	3.4	2.0	0.06	116.3	21	
		12.0	13.4	9.0	0.23	116.4	28	
		78.8					42	

TABLE 23 — Field Experiment 1B

Maris Huntsman — Flag Leaf (Values are means of 5 plants)

TL	LA	%I	TLN	TLN>3	L/cm^2	TL	T	
	81.4	0	0	0	0	5.5	0	(23.5.75)
	81.0	0.6	0.8	0	0.01	17.9	6	
	81.3	0.8	1.2	0	0.01	24.4	13	
Natural		1	1.2	0	0.01	24.6	20	
		1	1.6	0.4	0.02	25.6	27	
		1	2.2	0.8	0.02	25.2	34	
		1	2.6	0.8	0.03	25.2	41	
		1.8	2.4	1.2	0.02	25.2	48	
	73.6	0.6	0.8	0	0.01	24.2	-3	(6.6.75)
		1	2.0	0	0.03	28.0	3	
		1	2.2	0.6	0.03	28	10	
Artificial Inoculation		1	2.6	0.8	0.04	28	17	
		1	2.2	0.8	0.03	28	24	
		1	2.4	1.0	0.03	28	31	

TABLE 24

Maris Huntsman 2nd Leaf

TI	LA	%I	TLN	TLN >3	L/cm^2	TL	T	
	65.4	0	0	0	0	83.6	0	(15.5.75)
	65.1	0	0	0	0	96.0	8	
	64.3	0	0	0	0	107.8	14	
Natural	63.9	0.8	1.6	0.2	0.02	114	21	
		0.8	2.2	0.4	0.04	114	28	
		1.0	3.2	0.3	0.05	114	35	
		1.0	3.4	1.0	0.05	114	42	
		1.0	2.8	1.0	0.04	114	49	
		3.6	3.2	1.8	0.05	114	56	
	64.7	0.6	1.4	0	0.02	115.2	-3	(6.6.75)
		0.8	2.6	0	0.04	115.2	3	
		0.8	2.6	0	0.04	115.2	10	
Artificial Inoculation		1.8	4.6	0.8	0.07	115.2	17	
		1.8	5.2	1.0	0.08	115.2	24	
		6.2	10.4	4.2	0.16	115.2	31	

TABLE 25

Summary Table of Field Experiment 18 for 1974

(Compare with Table 16 in text)

Part of Plant + Variable	Cultivar / Infection Type					
	Natural Infection			Artificial Inoculation		
	Huntsman	Cappelle	Ranger	Huntsman	Cappelle	Ranger
Flag Leaf						
%I	1.3	9.2	5.7	5.4	13.7	9.7
'r'	0.019	0.053	0.084	0.055	0.061	0.069
L/cm^2	0.29	0.84	0.58	0.88	1.77	1.41
% >3	7.4	9.6	23.0	11.2	25.7	39.0
IL	28.1	25.7	24.1	22.4	25.5	21.7
Leaf 2						
%I	5.2	9.0	10.4	7.8	12.2	21.9
'r'	0.077	0.066	0.054	0.037	0.068	0.023
L/cm^2	0.37	0.73	0.96	1.38	1.18	2.83
% >3	20.1	30.4	34.8	22.4	26.7	52.0
IL	15.1	15.0	14.0	13.6	14.8	13.4
Ear						
%I	5.3	7.4	14.2	6.2	6.1	19.6
L/cm^2	6.18	5.03	9.03	6.39	4.90	12.20
% >3	39.9	31.2	55.7	27.6	31.0	62.9
IL	24.5	24.0	25.9	20.3	21.7	23.0

TABLE 26

Summary Table of Field Experiment 1B for 1975

(Compare with Table 16 in text)

Part of Plant + Variable	Cultivar	Infection Type				
		Natural Infection		Artificial Inoculation		
	Maris Fundin	Capitole	Goya	Maris Fundin	Capitole	Goya
Flag Leaf						
%I	4.4	2.1	1.9	6.7	11.3	11.1
'r'	0.064	0.015	0.058	0.064	0.048	0.119
L/cm^2	0.08	0.02	0.04	0.13	0.26	0.63
% > 3	31.6	32.1	20.1	22.8	31.8	11.6
IL	20.9	23.1	26.5	21.4	21.3	23.1
2nd Leaf						
%I	9.5	5.9	6.9	18.3	15.7	15.2
'r'	0.053	0.066	0.100	0.074	0.055	0.059
L/cm^2	0.14	0.10	0.10	0.29	0.35	0.42
% > 3	35.3	27.5	40.9	43.8	36.8	26.9
Plant Height	95.6	99.7	100.0	93.4	100.1	100.0

APPENDIX 2A

Tables 27-46

Weekly Mean % Septoria nodorum infection 1974

Spring Wheat

Values are means of up to 10 culms in a clump.

Key R1, R2 } Replicates

DAI - Days after inoculation

Treatments	1	inoculation at G.S.	6.7
	2	" " G.S.	8.9
	3	" " G.S.	10
	4	" " G.S.	10.5

TABLE 27 Field Experiment 2A

cv Bounty 208 Treatment 1

% Septoria nodorum infection

R1

DAI	Leaf			
	3	2	Flag	Ear
10	40.0	9,2	0.6	-
19	49.5	14.2	3.9	-
28	84.5	27.0	24.1	0.1
35	85.5	21.1	30.5	2.2
42	91.0	27.0	37.0	4.6
49	87.0	28.1	37.1	6.4
56		49.6	68.6	4.9

R2

10	30.0	8.9	0.1	-
19	56.1	17.5	10.7	-
28	90.0	18.0	10.0	0
35	81.5	42.0	26.6	3.5
42	84.0	54.0	44.0	7.2
49	99.9	62.1	55.7	12.5
56		71.9	58.9	8.9

Overall cultivar/treatment mean 35.5

TABLE 28 — Field Experiment 2A

cv Bounty 208 — Treatment 2

% Septoria nodorum infection

R1

DAI	Leaf			
	3	2	Flag	Ear
10	39.0	14.2	1.4	-
19	66.0	22.1	3.2	0.2
28	77.0	27.6	3.4	2.2
35	72.0	35.0	21.5	5.4
42	72.8	37.8	27.8	11.8
49	99.9	65.6	57.5	18.9
56		75.0	91.3	40.0

R2

10	45.0	14.6	6.1	-
19	70.0	22.0	10.0	0
28	68.1	22.1	20.6	7.2
35	76.0	32.5	41.6	15.2
42	81.3	26.3	60.0	15.8
49	95.6	49.4	52.9	36.1
56		56.0	68.0	33.5

Overall cultivar/treatment mean 39.5

TABLE 29 Field Experiment 2A

cv Bounty 208 Treatment 3

% Septoria nodorum infection

R1

DAI	Leaf			
	3	2	Flag	Ear
10	58.6	22.0	17.6	7.6
19	53.0	12.0	19.6	31.0
28	57.0	20.0	32.1	31.0
35	69.0	27.0	31.5	32.0
42		40.0	51.1	35.7
49		53.0	73.3	33.5

R2

10	60.0	24.0	30.0	0
19	66.5	26.5	23.1	27.7
28	78.5	39.5	31.6	26.6
35	83.9	36.5	48.9	40.0
42		44.3	60.8	37.5
49		52.8	82.1	30.5

Overall cultivar/treatment mean 45.0

TABLE 30 Field Experiment 2A

cv Bounty 208 Treatment 4

% Septoria nodorum infection

R1

DAI	Leaf			
	3	2	Flag	Ear
10	75.0	31.0	10.0	61.5
19	70.5	44.4	25.1	60.0
28	87.0	43.0	35.5	66.5
35	82.5	61.7	36.7	80.0
42		77.8	81.3	79.3

R2

10	73.9	35.5	30.1	59.0
19	78.4	54.5	64.5	73.5
28	88.5	87.0	86.0	78.5
35	99.9	99.9	99.9	66.5
42		99.9	99.9	79.0

Overall cultivar/treatment mean 69.1

TABLE 31 Field Experiment 2A

cv Bounty 208 CONTROL

% Septoria nodorum infection

R1

DAI	Leaf			
	3	2	Flag	Ear
1	47.0	7.3	0.3	-
9	53.5	25.0	4.5	-
18	64.5	40.6	6.0	-
25	70.5	36.7	6.2	2.6
32	85.7	30.0	29.3	9.3
39	96.4	62.9	52.1	20.1
46		69.2	66.7	30.8

R2

1	44.0	6.3	0.3	-
9	67.8	11.4	6.1	-
18	75.0	18.0	7.0	-
25	70.0	24.6	28.2	11.7
32	87.5	34.5	26.4	7.6
39	62.1	40.0	52.0	31.4
46		63.0	44.0	23.0

Overall cultivar/treatment mean 35.0

TABLE 32 Field Experiment 2A

B 519 Treatment 1

% Septoria nodorum infection

R1

DAI	Leaf			
	3	2	Flag	Ear
10	10.4	1.6	0.5	-
19	39.0	9.7	2.1	-
28	39.2	13.1	1.8	0
35	58.7	16.9	2.3	1.7
42	57.1	18.3	3.0	6.0
49	81.5	38.5	5.7	6.8
56		66.5	8.7	8.1

R2

10	4.3	2.0	0.6	-
19	57.8	6.0	3.1	-
28	58.0	10.0	3.0	0
35	95.0	25.3	17.1	5.0
42	97.2	34.4	19.4	10.1
49	99.9	54.5	20.5	10.7
56		75.0	13.3	17.7

Overall cultivar/treatment mean 25.7

TABLE 33 Field Experiment 2A

cv B 519 Treatment 2

% Septoria nodorum infection

R1

DAI	Leaf			
	3	2	Flag	Ear
10	13.3	8.2	2.5	-
19	50.1	16.6	1.7	0.2
28	23.9	10.4	1.9	1.2
35	29.0	11.8	3.3	3.9
42	51.8	51.1	7.7	15.8
49	81.5	35.7	13.7	16.0
56		56.4	32.1	18.8

R2

10	36.6	8.4	3.7	-
19	60.0	6.0	2.0	0
28	82.5	7.3	2.0	1.4
35	90.0	17.9	4.0	5.5
42	81.9	7.1	7.4	7.3
49	91.3	35.0	19.4	11.0
56		57.9	57.1	11.1

Overall cultivar/treatment mean 26.3

TABLE 34 Field Experiment 2A

cv B 519 Treatment 3

% Septoria nodorum infection

R1

DAI	Leaf			
	3	2	Flag	Ear
10	13.6	3.0	2.0	19.0
19	28.6	5.0	11.6	4.9
28	47.0	11.5	2.9	16.5
35	78.5	18.6	3.9	12.0
42		70.5	22.7	12.6
49		94.0	44.0	11.1

R2

10	10.0	5.0	4.0	0
19	73.0	20.6	3.0	2.4
28	79.2	28.5	10.8	15.2
35	97.1	25.0	17.0	17.5
42		70.0	37.5	16.6
49		99.9	50.0	30.0

Overall cultivar/treatment mean 33.9

TABLE 35 Field Experiment 2A

cv B 519 Treatment 4

% Septoria nodorum infection

R1

DAI	Leaf			
	3	2	Flag	Ear
10	5.5	6.1	10.5	3.3
19	21.0	10.1	15.4	8.7
28	62.0	17.1	4.4	10.6
35	99.9	33.0	18.2	16.7
42		92.5	49.0	16.6

R2

10	67.5	35.6	17.7	1.0
19	85.9	19.0	9.1	2.7
28	96.9	60.0	18.5	3.0
35	99.9	40.0	36.3	4.3
42		99.9	50.0	10.0

Overall cultivar/treatment mean 36.5

TABLE 36 Field Experiment 2A

cv B 519 CONTROL

% Septoria nodorum infection

R1

OAI	Leaf			
	3	2	Flag	Ear
1	5.0	3.9	1.2	0
9	19.4	7.8	2.6	0
18	31.2	26.3	6.5	0
25	83.3	36.7	25.8	13.2
32	47.5	32.6	16.8	13.7
39	83.0	37.0	19.0	20.0
46		60.0	43.3	22.9

R2

1	3.1	1.3	0.5	0
9	35.0	6.2	2.6	0
18	60.0	24.0	6.5	0
25	71.4	31.4	40.3	11.5
32	95.7	49.3	56.4	19.6
39	99.9	66.0	27.5	18.7
46		58.5	41.6	24.2

Overall cultivar/treatment mean 30.5

TABLE 37 Field Experiment 2A

cv Fortuna. Treatment 1

% Septoria nodorum infection

R1

DAI	Leaf			
	3	2	Flag	Ear
10	4.5	1.0	0	0
19	11.6	3.6	1.6	0
28	31.7	23.3	0.8	1.0
35	20.0	17.0	1.0	11.7
42	24.1	24.3	4.3	13.3
49	25.8	23.7	12.3	20.8
56	46.7	29.2	10.0	20.0

R2

10	22.5	3.9	0.1	0
19	98.4	16.8	3.7	0
28	70.0	10.0	7.0	0
35	73.9	26.3	3.8	3.3
42	75.6	28.4	9.0	7.7
49	72.5	38.0	8.4	8.8
56	86.4	35.3	12.8	12.9

Overall cultivar/treatment mean 20.0

TABLE 38 Field Experiment 2A

cv Fortuna Treatment 2

% Septoria nodorum infection

R1

DAI	Leaf			
	3	2	Flag	Ear
10	33.7	9.6	9.6	0
19	42.1	28.4	15.4	1.0
28	46.1	26.9	11.9	5.7
35	45.1	48.6	26.2	17.1
42	45.0	68.8	45.0	22.5
49	99.9	67.5	59.9	29.5
56		99.4	96.1	36.7

R2

10	14.5	18.7	3.3	0
19	20.0	20.0	4.0	0
28	19.1	22.7	8.1	14.6
35	34.4	31.5	20.2	30.5
42	54.4	41.9	35.9	28.8
49	97.2	81.1	67.8	27.1
56		99.9	99.9	22.8

Overall cultivar/treatment mean 38.5

TABLE 39 Field Experiment 2A

cv Fortuna Treatment 3

% Septoria nodorum infection

R1

DAI	Leaf			
	3	2	Flag	Ear
10	21.7	26.2	15.2	0
19	15.0	16.1	12.1	33.3
28	26.3	19.4	20.6	47.0
35	49.5	36.3	12.9	44.4
42		62.0	56.9	27.5
49			96.9	2.4

R2

10	30.0	20.0	17.0	0
19	34.4	14.4	11.6	7.1
28	46.3	27.5	19.5	12.8
35	40.8	15.8	25.0	18.3
42		29.2	46.7	21.7
49			99.9	30.0

Overall cultivar/treatment mean 38.1

TABLE 40 Field Experiment 2A

cv Fortuna Treatment 4

% Septoria nodorum infection

R1

DAI	Leaf			
	3	2	Flag	Ear
10	7.6	16.5	7.7	44.8
19	22.0	32.1	15.5	50.6
28	17.5	40.6	12.5	57.5
35	60.0	38.0	60.0	57.5
42		99.9	99.9	71.4

R2

10	25.8	3.6	2.0	46.7
19	21.9	10.6	12.4	57.5
28	10.8	15.0	6.9	51.9
35	67.9	35.0	26.7	55.8
42		82.1	52.9	57.8

Overall cultivar/treatment mean 38.9

TABLE 41 Field Experiment 2A

cv Fortuna CONTROL

% Septoria nodorum infection

R1

DAI	Leaf			
	3	2	Flag	Ear
1	7.0	0.5	0	0
9	29.4	4.4	0.4	0
18	41.0	12.8	3.8	0.8
25	39.4	12.6	4.9	6.0
32	56.4	27.8	6.8	9.7
39	67.1	50.0	12.0	13.3
46		69.2	59.2	26.8

R2

1	8.5	1.3	1.2	0
9	25.1	5.1	1.4	0
18	35.0	4.0	10.0	0
25	54.3	4.8	11.6	9.6
32	45.8	13.5	3.6	14.3
39	63.3	63.6	4.2	7.0
46		43.6	32.1	20.0

Overall cultivar/treatment mean 22.7

TABLE 42 Field Experiment 2A

cv Svenno Treatment 1

% Septoria nodorum infection

R1

DAI	Leaf			
	3	2	Flag	Ear
10	0.9	0.1	0	0
19	4.3	3.0	0.8	0
28	13.5	3.6	1.3	0
35	11.7	4.8	1.4	0.6
42	15.6	3.0	2.5	1.6
49	24.0	5.2	4.4	1.9
56	60.0	16.2	18.2	4.4

R2

10	2.2	0.2	0	0
19	27.5	3.4	3.0	0
28	40.0	4.0	5.0	0
35	48.5	3.7	3.9	0.5
42	49.0	6.9	7.3	2.1
49	65.5	5.3	4.9	2.6
56	92.5	13.8	18.7	5.9

Overall cultivar/treatment mean 11.1

TABLE 43 Field Experiment 2A

cv Svenno Treatment 2

% Septoria nodorum infection

R1

DAI	Leaf			
	3	2	Flag	Ear
10	5.2	2.6	2.7	0
19	24.8	4.1	2.7	0
28	30.8	2.2	1.9	0.8
35	37.0	5.3	10.0	1.8
42	59.0	16.3	11.4	2.5
49	70.0	33.8	35.5	6.4
56		91.0	59.0	13.5

R2

10	23.3	5.9	4.5	0
19	30.0	15.0	3.0	0
28	41.7	25.9	12.9	1.1
35	45.8	30.5	16.0	1.7
42	70.0	23.5	16.6	3.3
49	66.6	23.5	33.7	5.5
56		30.0	50.0	20.0

Overall cultivar/treatment mean 23.7

TABLE 44 — Field Experiment 2A

cv Svenno — Treatment 3

% Septoria nodorum infection

R1

DAI	Leaf			
	3	2	Flag	Ear
10	36.2	11.0	1.3	0.7
19	36.6	19.2	4.8	0.9
28	47.5	43.2	7.4	2.2
35	54.5	52.2	26.1	5.1
42		63.9	43.7	2.9
49		63.9	43.7	2.9

R2

10	33.7	11.9	1.4	0.7
19	43.0	13.6	5.7	2.1
28	61.3	5.3	5.6	3.6
35	61.3	54.5	33.3	4.0
42		71.5	60.5	14.0
49		71.5	60.5	2.4

Overall cultivar/treatment mean 33.1

TABLE 45 Field Experiment 2A

cv Svenno Treatment 4

% Septoria nodorum infection

R1

DAI	Leaf			
1	3	2	Flag	Ear
10	15.4	2.7	1.8	0.5
19	27.0	9.7	2.0	1.2
28	51.1	10.2	1.5	2.0
35	66.1	24.6	15.6	3.4
42		53.0	26.0	15.7

R2

10	47.2	4.4	1.8	0.5
19	46.8	8.3	1.4	1.6
28	55.1	4.1	1.4	2.4
35	67.0	22.8	17.4	3.0
42		26.2	14.6	4.1

Overall cultivar/treatment mean 21.7

TABLE 46 — Field Experiment 2A

cv Svenno — CONTROL

% Septoria nodorum infection

R1

DAYS	Leaf			
	3	2	Flag	Ear
1	3.5	0.4	0	0
9	26.3	3.0	0.1	0
18	47.9	6.1	2.3	0
25	63.0	9.8	2.3	0.5
32	58.9	11.8	6.0	2.3
39	60.0	15.3	7.8	2.9
46		18.8	7.0	4.3

R2

1	2.0	0	0	0
9	38.6	3.4	0.3	0
18	40.0	7.0	3.0	0
25	63.0	11.7	3.3	0.4
32	77.8	16.2	3.5	1.6
39	73.9	16.9	2.3	1.8
46		18.0	2.8	4.6

Overall cultivar/treatment mean 17.0

APPENDIX 2B

TABLES 47-58

DETAILED STUDY OF INFECTION, SPRING WHEAT 1974

Values are means of up to 10 culms in a clump.

Key as for Appendix 1B P. 263

and PL = peduncle length

TABLE 47 — Field Experiment 28

Bounty 208 — Flag

TI	LA	%I	TLN	TLN >3	L/cm2	IL	T
	12.4	1.0	3.0	0.6	0.26	13.8	1
		15.2	9.0	4.6	0.78	16.6	5
		19.2	11.4	5.0	1.02	16.4	12
Natural		15.0	13.3	6.0	1.10	17.0	19
		40.0	19.0	8.5	1.35	17.0	27
		50.0	23.8	12.3	2.00	17.0	33
		60.0	25.0	19.5	2.35	16.5	40
	11.6	5.0	12.0	5.0	1.02	16.6	1
Artificial	13.8	5.0	11.0	5.6	0.86	16.8	5
Inoculation		35.0	14.4	8.0	1.12	16.2	12
		32.5	23.0	9.3	1.88	16.6	19
		32.0	21.5	11.8	1.78	16.6	27
		42.5	22.5	14.0	1.83	16.6	33
		60.0	31.7	19.3	2.63		40

TABLE 48

B 519 — Flag

TI	LA	%I	TLN	TLN >3	L/cm^2	IL	T
	15.0	4.4	12.8	1.6	1.24	17.0	1
	15.1	8.6	14.6	2.4	1.16	17.3	5
Natural		19.2	15.4	4.2	1.22	16.6	12
		17.2	17.8	3.8	1.36	16.8	19
		11.5	16.5	3.5	1.18	16.5	27
		16.3	21.5	4.5	1.63	16.5	33
		22.5	30.5	6.3	2.20	16.5	40
	10.1	4.4	11.0	3.6	0.98	17.4	1
Artificial		7.6	17.4	3.2	1.78	16.9	5
Inoculation		7.6	18.2	3.4	1.84	17.1	12
		10.6	14.6	2.8	1.50	17.1	19
		6.8	15.0	3.4	1.20	17.1	27
		13.8	32.5	4.2	2.93	17.4	33

TABLE 49 Field Experiment 28

Fortuna Flag

TI	LA	%I	TLN	TLN > 3	L/cm^2	IL	T
	12.2	0.4	1.2	0.2	0.14	19.5	1
		3.8	4.0	1.4	0.38	19.2	5
Natural		20.2	9.2	4.2	0.84	19.7	12
		11.5	9.8	4.3	0.85	18.8	19
		23.8	13.5	6.3	1.13	18.8	27
		25.0	26.0	19.0	1.60	21.0	33
	16.8	5.2	14.8	7.2	0.92	24.3	1
Artificial Inoculation	17.6	6.0	19.0	7.6	1.06	24.9	5
		13.0	25.4	10.8	1.40	24.6	12
		14.0	25.8	9.4	1.48	25.2	19
		15.0	24.6	10.2	1.38	25.2	27
		32.0	32.4	14.2	1.98		33

TABLE 50

Svenno Flag

TI	LA	%I	TLN	TLN > 3	L/cm^2	IL	T
	11.4	1.6	2.0	0.4	0.16	15.8	2
Natural		7.6	5.0	2.8	0.42	15.6	9
		9.2	5.4	2.4	0.46	16.1	16
		17.2	9.2	3.2	0.76	16.1	24
		17.0	13.4	4.4	1.10		30
		8.8	17.5	2.8	1.12		37
	9.7	1.2	2.6	0	0.28	16.7	2
Artificial Inoculation		3.8	7.0	1.6	0.70	16.2	9
		11.6	10.0	2.8	1.02	16.3	16
		4.5	9.5	1.5	0.98	16.6	24
		8.3	17.3	4.7	1.70	16.2	30
		10.3	14.3	4.5	1.40	16.6	37

TABLE 51 Field Experiment 28

Bounty 208 2nd Leaf

TI	LA	%I	TLN	TLN >3	L/cm^2	IL	T
	11.4	7.0	7.2	2.8	0.66	8.6	1
		14.0	12.2	4.8	1.10	7.3	5
Natural		22.0	15.2	6.6	1.36	7.4	12
		25.0	20.0	8.8	1.90	7.4	19
		38.8	20.8	14.8	2.45		27
		45.0	18.5	11.0	2.55		33
	10.0	15.0	4.0	2.2	0.42	7.4	1
		15.2	6.4	3.4	0.70	8.2	5
Artificial Inoculation		15.2	10.0	4.4	1.04	8.3	12
		31.0	13.0	8.2	1.30	8.3	19
		32.0	13.4	10.6	1.42		27
		20.0	11.0	8.3	1.37		33

TABLE 52

B 519 2nd Leaf

TI	LA	%I	TLN	TLN >3	L/cm^2	IL	T
	10.0	14.2	9.6	5.4	0.96	9.0	1
		28.2	12.8	7.4	1.28	7.8	5
Natural		22.5	16.5	4.8	1.98	8.6	12
		30.0	19.3	8.3	2.38	8.6	19
		45.0	26.7	17.3	2.67		27
		42.5	31.0	19.0	2.39		33
	10.0	3.6	6.4	2.4	0.70	8.2	1
		10.0	14.8	5.4	1.66	8.4	5
		15.0	18.2	7.4	2.00	8.4	12
Artificial Inoculation		23.0	26.4	9.0	3.12		19
		10.0	28.0	5.5	2.57		27
		55.0	46.5	20.5	4.28		33

TABLE 53 Field Experiment 28

Fortuna 2nd Leaf

TI	LA	%I	TLN	TLN>3	L/cm^2	IL	T
	14.5	10.4	1.6	0.2	0.40	9.8	1
		3.0	6.5	1.5	0.48	9.4	5
Natural		15.0	16.3	8.0	1.15	9.7	12
		15.0	17.7	8.0	1.33	9.7	19
		40.0	23.0	15.0	1.80		27
		30.0	25.0	12.0	2.10		33
	15.9	14.0	25.6	14.8	1.60	11.2	1
		17.0	26.2	14.6	1.78	11.2	5
Artificial Inoculation		21.0	31.2	16.4	1.98		12
		25.0	34.8	17.6	2.36		19
		40.0	41.3	19.7	2.80		27
		50.0	42.0	27.0	3.10		33

TABLE 54

Svenno 2nd Leaf

TI	LA	%I	TLN	TLN>3	L/cm^2	IL	T
	16.2	0.4	2.2	0.8	0.14	9.8	2
		12.2	6.4	3.0	0.40	9.2	9
Natural		14.2	8.4	3.6	0.50	9.4	16
		17.0	10.0	5.2	0.64	9.4	24
		20.0	14.2	6.6	0.86		30
		21.0	20.8	9.8	1.30		37
	12.9	4.0	1.0	0.4	0.10	9.4	2
		21.4	7.0	4.4	0.50	8.8	9
		24.2	13.2	4.6	1.00	8.9	16
Artificial Inoculation		15.3	10.3	7.0	0.80	8.9	24
		10.5	8.5	6.0	0.60		30
		17.5	14.5	9.5	1.05		37

TABLE 55 Field Experiment 28

Bounty 208 Ear

TI	%I	TLN	TLN>3	PL	T
Artificial Inoculation	0.2	0.2	0	16.8	1
	15.0	4.8	4.6	19.2	5
	14.0	9.0	6.2	19.6	12
	21.0	10.8	6.6	19.6	19
	28.0	13.0	8.6	19.6	27
	36.0	14.4	8.6	20.0	33
Natural	1.0	0.4	0	19.2	1
	5.4	2.6	2.0	18.8	5
	7.2	5.2	3.8	18.8	12
	22.0	10.6	5.8	18.8	19
	22.0	10.8	6.6	19.4	27
	23.0	15.0	7.4	19.4	33

TABLE 56

B 519 Ear

TI	%I	TLN	TLN>3	PL	T
Natural	1.0	1.4	0	29.2	5
	3.6	4.8	0.2	29.4	12
	20.3	10.0	5.0	29.4	19
	18.7	8.0	3.3		27
	23.7	12.3	4.7		33
Artificial inoculation	8.0	5.4	2.6	30.8	5
	11.5	8.0	3.2	31.0	12
	14.0	10.4	3.4	31.0	19
	15.0	13.4	4.2		27
	24.0	13.8	6.2		33

TABLE 57 Field Experiment 28

Fortuna Ear

TI	%I	TLN	TLN >3	PL	T
Natural	5.0	3.0	1.8	26.6	5
	9.4	6.0	3.0	27.0	12
	13.4	7.6	3.8	27.0	19
	14.4	8.2	4.2	27.2	27
	18.0	9.0	5.2	27.2	33
Artificial Inoculation	12.2	5.2	3.2	34.8	1
	29.2	15.2	6.0	34.6	5
	35.2	15.8	15.2	35.0	12
	44.0	18.0	16.4	35.0	19
	43.0	20.2	16.8		27
	45.0	27.0	17.0		33

TABLE 58

Svenno Ear

TI	%I	TLN	TLN >3	PL	T
Natural	0	0	0	13.2	9
	1.0	1.6	0.6	13.5	16
	1.0	1.5	0.5	13.5	24
	2.3	2.0	1.0		30
	2.6	2.6	1.0		37
Artificial Inoculation	0.4	1.0	0	17.6	9
	0.8	1.8	0	19.1	16
	0.8	1.8	0	19.1	24
	1.8	2.0	0.6	19.2	30
	1.8	2.8	0.4	19.2	37

TABLE 59 % Germination of S. nodorum in eluted Cladosporium inhibitors from maximally and minimally hydrolysed infected and control field grown wheat plants at various growth stages

Growth stage	Infection	Hydrolysis	Part	Maris Huntsman				Maris Ranger			
				x 2 Tissue conc.	Tissue conc.	x ½	C	x 2 Tissue conc.	Tissue conc.	x ½	C
6-7	HEALTHY	MINIMUM	2nd leaf	90	85	93	90	-	-	-	-
11			Ear	90	97	90	90	-	-	-	-
10	30%	MINIMUM	Flag	-	93	97	97	-	95	90	97
11	50%		Flag	-	83	90	87	-	90	90	87
11	50%		Ear	87	-	-	-	-	87	93	97
10	HEALTHY	MAXIMUM	Flag	67	70	90	90	90	97	100	90
10			Flag proximal portion of infected leaf	-	70	93	97	-	-	-	-
10			Ear unemerged	87	87	90	90	85	97	100	90
11			Flag	80	93	100	90	87	90	100	90
11			Ear	80	73	100	95	77	80	90	95
10	30%	MAXIMUM	Flag	-	73	80	87	-	-	-	-
11	50%		Flag (A)	-	97	100	100	A & BC -	0	63	100
			Flag (BC)	-	83	88	100				
11	50%		Ear	-	90	93	97	-	67	85	97

TABLE 60. Germ tube growth of S. nodorum in eluted Cladosporium inhibitors from maximally and minimally hydrolysed infected and control field grown wheat plants at various growth stages.

Growth Stage	Infection	Hydrolysis	Part	Maris Huntsman				Maris Ranger			
				x 2 Tissue conc.	Tissue conc.	x ½	C	x 2 Tissue conc.	Tissue conc.	x ½	C
6-7	HEALTHY	MINIMUM	2nd leaf	133 ± 6.7	138 ± 1.0	142 ± 6.1	143 ± 8.4	-	-	-	-
11	HEALTHY	MINIMUM	Ear	146 ± 8.3	153 ± 8.1	149 ± 4.6	143 ± 8.3	-	-	-	-
10	30%	MINIMUM	Flag	-	65 ± 4.0	70 ± 3.7	90 ± 5.2	-	65 ± 3.9	57 ± 5.8	90 ± 5.2
11	50%	MINIMUM	Flag	-	52 ± 4.1	61 ± 4.4	81 ± 4.6	-	58 ± 5.2	82 ± 4.6	81 ± 4.6
11	50%	MINIMUM	Ear	-	-	-	-	-	55 ± 3.1	81 ± 6.5	90 ± 5.2
10	HEALTHY	MAXIMUM	Flag	91 ± 7.0	41 ± 4.8	83 ± 8.4	130 ± 12.4	109 ± 6.4	134 ± 8.7	155 ± 8.9	130 ± 12.4
10	HEALTHY	MAXIMUM	Flag proximal portion infected leaf	-	43 ± 3.3	57 ± 5.4	90 ± 5.2	-	-	-	-
10	HEALTHY	MAXIMUM	Ear unemerged	109 ± 7.3	138 ± 4.8	158 ± 7.3	154 ± 7.8	149 ± 10.9	144 ± 7.8	154 ± 9.9	154 ± 7.8
11	HEALTHY	MAXIMUM	Flag	106 ± 8.4	129 ± 7.1	139 ± 8.7	136 ± 17.7	115 ± 9.2	129 ± 9.1	149 ± 7.1	136 ± 17.7
11	HEALTHY	MAXIMUM	Ear	59 ± 5.2	83 ± 6.2	125 ± 6.2	162 ± 8.9	53 ± 4.9	116 ± 11.2	133 ± 8.3	162 ± 10.8

Cont'd.....

TABLE 60 (Continued) Germ tube growth of S. nodorum in eluted Cladosporium inhibitors from maximally and minimally hydrolysed infected and control field grown wheat plants at various growth stages.

Growth Stage	Infection	Hydrolysis	Part		Maris Huntsman				Maris Ranger			
					x 2 Tissue conc.	Tissue conc.	x ½	C	x 2 Tissue conc.	Tissue conc.	x ½	C
10	30%	M	Flag		-	26 ± 2.6	28 ± 2.7	81 ± 4.6	-	-	-	-
11	50%	A	Flag	(I)		67 ± 4.6	65 ± 3.7	82 ± 3.7	-	0	26 ± 2.3	82 ± 3.7
				(II)		40 ± 2.7	64 ± 3.5	(I and II)				
		X										
11	50%		Ear			50 ± 4.6	70 ± 3.1	90 ± 5.2	-	47 ± 2.3	64 ± 6.3	90 ± 5.2
		I										
		M										
		U										
		M										