"A PRELIMINARY STUDY OF FUNGI ASSOCIATED WITH DISEASES OF WILD RICE IN MANITOBA"

by

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ABSTRACT

Fungal isolates were obtained from the aerial parts of wild rice. These were used for greenhouse and field inoculation trials in which seed yield, seed germinability and lesion indices were the parameters applied to assess pathogenicity. Selected isolates which showed pathogenic potential were tested for foliar or culm rot tendencies. Seed borne studies were conducted using seed from field inoculated plants.

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INTRODUCTION AND LITERATURE REVIEW

Wild rice, Zizania aquatica L., native to Manitoba and widespread throughout North America, has been a traditional staple food of native Indians for many years (Steeves, 1952). Today, while still remaining of importance to the Indian, it has also become a widely marketed specialty food. This has been made possible by the development of techniques in paddy production which in 1970, raised total production to between 1.4 and 1.5 million pounds of finished rice (Brooks, 1971). Approximately 60 per cent of the crop is retailed via restaurants and caterers and about 40 per cent via stores and private sales or consumed by the original grower or processor (Little, 1968).

Objectives of the Study

In recent years fungal disease has frequently severely damaged paddy-grown crops of wild rice. Disease losses are of particular concern to paddy operators because of the relatively high capital costs of establishing this crop.

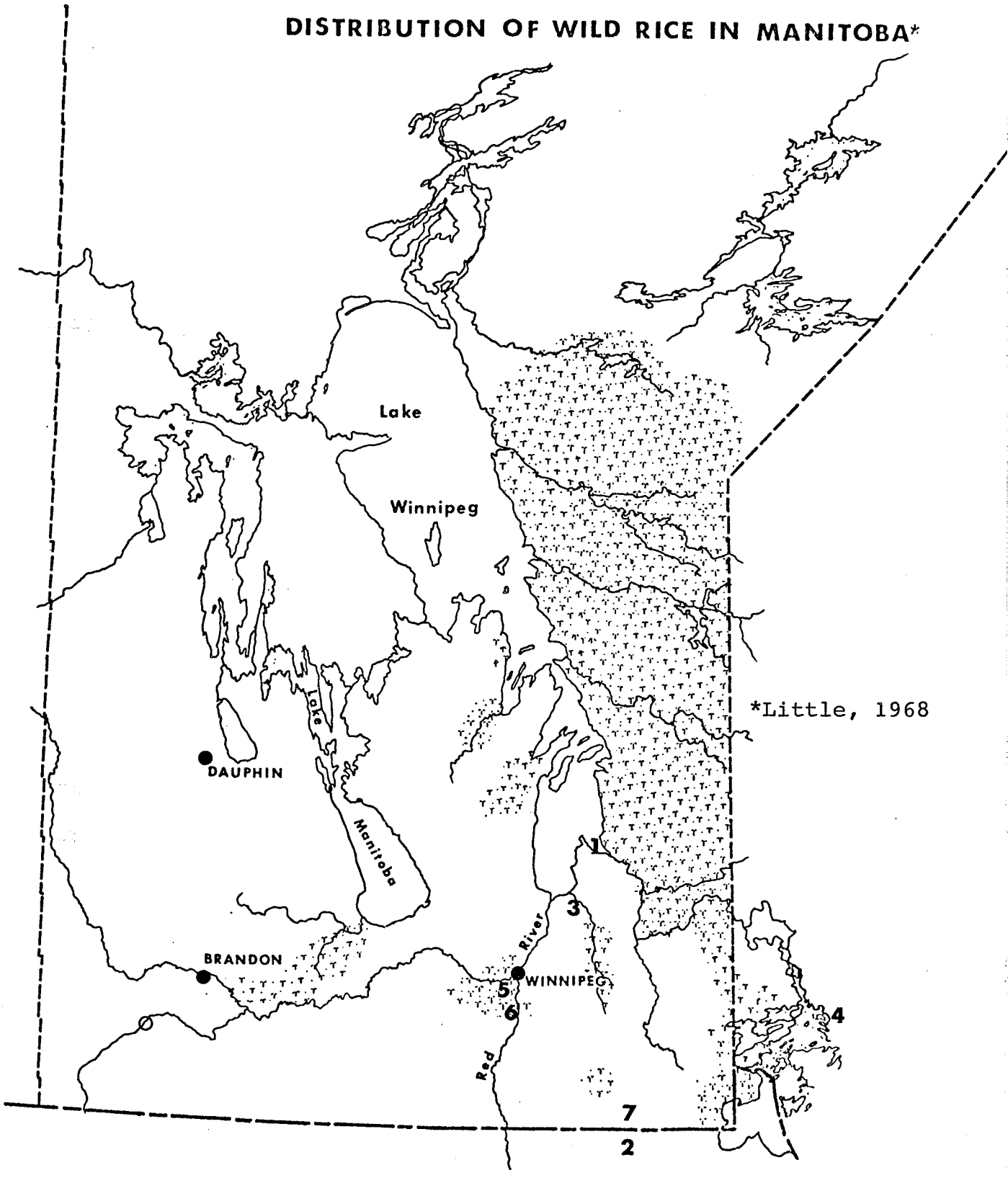
The present study concentrated upon the fungal pathogens of wild rice. In view of the scarcity of data related to fungal disease of wild rice it was apparent that a survey and screening program would be necessary. Special attention

was given to pathogens of aerial parts as they seemed to be the most abundant and damaging. An attempt was made to isolate and identify, at least to generic level, and to determine the pathogenicity of as many as possible of the potential fungal pathogens involved. By greenhouse and field experiments a study was made of the losses to be expected and the nature of the infection. A loss in the vigour of the plant with respect to specific pathogens was determined by the assessment of lesion densities relative to the leaf area (lesion indices), the dry weight yield of the seed and the germinability of the seed after-ripened for six months. In addition a separate study of the after-ripened seed was conducted to determine whether or not the pathogens were seedborne; their ability to overwinter via the seed and re-infect the plant the following spring. The results of these tests are presented in the following chapters.

The Natural Distribution of Wild Rice

Fig. 1.1 shows the natural distribution of Z. aquatica as well as the sources of isolates used in subsequent experiments. Four varieties are recognised. These include Z. aquatica angustifolia Hitchcock (Z. palustris var palustris (Fassett) Dore) or var. interior Fassett (Z. palustris var interior (Fassett) Dore), Z. aquatica var. brevis Fassett, Z. aquatica var texana and Z. aquatica

FIG. 1.1


DISTRIBUTION OF WILD RICE IN MANITOBA*
Lake
Winnipeg
Lake
Manitoba
DAUPHIN
BRANDON
WINNIPEG
Red River
1
2
3
4
5
6
7
*Little, 1968

(Edman, 1969). The varietal notations though are not fully agreed upon and it should be mentioned that stands in the central United States and Canada, including Manitoba, are of a mixed intermediate nature (Rogosin, 1951).

Z. aquatica var. angustifolia or var interior is the variety most commonly grown in commercial paddies and was the variety used throughout this study. The predominant harvest regions include parts of Minnesota, Wisconsin and Michigan in the United States and Ontario and Manitoba in central Canada (Little, 1968; Edman, 1969). Seeded natural stands in parts of northern Saskatchewan are also important (Neilson, 1964). Within the wider spreading natural distribution of wild rice there are thus a number of localised areas where wild rice has become of agricultural importance because of the employment of cultural techniques for paddy production.

Another species of wild rice not found in North America but which occurs naturally in parts of Russia (Ivanov and Ivanova, 1967) and China (Teng, 1932) is the broadleaved and perennial species, Z. latifolia Turcz (Sorokina, 1970). While a map of its distribution was not found in the literature, it is reported to be newly invading lake habitats as, for example, in the overgrowth of the Kripetsk swampy forest lakes (Ivanov and Ivanova, 1967). These authors also make reference to the artificial seeding of lake shallows in

certain areas. The introduction of broadleaved wild rice (Z. latifolia) into the Kremenchug Reservoir on the Dneiper River is also reported (Potul' Nyts' and Molyaka, 1968). This species also suffers from leaf spot symptoms probably similar to those found in North America on Z. aquatica (Yamamoto, 1956). Unfortunately it is not always clear from the literature which species of wild rice is involved, (Z. latifolia or Z. aquatica), since these names are frequently treated as synonyms as in the Review of Applied Mycology (Teng, 1932).

THE GROWTH CONDITIONS OF WILD RICE

Water

Quality

The nutrient requirements of wild rice (Z. aquatica) have not been determined (Grava, 1973). However, the possible existence of certain biological and physico-chemical factors influencing the distribution of wild rice in North America, may be mentioned briefly.

In Manitoba, Z. aquatica var. angustifolia, is more or less confined to the region immediately east of Lake Winnipeg, as shown in Fig. 1.1. Although Little (1968) in

reference to Moyles' work (1945) points out that wild rice grows better in water of less than 10 p.p.m. sulphate ion, greater than 40 p.p.m. carbonate ion and greater than 7 p.p.m. hydrogen ion, little is really known regarding the factors which restrict the natural growth of wild rice in the area to the west of Lake Winnipeg; it has been suggested (Little, 1968) that water characteristics are not especially favourable to its growth in this area. However, J. Dean (personal communication) has shown that in greenhouse conditions, wild rice will grow vigorously in sulphate concentrations exceeding the figures quoted. With reference to the paddy production of wild rice in Manitoba, Rogalsky et al (1971), state that water quality can be of importance in alkaline regions. "The sulphate ion concentration should be under 200 p.p.m., while the carbonate ion should be less than 60 p.p.m. The desired pH range of water is from 6.0 to 8.0 and the calcium and magnesium content each less than 70 p.p.m."

The turbidity of the water influences the growth of germinated seed by controlling the light available to the submerged leaves. Turbid water is thought to favour the growth of competitors such as Ceratophyllum demersum L. (coontail), Potamogeton zosteriformis Fern. (flat-stemmed pondweed), Lemna trisulca L. (star duckweed) and water lilies (Little, 1968).

Depth

The data available on the effect of water depth on wild rice growth sometimes appears contradictory and this should be interpreted with caution as quite often a favourable depth is quoted with reference to different parameters, e.g. plant survival (Chambliss, 1940), seed number (Rogosin, 1957) or final dry weight (Weber and Simpson, 1967). An assessment of the effect of different water depths on Z. aquatica in terms of morphological and dry weight changes which occur during a complete life cycle, however, is probably a more accurate method for determining optimal water depth (Thomas and Stewart, 1969). It was found that while duration of the submerged leaf and aerial stages was unaffected by water depth, the time spent in the floating leaf stage increased with water depth and resulted in plants flowering later than those at shallower depths. Water was required to cover the submerged leaves and to support the floating leaves while leaves, sheaths and stems were liable to breakage as a result of drop in water level. However, plants with aerial leaves survived with no free water above the rooting medium. Yield in terms of the dry weight of the total plant was decreased by water depths less than eight cm and greater than 110 cm. The decline of wild rice in many areas of Ontario was attributed to either excessively low or high water levels during the critical submerged and floating leaf stages.

In native stands a water level of 60 cm is thought to be conducive to high yields while in controlled experiments, a water depth of 15 cm has proven best for paddy production (Little, 1968). A disadvantage of shallower water, however, may be the resulting greater development of weeds (Rogalsky et al., 1971) although maintenance of water to a depth of 15 cm or more will control most of them except cattails, Typha latifolia L.

It is also reported that the water regime may influence the formation of ultrastructural wax on the cuticle of floating and aerial leaves. As an emergent and hydrophytic grass, wild rice in nature may be subjected to rapidly fluctuating water levels, severe wind and wave abrasion, as well as temporary drought. Under these conditions the rodlets and platelets of epicuticular wax may contribute to the plant's survival by virtue of their hydrophobic nature and by contributing to the regulation of the water balance within the leaf. The deposition of wax is in turn possibly regulated by cuticular tension (Martin, 1966) which may influence the diameter of possible wax extruding pores (Hawthorn and Stewart, 1970).

Although wild rice is known to grow satisfactorily in saturated soil lacking free water above the rooting medium (J. Dean, personal communication), such plants are reported to have reduced leaf area, an overall increased production

of epicuticular wax, but a total absence of wax on first leaves (Hawthorn and Stewart, 1970).

Other Aspects

Waves or water currents passing through a wild rice stand are thought to help induce seed germination in spring. However, the significance of the water flow is disputed (Little, 1968). Natural stands of wild rice are seldom if ever found in the presence of continuous unidirectional currents of any magnitude, where ice scouring prevents stand establishment.

Prolonged rain during flowering may result in reduced pollination and unfilled hulls. Rain during or after the harvest season may cause lodging and increased shattering.

Soil

Wild rice has the potential to grow on a wide range of soils. In nature its substratum ranges from "fermenting muck oozes" common on lake floors, to hard sand or sand loam found on some river beds. However, its optimal substrate appears to be a slightly acid muck soil from several to 60 or more centimetres thick, overlying clay (Little, 1968).

Plants Associated with Wild Rice

It is likely that more natural stands are reduced by weeds than by any other cause (Little, 1968). Aquatic competitors include the species mentioned previously (p.16) and in addition the following:

Najas flexilis (Willd.) Rostk. & Schmidt (bushy pondweed)

Lemna minor L. (duckweed)

Spirodela polyrhiza (L.) Schleid. (duckweed)

Utricularia macrorhiza Le Conte. (bladderwort)

Phragmites communis Trin. (reed)

(Moyle, 1945)

Algal blooms sometimes reduce light availability to wild rice seedlings by forming mats on the water surface. They may also interfere with normal plant respiration and gas exchange. Algae affect the plant in the floating leaf stage also. Hawthorn and Stewart (1970) report that algae adhered to the surfaces of still submerged floating leaves, which in turn were bleached and this caused the death of the plant. This suggested that the disappearance of wild rice from many of its former habitats might have been caused by the algal blooms resulting from increased eutrophication.

Animals Associated with Wild Rice

Water fowl such as ducks eat the dormant or germinated seed and shoot in the spring and the dormant seed in the fall. Blackbirds and other small birds may strip large areas of the ripened crop by landing on the stalks near the seed-head and shattering the ripened seed.

Muskrats eat young shoots, leaves and seeds. Deer, moose and cattle eat the foliage. Some fish such as the german carp are also thought to contribute to the loss in certain natural wild rice stands by feeding on the seed, uprooting the young plants and disturbing the water, though their role here is in doubt (Rogosin, 1951).

DISEASES AND PESTS

There are a few reports of disease in the literature, mostly in relation to Z. aquatica in the areas previously outlined, though there is only one reference specific to Manitoba. Some references to diseases of Z. latifolia are also considered briefly.

Fungi and Bacteria

Zizania aquatica

Shoemaker (1959) identified the causal agent of leaf spot on wild rice as Bipolaris zizaniae (Nisikado) comb. nov. Shoemaker, a grass parasite separated from the Helminthosporium (≡ Drechslera, Ito) group.

Bean and Schwartz (1961) reported a severe epidemic of brown spot disease on cultivated wild rice in northern Minnesota. This had increased each year since the fields were established, so much so that in one 10 acre field where wild rice had been grown for 3 consecutive years, there was a complete crop failure resulting from defoliation and lodging. The fungus isolated was H. oryzae, B. de Haan (≡ D. oryzae (B. de Haan) Subram. and Jain), though this may not have been the only fungus involved. The disease severity appeared to be also related to the density of the plant population because in younger fields nearby with thinner stands, brown spot was less severe. This association with thickly seeded stands is now well recognised. Also it is stated that drought during the 1961 growing season may have been important, as Hemmi and Suzuki (1931) found that cultivated rice (Oryza sativa) grown under dry conditions was more susceptible to attack by D. oryzae. (Presumably the reference is to the water level in the paddies and not

the humidity regimes of the aerial parts, though this would be affected, as the Japanese workers were concerned with the relationship between soil moisture and the development of D. oryzae affecting rice seedlings).

Similar brown spots and lodging symptoms were reported later in Minnesota. H. sigmoideum Cav., (= Nakataea sigmoidea Hara), the fungus that causes stem rot of rice (Oryza), and Sclerotium sp. were isolated from wild rice (Morrison and King, 1971). Again there were indications that the disease was progressive from year to year. Both fungi were found on plants with stem rot symptoms in five paddies which had been previously cropped for two to six years, but were not found on plants in eight paddies without stem rot and in which wild rice had been grown for only one to two years. Spread of the pathogens to new paddies was thought to be by Sclerotium-infested seed or by plant debris which is commonly found in seed used for planting. King (1971) has further reported on an association between specific fungi and leaf stem spots on the one hand (Drechslera sp.) and root and basal stem roots on the other (Pythium sp., Fusarium sp., Phytophthora sp., Sclerotium sp. and H. sigmoideum).

Attempts at control of the brown spot disease have been made in Minnesota by fungicide application as dormant and foliage sprays, removal of crop debris and the selection of

resistant plants (Bean and Schwartz, 1961). In 1971, tests were made to determine if fungicides would provide effective control of 'Helminthosporium' foliage and stem diseases. Although the fungicides provided a certain degree of protection it was reported that they were not economically feasible for use with wild rice (Kernkamp, 1972).

There are only two references in the literature to fungal diseases of wild rice in Manitoba and both refer to ergot, Claviceps purpurea (Fr.) Tul. In 1938 C. purpurea became a problem on wild rice in Manitoba, where the crop, owing to its failure in Minnesota, was of considerable commercial value (Conners, 1939). Inoculations with pure cultures of C. purpurea showed that in Manitoba a single strain attacks rye, barley, wheat, oats and grasses. Another strain found on Z. aquatica was unable to attack cereals and other grasses and its sclerotia floated on water, unlike the former strain (Brown, 1948).

In 1972 Sclerotium sp. was isolated from a few plants from the three-year-old paddies of Fort Alexander, Manitoba (D. Punter, personal communication), though it was absent in the fall 1970, when the paddies were only one year old (Cf. p.23 and Morrison and King, 1971).

Zizania latifolia

A few fungal pathogens have been reported on Z. latifolia, namely, H. zizaniae, H. oryzae, Piricularia oryzae (Sacc.) Cav., Sclerotium oryzae-sativae (Catt.) Sawada, S. hydrophilum Nakata and Ustilago esculenta (Pers.) Roussel, as well as a bacterium, Xanthomonas oryzae Dowson (Ou, 1972).

It was found that H. oryzae caused a brown spot of Z. latifolia, overwintering on it in ponds and ditches in western Japan and this formed a primary source of infection for rice plants (Oryza) in spring (Yamamoto, 1956).

Greenhouse inoculation trials on Oryza as well as Z. latifolia revealed two strains of Z. latifolia which were highly resistant to P. oryzae (Katsuya, 1960 and 1961).

U. esculenta was reported on Z. latifolia from Canton in China (Teng, 1932). Cultural studies of U. esculenta were conducted later. These involved its physiological growth factors, the nuclear behaviour of germinating spores and the diploid phase in vitro (Su, 1961). Enkina (1968) also reported on the symptoms resulting from the smut infection of Z. latifolia. These consisted of dwarfed stems which had only two to five shortened internodes (normally about 10) and deformed and hypertrophied inflorescences in

which no seeds were being formed. Su (1961) also referred to hypertrophied culms. "The chlamydospores were spherical, elongated or elipsoid, 5.5-8 X 5-7 μ, with a smooth wall."

The bacterium, Xanthomonas oryzae, pathogenic on rice (Oryza) and grasses (including wild rice) in Japan was reported to overwinter in diseased rice straw and seeds, but probably not in the soil. Primary outbreaks of rice leaf blight seemed to originate from material which had overwintered in grasses especially Leersia oryzoides (L.) Swartz var. japonica (Makino)Hack..Isolates from rice plants (Oryza) were pathogenic to wild rice and also to additional species including Phalaris arundinacea L., L. oryzoides, Phragmites communis Trin., Isachne globosa (Thunb.) O.Ktze.and other grasses. Strains of the organism from naturally infected plants of wild rice and L. oryzoides also attacked rice (Oryza). (Goto et al., 1953).

One of the few citations of work involving the roots of wild rice has shown that it has an active rhizosphere. It was reported that the formation of a root microflora in aquatic plants depended on their physiological condition. "Weak plants have less abundant microflora than the strong ones. This is related to the number of organic substances escaping through the roots. The roots of Zizania are not only infected on the surface but also inside (in the primary root core). The genera Pseudomonas, Bacterium, Bacillus and

Achromobacter are representative of the aquatic root microflora." (Makulova, 1970). In the present study Bacillus sp. was isolated from the overwintered seed (p.178).

It should be noted that the references of Teng (1932), Su (1961) and Goto et al (1953) cite the host plant as Z. aquatica. It is not clear whether this has arisen out of the initial synonymous use of the names Z. aquatica and Z. latifolia as a result of a possible reviewer's error (Cf. Teng, 1932, R.A.M.) or whether Z. aquatica was truly the host plant involved.

Nematodes

The morphology of the nematode Radopholus gracilis (de Man) Hirschmann parasitic on the roots of Z. aquatica in Ontario, has been described by Sanwal (1957). The nematodes appeared to be adapted to an aquatic environment and seemed unaffected by low temperatures and low oxygen content in their habitat. It is known that Radopholus spp. are endoparasitic burrowing nematodes and are perhaps the most important of plant parasitic nematodes which occur in the tropics where they attack important food crops such as the cultivated rice (Oryza). The damage to plant roots resembles that of the lesion nematode group, which form conspicuous necrotic lesions on the roots of susceptible plants.

Insects

The following insects have been reported to attack wild rice (Little, 1968; Noetzel, 1971):

Rhopalosiphum fitchii Sanderson	(aphid)
R. prunifoliae Fitch	(aphid)
Hydrellia griseola Fall.	(shore fly)
Eribolus longulus Loew	(frit fly)
Apamea apamiformis Guenee	(rice worm)
Chilo ?plejadellus Zincken	(stalk borer)
Pseudaletia spp.	(army worm)
Septis sp.	
Agrotis sp.	(cut worm)

The aphids are reported to have caused severe losses in the past (Adams, 1945), though no recent reports of excessive damage have been found. Control methods include a sustained spray schedule with parathion, color traps, use of biological predators compatible with wild rice and various systemic insecticides (Little, 1968). *H. griseola* causes damage by the larvae burrowing into the mesophyll of floating leaves; 80 per cent loss to experimental paddies in St. Paul, Minnesota occurred in this way. *E. longulus* is the "rice stem maggot" which causes damage to the developing inflorescence while still within the inflorescence sheath resulting in dramatic losses in yield of individual heads.

A. apamiformis feeds on the developing grain and is the major insect pathogen. Its life history in connection with wild rice is well known (Mackay and Rockburne, 1958). The pathogenic status of C. ?plejadellus, a stalk borer, is not yet clear. The larvae burrow into the culm until below the water level, prior to paddy drainage (Noetzel, 1971). Pseudaletia, Septis and Agrotis spp. are on record as having caused damage to wild rice occasionally (Little, 1968).

Preventative studies of A. apamiformis have shown that malathion and carbaryl (Sevin) gave excellent insect control. It was found that applications were most effective 3 weeks after initial rice worm oviposition. The eggs can be observed in clusters from three to 50 within the empty glumes as the seed is developing. A possible means of biological control of A. apamiformis by way of Bacillus thuringiensis is also at present under investigation (Noetzel, 1971).

CHAPTER ONE

EXPERIMENT I

PRELIMINARY PATHOGENICITY STUDIES

In the fall of 1970, research commenced with the isolation of as many fungi as possible from the aerial parts of wild rice growing in the year-old paddies of the Fort Alexander Indian Reservation near Pine Falls, Manitoba. The seed used for this paddy had been obtained from a Wisconsin processor, Ned Fennel (J.M. Stewart, personal communication) and was identical to Zizania aquatica var. angustifolia found in Manitoba.

The most abundant symptom was the occurrence of localised and spreading brown lesions on leaves and leaf sheaths. This was accompanied at times by black sori on the inflorescence stalks and heads of more mature plants. Invariably such plants also had a black flecking of their upper leaf sheaths. The brown spotting was known to be caused, at least in part, by Drechslera spp., as isolates had been made during the previous summer. Similarly the cause of the black sori had been previously identified as Entyloma lineatum Davis (J. Reid, personal communication). Root symptoms were noticeably absent and basal culm regions

appeared healthy at the time of isolation.

ISOLATION OF POTENTIAL PATHOGENS

MATERIALS AND METHODS

Fungi were isolated by the damp chambering method with the exception of E. lineatum (Appendix 1.1). Portions of leaf and leaf sheath bearing a representative selection of lesions were photographed and then surface-sterilised for three minutes in one per cent sodium hypochlorite solution (diluted commercial Javex bleach) to kill surface contaminants. They were then placed in Petri dishes on filter paper moistened with sterile distilled water. These were incubated at 25°C for 48 hours or until mycelial growth or sporulation occurred. The cultures were checked daily to ensure that slow-growing organisms were not obscured by overgrowth.

The plates were removed from incubation and the leaf surfaces scanned in sterile conditions with a binocular dissecting microscope. Where possible, spores were picked off singly or in clumps from conidiophores and inoculated onto agar plates. Single-spore cultures were then obtained from these initial cultures. Where mycelial growth alone occurred on the leaf surfaces, mass transfer of the mycelium

TABLE 1

PERIODS OF GROWTH REQUIRED FOR ADEQUATE SPORULATION OF FUNGAL ISOLATES

ISOLATE	MEDIUM	°C	LIGHT (h:hours)	GROWTH PERIOD IN DAYS 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
ENTYLOMA LINEATUM	LIQUID PYG	18		+
Mucorales	PYG	20	WHITE 12h	+
DRECHSLERA 1	PYG	20	WHITE 12h	+
DRECHSLERA BICOLOR	PYG	30	NR.UV 24h	+
ALTERNARIA 1	PYG	20	WHITE 12h	+
ALTERNARIA 2	PYG	20	WHITE 12h	+
ALTERNARIA 3+G.*SIMPLEX	PYG	20	WHITE 12h	+
PEYRONELLAEA SP.	PYG	20	WHITE 12h	+
EPICOCCUM NIGRUM	PYG	20	WHITE 12h	+
FUSARIUM 1	PDA	20	NR.UV 12h	+
FUSARIUM 2	PDA	20	NR.UV 12h	+
CLADOSPORIUM 1	PYG	25	---	+
CLADOSPORIUM 2	PDA	25	---	+
CLADOSPORIUM 3	PYG	25	---	+
FUSARIUM 3	PDA	20	NR.UV 12h	(+)
Basidiomycete	PYG	20	NR.UV 12h	(+)
unidentified fungi # 1&2	PYG	25	---	(+)

+: GOOD SPORULATION
(+): INADEQUATE SPORULATION
*: GONATOBOTRYS

was used for initial isolation and pure cultures were established by further transfer of hyphal tips.

Each isolate was grown on four agar media to determine its nutritional preferences, particularly with regard to conidium formation (Table 1, p.32). These included potato dextrose agar (PDA), peptone yeast-extract glucose agar (PYG), Czapek Dox agar (CDA) and "V-8" juice agar (V8) (Appendix 1.3,p.210). Cultures were incubated either at 25°C in the dark or at 20°C under alternating 12 hour periods of dark and fluorescent light. Under these conditions PYG agar proved to be generally conducive to sporulation with a minimum of vegetative growth, and was therefore adopted as the general growth medium for the remainder of the study. Those few isolates which failed to sporulate (essential for adequate identification and desirable for the preparation of inoculum) were subjected to near-UV irradiation following the method of Leach (1962). Cultures on PYG agar were exposed to continuous illumination from two 40 watt "Black Light" tubes (G.E. F20T12.BLB Black Light, U.S.A.) at a range of 56 cm and a temperature of 30°C and also to near-UV and fluorescent light (12 hour day) at 20°C. In addition a further attempt to induce sporulation in two persistently sterile isolates (Unidentified fungi # 1 & 2) was made. Fresh leaves of wild rice, autoclaved at 120°C and 15 p.s.i. for 20 min. were placed in Petri dishes and covered with a thin film of potato carrot agar (PCA) (Tuite, 1969). These

were inoculated with the fungi and incubated under the light and temperature regimes described. Fungal isolates which produced recognisable spore forms were identified to the generic level and grouped under taxonomic species. Non-sporulating isolates were characterised as far as possible by macro- and microscopic features of the colony.

The majority of fungal isolates were maintained in the form of single-spore or single hyphal-tip cultures on slants of a weak potato carrot or V8 agar in sealed tubes. These were incubated at 20°C under an appropriate light regime until satisfactory vegetative growth and sporulation had occurred. They were then stored at 2°C. Fusarium spp. were maintained in soil (Toussoun and Nelson, 1968). E. lineatum could be stored in yeast form (monokaryon) on slants for no more than three months before subculturing.

RESULTS

Table 2, p.35, provides a summary of the lesion types which were recognised along with a record of the fungi isolated from each. These lesion types are illustrated specifically in Plates 2#1 and 2#2 (lesion type 1.0), 1#5 and 4#7 (2.0), 5#6 (3.0), 1#1, 1#2 and 1#4 (4.0), 1#8 (5.0), 2#9 (6.0), 1#10 and 3#6 (6.1), 3#7 (6.2) and 1#7 (7.0), respectively. They are also cited generally throughout Plates 1-6, as tabulated.

TABLE 2

FUNGI ISOLATED FROM LESIONS ON AERIAL PARTS OF WILD RICE GROWN AT FORT ALEXANDER, MANITOBA

LESION TYPE			FUNGUS ISOLATED																	
LESION CODE	PLANT PART		DRECHSLERA 1	DRECHSLERA BICOLOR	FUSARIUM 1	FUSARIUM 2	FUSARIUM 3	ALTERNARIA 1	ALTERNARIA 2	ALTERNARIA 3 & GONATOBOTRYS SIMPLEX	ENTYLOMA LINEATUM	PEYRONELLAEA SP.	EPICOCCUM NIGRUM	CLADOSPORIUM 1	CLADOSPORIUM 2	CLADOSPORIUM 3	Basidiomycete	Mucorales	Unidentified fungi # 1 & 2
1.0	lf	Localised necrosis (shot hole) 0.1 X 0.1 cm	+		+	+		+	+										
2.0	lf	Necrotic areas 0.5-1.0 X 1.0-10.0 cm						+	+	+		+	+	+	+	+	+		+
3.0	lf	Brown discolouration 0.5-1.5 X 1.0-10.0 cm	+		+		+												
4.0	lf	Localised brown spot 0.1-0.4 X 1.0-0.5 cm	+	+	+							+	+						
5.0	st	Internodal/Nodal culm rot (width of stem)			+													+	
6.0	lf	Elongate black-brown spot 0.05-0.1 X 0.2-0.4 cm	+																
6.1	st	Black to lead-grey sori 0.05-0.4 X 0.2-0.4 cm									+								
6.2	sh	Black-brown flecking 0.1 X 0.2 cm																	
7.0	lf	Chlorotic depressions (Insect damage) 0.1-0.4 X 0.1-10.0 cm						+						+					

lf: leaf st: stem sh: leaf sheath

Several lesion types yielded a wide variety of fungi and several fungi were isolated from more than one lesion type. Although the black-brown flecking of the leaf sheath (type 6.2, Plate 3#7) was the only lesion type from which fungi were consistently absent, occasional examples of other lesion types also appeared to be devoid of fungi.

DISCUSSION

These preliminary results indicated the unreliability of lesion type as an indicator of the fungus present. Those organisms, Drechslera spp. and Fusarium spp. which were considered likely to be primary parasitic invaders, were commonly but not exclusively, associated with smaller localised lesions. However, several of these organisms were often isolated from a single lesion type. Those fungi which were suspected of being secondary invaders and perhaps only saprophytes were mainly isolated from the larger spreading lesions (type 2.0, Plate 1#5), though they sometimes occurred alone on localised lesions.

The small dark-brown lesions (type 6.0, Plate 2#9) associated with Entyloma occasionally yielded Drechslera spp. This points to the fact that the early stages of Drechslera lesion development, if severe enough when the darker lesions result (Plate 4#1) can be confused with the typical smut lesions of wild rice.

Black flecking of the upper leaf sheaths (type 6.2, Plate 3#7), occurring regularly on plants with fairly advanced soral development, was correlated to some extent with smut infection. Plating out on agar, however, did not yield the fungus. It is conjectured that a toxic effect of the possible systemic invasion of E. lineatum or a combined effect of that and some other factor(s) may be operative. Alternatively the flecking may represent a pathogen which escaped the method of isolation used; e.g. a bacterium or virus.

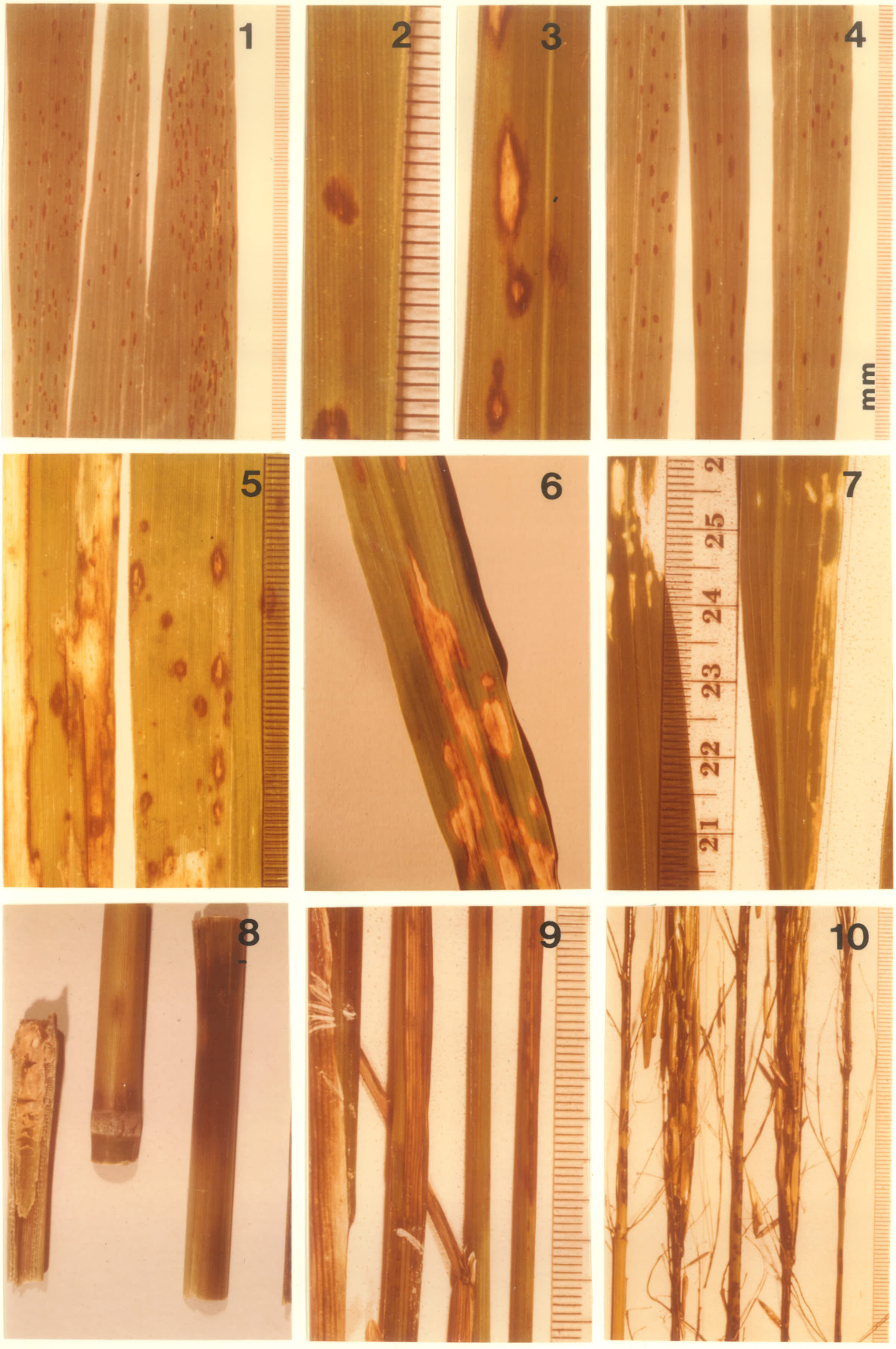
The nodal culm rot (type 5.0, Plate 1#8) was probably part of the ordinary decay of senescent plants, isolations being conducted late in the season. This is borne out by the fact that the fungi isolated included a member of the Mucorales and Fusarium 1 which subsequently did not show culm rot tendencies of the type found in the field, though Fusarium 1 did show some culm invasion under ideal conditions (Expt. IV).

From these initial observations on lesion type it was apparent that lesion type is more a response of the wild rice plant than a specific symptom of a given pathogen. Secondly it should be pointed out that fungi were not isolated invariably from the lesion itself, but often from the neighbouring tissues of the leaf. This implies that the fungus need not have developed initially within the lesion,

Plate 1

Symptoms from Natural Infection

1	Lesion type 4.0	St. Adolphe
2	Lesion type 4.0	Fort Alexander
3	Lesion type 4.1	
	2.0 early stages	Fort Alexander
4	Lesion type 4.0	St. Adolphe
5	Lesion type 2.0	
	4.1	Fort Alexander
6	Lesion type 3.1	Fort Alexander
7	Lesion type 7.0	Fort Alexander
8	Lesion type 5.0 culm rot	Fort Alexander
9	Lesion type 5.0	Fort Alexander
10	Lesion type 6.1	La Salle River
	(Note premature	
	seed shattering)	


1
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mm
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21
22
23
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25
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10

but may have spread in the mesophyll from some other part. Thus inoculation trials were carried out in the greenhouse to clarify the pathogenic status of each isolate.

GREENHOUSE PATHOGENICITY TRIALS

The isolates from the field were tested for their pathogenicity by inoculation on cultured wild rice grown in the greenhouse in the winter of 1970-71. The results were to be compared with later field inoculation studies.

MATERIALS AND METHODS

Seed from the paddies of Fort Alexander was germinated, under water, in 250 ml beakers in a growth chamber. It received a 15 hour day of fluorescent and incandescent light at 22°C and a night temperature of 17°C. Following germination and growth to the early submersed leaf stage (approximately one week), seedlings were removed and stored in bulk under water at 2°C in a polystyrene tank until enough uniform seedlings were obtained. The day-length in the greenhouse was 17 hours as a shorter day-length tended to promote premature flowering (J. Dean, personal communication).

Square tanks (60 X 60 X 30 cm) were constructed from wood and lined with a double thickness of polyethylene (.0147 cm or .006 inch) secured by masking tape (Plate 2 # 10). A black soil, sand and peat mixture (2: 1 : 1), steam sterilised for 15 hours at 120°C and 12 p.s.i., provided a rich soil substratum. This was carefully compacted in each tank to a depth of 12 cm. It was then covered with a layer of sterilised sand (2 cm). This helped to prevent soil disturbance when the tanks were flooded and provided a satisfactory substrate for planting. Clouding of the water must be minimised as light availability is fairly critical for seedling growth.

The tanks were randomised with the random numbers of Fisher and Yates (1957) and three planted daily over a period of 12 days. The fluorescent lights were lowered to within 15 cm of the water to give maximal light to the seedlings. Tanks were replenished with water on a daily basis. This helped to control algal growth during the submersed leaf stage. If algal growth was sufficient to reduce the light reaching the plants it was necessary to replace the water by syphoning it off and simultaneously maintaining a steady inward flow. Replacing the water by this method avoided fracture of the stems, which are very weak at this stage. The pH of the water in the tanks was generally alkaline and varied from 7.6 to 8.6 at the floating leaf stage. The water and air temperatures were maintained at 20-21°C.

Preparation of the inoculum

For purposes of preparing a spore suspension all isolates were grown on PYG agar except the Fusarium spp. which were grown on PDA (Tousson and Nelson, 1968). The agar was used at a strength of approximately three per cent, so as to prevent it from breaking up during the harvesting of the spores. The growth periods for the staggered preparation of the inocula are shown in Table 1, p.32.

Spores were harvested in sterile conditions immediately before inoculation by scraping the plates individually with a sterile wire loop or bent glass rod after the addition of 10 to 15 ml of sterile distilled water and one drop per plate of the surfactant Tween 80. This procedure helped to dislodge the spores and to prevent clumping, both in the haemocytometer cell and in the nozzle of the spray gun used for inoculation. The suspensions for each isolate were decanted through a double layer of cheese-cloth to exclude mycelial fragments, and then collected in a 250 ml Erhlenmeyer flask.

Accurate counts of spore germinabilities and spore densities were not made at this stage, though for the purposes of these preliminary inoculation trials rough estimates with a haemocytometer cell were made for Fusarium spp., Cladosporium spp. and E. lineatum. These were in

the order of 1 X 10^6 spores per ml. They were adjusted by dilution to approximately 2 X 10^2 spores per ml. One hundred ml of spore suspension per isolate was adequate for each inoculation of the plants in one tank.

Inoculations

After eight to nine weeks of growth, plants were prepared for inoculation by the removal of dead and moribund submersed and floating leaves. Under-sized plants were also removed, resulting in 20 to 25 uniform-sized plants in the early aerial leaf stage per tank (Plate 2#11). The ambient temperature was raised to 30°C. The spore suspensions were applied by means of a spray gun (Dahlia Sprayer No. A-211 Form., Maruhachi Industry Co., Tokyo) used at maximum pressure to ensure proper impaction on the leaves and leaf sheaths. It was held at approximately 30 cm from the plants, so that they were enveloped by a uniform mist.

To prevent spores from drifting on to plants in neighbouring tanks each was surrounded by a wire-framed polyethylene hood which extended 60 cm above the water level and enclosed the tank above and on either side, but was open at the front to allow access to the plants. Inoculated plants were treated in one of two principal ways. In one treatment, the polyethylene hoods were removed immediately after the plants were inoculated and the tanks left at 30°C

and 35 per cent relative humidity. In the other treatment, inoculations were carried out in the same way but the polyethylene hoods retained and a front piece added immediately after inoculation. This gave a cubical chamber that was completely closed. Spray from humidifiers was directed through an orifice cut 15 cm from the top of each front piece resulting in a circulation of mist which saturated leaf and leaf sheath surfaces. Humidification was maintained for 24 hours following which the hoods were removed. This was considered long enough to allow infection.

Infection by the smut E. lineatum, however, was investigated beyond the use of these two basic treatments, to help determine whether or not it invaded the plant systemically. Three further methods of inoculation were used. The inflorescences of wild rice with young plumose stigmas (Plate 2#8) were inoculated independently of the leaves. In order to achieve this the head was enclosed within a conical flask during inoculation. Stigmas which emerged later were inoculated using a fine-haired paint brush. Supplementary humidification was not provided as it was thought that the receptive stigmatic surface would of itself remain sufficiently moist to allow spore germination and infection. A fourth method of inoculation involved simply spraying the spore suspension (without inclusion of the surfactant) on the water surface. Finally the seed of wild rice, after-ripened for 120 days, was inoculated to

find out whether or not the fungus could maintain itself within the growing plant. One hundred and fifty seeds were individually dehulled with a scalpel and surface sterilized. An equal sample was surface sterilized without dehulling. Two hundred and fifty ml of 95 per cent ethanol and four per cent sodium hypochlorite (commercial Javex bleach) (1 : 2) was used as a surface sterilant for three minutes for each batch of seed (Frank and Larson, 1970). Each was then washed in four changes of sterile distilled water and immersed in 150 ml of the smut spore suspension at 22^{o}C for 24 hours under vacuum. Following this treatment the suspension was decanted off and the seed left under water to germinate at 22^{o}C under laboratory lighting conditions. Germinated seedlings were planted out in the usual way and the plants monitored for smut infection. It should be pointed out that in all five treatments involving the smut spore suspension, the spores were dikaryotic (Appendix 1.1).

RESULTS

Table 3, p.46, summarises the occurrence of infection in these preliminary trials. In the initial treatment with a relative humidity as low as 35 per cent, infection was not achieved by most isolates tested. However there were two notable exceptions, namely, Fusarium 1 and Fusarium 2. Fusarium 1 achieved a higher degree of infection than

Table 3

RESULTS OF PRELIMINARY GREENHOUSE INOCULATIONS

ISOLATES	WITHOUT HUMIDIFICATION 35%R.H.&30°C		WITH HUMIDIFICATION 100%R.H.&30°C	
DRECHSLERA 1	+	r	+++	r
DRECHSLERA BICOLOR	+	r	++++	r
FUSARIUM 1	++++	r	+	r
FUSARIUM 2	+++	r	+	r
FUSARIUM 3	+			
ALTERNARIA 1				
ALTERNARIA 2			+	r
ALTERNARIA 3 & GONATOBOTRYS SIMPLEX				
ENTYLOMA LINEATUM (leaves)			++	
ENTYLOMA LINEATUM (stigmas)			———	
ENTYLOMA LINEATUM (water)			———	
PEYRONELLAEA SP.			+	r
EPICOCCUM NIGRUM			+	r
CLADOSPORIUM 1	+			
CLADOSPORIUM 2			+	
CLADOSPORIUM 3	+			
Mucorales			+	
Unidentified fungus #1			+	
Unidentified fungus #2	+			
Basidiomycete				
CONTROL				
ENTYLOMA LINEATUM (inoculated whole seed)			———	
ENTYLOMA LINEATUM (inoculated dehulled seed)			———	

++++: severe infection
+++ : moderate infection
++ : light infection
+ : very light infection (1-2 lesions)
r : successful re-isolation

Fusarium 2 by a general 'shot—hole blasting' of the leaves, although the leaf sheaths remained relatively free of infection (Plates 2#1 and 2#2). The symptoms were similar for Fusarium 2 (Plate 2#3) but were more blotch-like and spreading in nature. Drechslera 1 and D. bicolor produced only one or two lesions. Among the group of suspected saprophytes Cladosporium 1, Cladosporium 3 and Unidentified fungus #2 gave slight evidence of infection but none of these was recovered by the damp chambering method. E. lineatum also produced no infection at this humidity.

With the use of 100 per cent humidification, however, the results were to some extent reversed. Fusarium 1 and Fusarium 2 produced only a few lesions, but infection was moderate in the case of Drechslera 1 (Plate 2#4 and 2#5) and severe with D. bicolor. After four days, spread of the lesions of D. bicolor had totally browned some leaves and caused others to wither (Plate 2#6 and 2#7). E. lineatum produced slight aerial leaf infection with the occurrence of a few lesions on some plants (Plate 2#9). Smut infection was verified three weeks later by the presence of mature sori on the inflorescence stalks of nine out of 25 plants. No symptoms appeared on plants whose stigmas had been inoculated or those in which the inoculum had been applied to the water surface. Plants which had been inoculated as seed, showed faint lesion development on the young aerial leaves but attempts at reisolation did not yield the

Plate 2

The Preliminary Greenhouse Inoculation Trials

1 *Fusarium 1* (no initial humidification)

Lesion type 1.0

2 *Fusarium 1* (no initial humidification)

Lesion type 1.0

3 *Fusarium 2* (no initial humidification)

Lesion type 1.1

4 *Drechslera 1*

Lesion type 4.0; flag leaf sheath
4.1

5 *Drechslera 1*

Lesion type 1.0; occasional
4.0
4.1

6 *Drechslera bicolor*

Lesion type 4.0
2.0; sparse

7 *Drechslera bicolor*

Lesion type 2.0
4.0; sparse

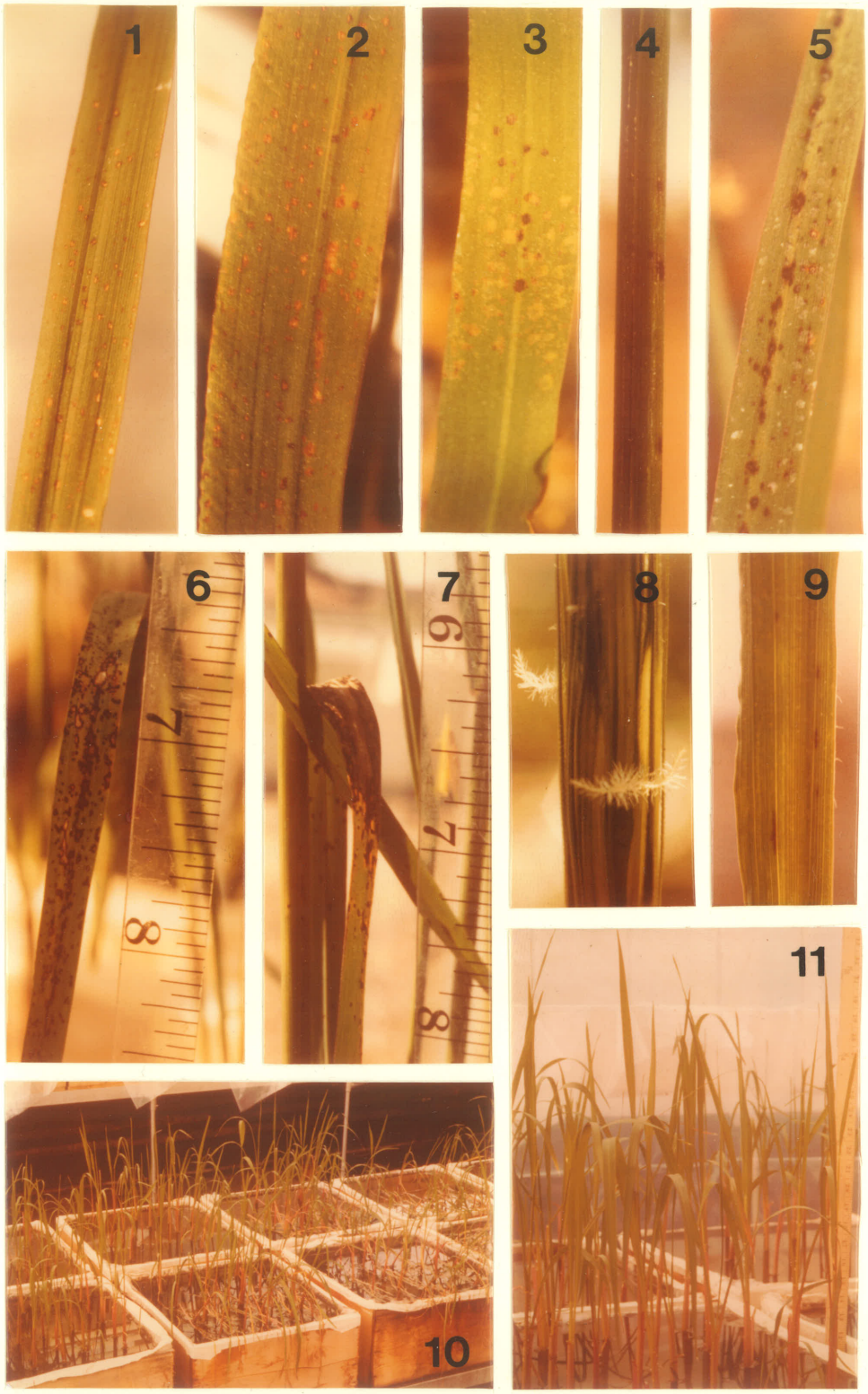
8 Receptive stigmas of wild rice

9 *Entyloma lineatum*

Lesion type 6.0

10 Part of a block of wild rice tanks ("plots") at floating and aerial leaf stages.

11 Wild rice plants at the time of inoculation (early aerial leaf stage).


1
2
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11

smut neither were any sori recorded on mature inflorescence stalks.

Occasional extremely limited lesion development appeared after inoculation with a number of other fungi grouped as probable saprophytic or weakly parasitic types. These included Cladosporium spp., Alternaria 2, Peyronellaea sp., E. nigrum, a member of the Mucorales and Unidentified fungus #1. However only Alternaria 2, Peyronellaea sp. and E. nigrum were recovered by re-isolation.

DISCUSSION

The success of the tests employing both low and high relative humidity levels underlined one way in which environmental factors have differential control over fungal infection. It was clear that a low relative humidity (R.H.) accompanied by relatively high temperature favoured infection by Fusarium 1 and Fusarium 2 and discouraged infection by Drechslera 1, D. bicolor and E. lineatum. However a high relative humidity accompanied by a relatively high temperature reversed this trend. Both these sets of conditions may be duplicated in the field within a given 24 hour period. High R.H. and temperature may occur at dusk and with rain in summer, whereas a low R.H. and high temperature during the day will be limited by external conditions for relatively short periods of time only.

CHAPTER TWO

EXPERIMENT II

FIELD PATHOGENICITY TRIALS

In the summer of 1971, field trials were carried out in order to test the isolates for their pathogenicity under natural environmental conditions. Of particular interest were those genera which had resulted in significant infection in the greenhouse. For this reason three additional Drechslera isolates were tested. These had been obtained during the summer of 1970 by Dr. J. Reid, and all came from within a 20 mile radius of Fort Alexander. In order to provide room for these in the experimental design, three isolates which had showed little or no infection in the greenhouse trials (Chapter One), namely the member of the Mucorales, the Basidiomycete and E. nigrum, were omitted from the field experiment.

A randomised block design was chosen for the inoculations. This facilitated statistical treatment of the results by the Analysis of Variance and Linear Regression Analysis. The four criteria used to determine disease development were: (i) the estimation of lesion density on aerial parts, (ii) the seed yield (dry weight) of the plant, (iii) the

germinability of the seed and (iv) the persistence of the disease organism on or in the seed. The latter is dealt with separately in Chapter Four.

MATERIALS AND METHODS

Two experimental paddies were constructed at a site 100 metres north of the commercial paddies of Fort Alexander near Pine Falls, Manitoba. Spruce boarding was staked out to form a square enclosure with sides 15 metres in length and one metre in height for each paddy. These were lined with a double thickness of six mil (0.015 cm) polyethylene buried at the base to a depth of 14 cm. Soil was heaped against the outer sides of the paddy to prevent water seepage and water from a near-by creek was pumped into each to a depth of 30 to 42 cm.

A total of 96 plots, each 1.2 by 1.2 metres, were marked out. Paths 30 cm wide were left between each plot. The plots were grouped into four blocks; three in the first paddy and one in the neighbouring paddy and were randomised with the use of tables of random numbers (Fisher and Yates, 1957). This lay-out was necessitated by some difficulties which were encountered during transplantation and also required the use of edge plots (Plate 3#8 and 3#9). The end result was 24 plots per block, three of which served as uninoculated controls. A fourth control was derived from

randomised and uninoculated spare plants in plots reserved for point inoculation (one per block). This relatively small number of controls proved adequate as the plants were selected for uniformity at the time of transplantation.

Transplantation

Wild rice plants in the early aerial leaf stage were carefully uprooted from the commercial paddies of Fort Alexander. Each plant was individually transplanted avoiding damage to the roots and stem as far as possible. An earlier attempt had been made to transplant at the floating leaf stage but this had failed owing to the weakness of the stems; the slightest fracture was often fatal to the plant. Transplantation was carried out, one block at a time, over a period of three to four weeks. Approximately five days were required to complete each block.

Immediately following transplantation, the plants suffered a set-back lasting four to five days. However, this was limited to withering of the first aerial leaves. Examination of a few random plants revealed new adventitious roots appeared within 48 hours. Once these were well developed -- it seemed that none of the old roots continued to function after transplanting -- the aerial leaves soon started to push through the leaf sheaths (five to seven days). Seventeen days after transplantation, it was

Table 4

SUMMARY OF FIELD INOCULATIONS/TREATMENTS 1-22

	ISOLATE	METHOD OF INOCULATION
1	DRECHSLERA 1	SPRAY
2	DRECHSLERA 2	SPRAY
3	DRECHSLERA 3	SPRAY
4	DRECHSLERA 4	SPRAY
5	D. BICOLOR	SPRAY
6	ALTERNARIA 1	SPRAY
7	ALTERNARIA 2	SPRAY
8	ALTERNARIA 3+GONATOBOTRYS SIMPLEX	SPRAY
9	E. LINEATUM	SPRAY
10	E. LINEATUM	INJECT CULM
11	E. LINEATUM	INJECT INFLORESCENCE STALK
12	PEYRONELLAEA SP.	SPRAY
13	FUSARIUM 1	SPRAY
14	FUSARIUM 2	SPRAY
15	FUSARIUM 3	SPRAY
16	unidentified fungus #1	SPRAY
17	unidentified fungus #2	SPRAY
18	unidentified fungus #2	INJECT CULM
19	CONTROL 1	SPRAY
20	CONTROL 2	SPRAY
21	CONTROL 3	SPRAY
22	CONTROL 4	SPRAY

Treatments 23 and 24 (Cladosporium spp. - point inoc.) are omitted as insufficient data was obtained for disease assessment.

These treatment numbers (1-22) apply in Figs. 2.2, 2.3, 2.4, 2.5 and 2.6 and Figs. 4.1, 4.2, 4.3 and 4.4.

noticeable that there was not only within-block plant uniformity, but also between-block uniformity, despite the staggered planting times. At this stage the plants were ready for inoculation (Plate 3#10).

Field inoculations

The isolates with the respective method of inoculation for each are listed in Table 4,p.54. Except in certain cases where inoculum was injected into the culm or inflorescence stalk, each plant was inoculated twice. The first inoculations, aimed at aerial parts (in the case of the spray method) were started on July 28, 1971 and completed by August 13, at which time flower heads had begun to appear generally throughout the plots. The vigorous growth and tillering (three to four tillers per plant) had markedly increased the stand density. The second spray inoculations, begun on August 22 and completed by September 1, were aimed at aerial leaves which had emerged since the first inoculations.

The inoculum was prepared in the same manner and applied at the same dosage per unit area as for the greenhouse inoculation trials (Chapter One). For the spray inoculations the procedure was similar to that used in the greenhouse except that the use of wire-framed polyethylene hoods was omitted and allowances had to be made for the wind. Inoculated plots were marked with a stake on which were

written the details of inoculation procedure.

The point inoculation method was used as an alternative to the spray method when the latter had been found to be unsuccessful in the greenhouse. It was also used to help determine whether E. lineatum could develop systemically. The usual method was to inject 0.5 ml of spore suspension into the culm, at a point about 7 to 10 cm above the water level, though E. lineatum was also injected into the inflorescence stalks at a point just above the flag leaf. In any given plot point inoculum was observed to travel up into the tissues of the leaf sheaths and lower midrib regions of the laminas; some also moved downward into lower leaf sheath areas. In a few plants the inoculum flowed between leaf sheaths and flooded out around the ligule of the first leaf.

Determination of the Yield

It was necessary to collect the seed in order to determine the effect of specific isolates on the yield of the plants. Normally, wild rice plants shed their seed into the water, the moment that it matures. In order to overcome this shattering of the head, bags were stapled over individual heads approximately nine days after completion of the second inoculations. The bags used in Block A and B were air-porous, translucent pollinating bags

of the plastic coated variety (Bag stock No. 571, Cenco Pollinating Supplies, Corn States Hybrid Service Inc., Des Moines, Iowa). Unfortunately these bags were in short supply and so a lighter, slimmer type of bag had to be used in Block C and D (Bag stock No. 317 of the same manufacturer). As the bags caused the plants to bend over quite significantly, a supporting string was tied at a height of two meters between stakes placed at the corners of each plot in order to prevent breakage of the stems by wind and rain (Plate 3#9, p.64). Later observations showed that the plants stood up well despite the bags; little advantage was gained by the use of strings (Plate 3#8). Once the stringing was completed the bags were attached as quickly as possible, before shattering commenced; 25 plants per plot were bagged.

The heads were collected 14 days after bagging and the seed extracted from individual bags on a per-plot basis. It was cleaned by the removal of extraneous matter and larvae of the moth Apamea apamiformis, very common on the wild rice (Introduction: p.29). Each plot sample was weighed fresh and divided into two equal subsamples. One subsample was oven-dried at 150° for 48 hours and then re-weighed. By this time weights were constant. These dry weights represent the yield determinations and are shown in Appendix 2.1. The other subsample was stored under water in polyethylene bags at 2°C for germinability

determinations and an investigation of disease 'carry-over' via seed.

Determination of the Lesion Index

An estimate of leaf tissue damage was made by comparing harvested leaves with a series of cards bearing uniform dots in varying densities. The dots were marked on graph paper to percentage cover values of 0.10, 0.25, 0.50, 1.00, 2.50, 5.00, 10.00, 25.00 and 50.00 per cent. These round figures were chosen to simplify preparation of the cards and were numbered from 2 to 10 respectively. The card series approximates to a straight line when plotted logarithmically (Fig. 2.1, p.61). The flag leaves and next youngest leaves of the plants harvested from each plot were examined with reference to the cards. A lesion index number was then assigned to each plot on the basis of the closest match to a standard card, or an approximate decimalised number if their lesion density fell between two cards. Lesion indices represent the effect of both first and second inoculations.

Determination of the Seed Germinability

Seed which had been stored in the cold for six months at $2^{o}C$ was removed in May 1972 and germinated in 120-seed aliquots per plot sample in petri dishes containing sterile

distilled water. These were incubated in a growth chamber at 22°C with a 16 hour day of fluorescent and incandescent lighting and at 17°C during the night period. Germinated and ungerminated seedlings were counted after 10 days under these conditions. Ungerminated seed was not discarded at this stage but kept in storage for further experiments (V & VI) to be described in Chapter Four (p.170).

RESULTS

Plate 3, p.64 , illustrates the field plots and some of the disease symptoms which occurred.

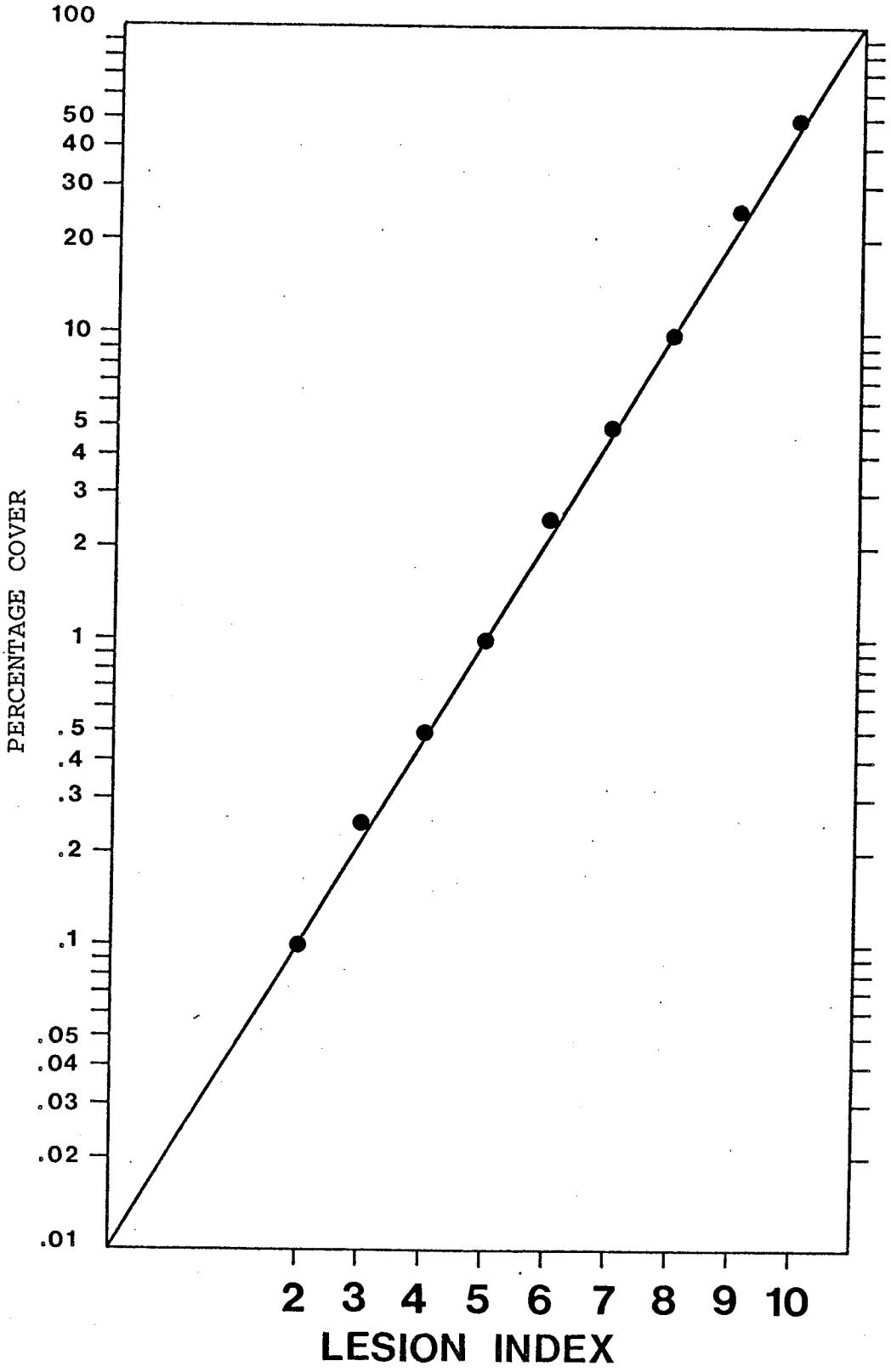
Yield

The mean data for seed yield, lesion index and seed germinability in Blocks A and B are presented in Fig. 2.2. An assessment of seed production in Blocks C and D proved impossible as the lightweight bags were destroyed by black-birds. The yield of inoculated plants is expressed as a percentage of control values in Fig. 2.3. Fig. 2.4 shows the variance (F ratio values) of yield and lesion index for the various treatments. The raw data and statistical analyses are summarised in Appendices 2.1 to 2.8. Statistical computations were carried out with the help of an Olivetti Programma 101 and statistical tables (Fisher and Yates, 1957). The five per cent level of

FIG. 2.1

A plot of percentage leaf tissue damage against the standardised card series (lesion index). Dots represent actual points used. The straight line is the linear plot for 0.01 to 100 per cent lesion cover.

FIG. 2.1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50
40
30
20
10
5
4
3
2
1
.5
.4
.3
.2
.1
.05
.04
.03
.02
.01
PERCENTAGE COVER
2 3 4 5 6 7 8 9 10
LESION INDEX

significance was accepted unless otherwise indicated. It should be pointed out that the yield variance and the percentage loss in yield are not entirely comparable indicators of yield reduction owing to the fact that the variance is a measure of within-block as well as between-block differences whereas the percentage loss in yield is a measure only of the difference in the means of two between-block replicates relative to the means of their controls.

Of all the isolates tested in the field, eight caused a significant loss in yield (Fig. 2.3 and 2.4). Three isolates of Drechslera spp. individually reduced the yield by 30, 23 and 20 per cent dry weight respectively. These were Drechslera 2, Drechslera 3 and Drechslera 4 obtained in the summer of 1970 by Dr. J. Reid. A fourth isolate, Drechslera 1 obtained in the fall of 1970 did not reduce the yield enough for it to be significant at five per cent though this was only marginal (Fig. 2.4 & Appendix 2.5). However if the dry weight data for all four isolates were treated as a whole, an F ratio resulted which was still significant at five per cent (Appendix 2.5).

D. bicolor produced by far the greatest loss (38 per cent), significant at 0.1 per cent (Appendix 2.5), and also a high variance (Fig. 2.4). Alternaria 2 also caused significant reduction in yield with a high F ratio.

Plate 3

Field Inoculation Trials

1 Drechslera bicolor

Lesion type 4.0

2.0

2 Drechslera 4

Lesion type 4.0

2.0

3 Drechslera 4

Lesion type 4.0

2.0

4 Fusarium 1

Lesion type 4.0 limited

Note absence of type 1.0; p.49.

5 Entyloma lineatum

Lesion type 6.1, inoculated inflorescence stalk showing early soral development and point of inoculation.

6 Entyloma lineatum

Lesion type 6.1; natural infection

7 Entyloma lineatum

Lesion type 6.2; black flecking of flag leaf sheath on plant with smut sori.

8 Wild rice plants with bags to record seed yield.

9 General view of experimental paddy (Fort Alexander) showing inoculated and bagged plots.

10 Wild rice plants at time of first spray inoculation (early aerial leaf stage).


1


2


3


4


5


6


7


8


9


10

FIG. 2.2

The seed yields (histograms), lesion indices (closed circles) and germinabilities of the resulting seed (open circles) from wild rice plants inoculated in the field with various fungal isolates. Bars represent standard deviations of the means. Where bars are absent, the standard deviation is zero. For treatments 1-22, see Table 4, p. 54.

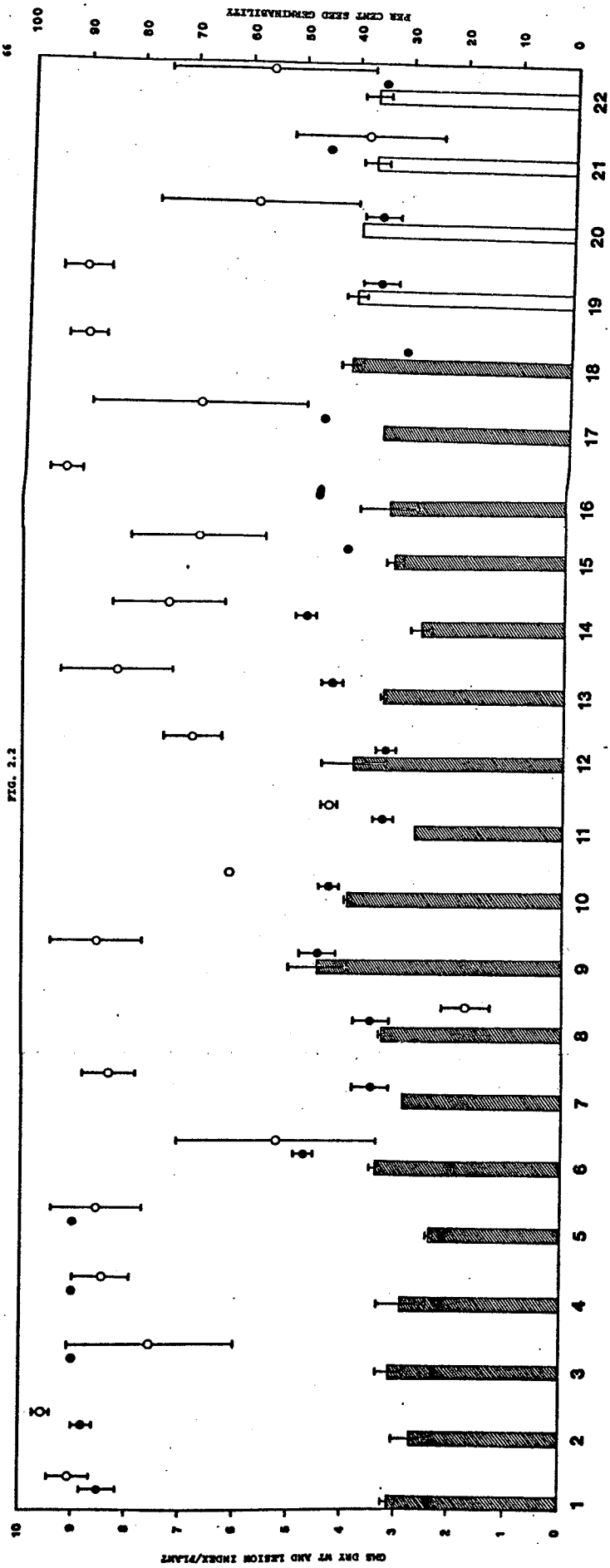

PER CENT SEED GERMINABILITY
100
90
80
70
60
50
40
30
20
10
0
22
21
20
19
18
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
10
9
8
7
6
5
4
3
2
1
0
GMS DRY WT AND LESION INDEX/PLANT

FIG. 2.2

FIG. 2.3

The percentage seed yields of wild rice plants inoculated with various fungal isolates under field conditions (Cf: Fig. 2.2). For treatments 1-18, see Table 4, p. 54.

FIG. 2.3

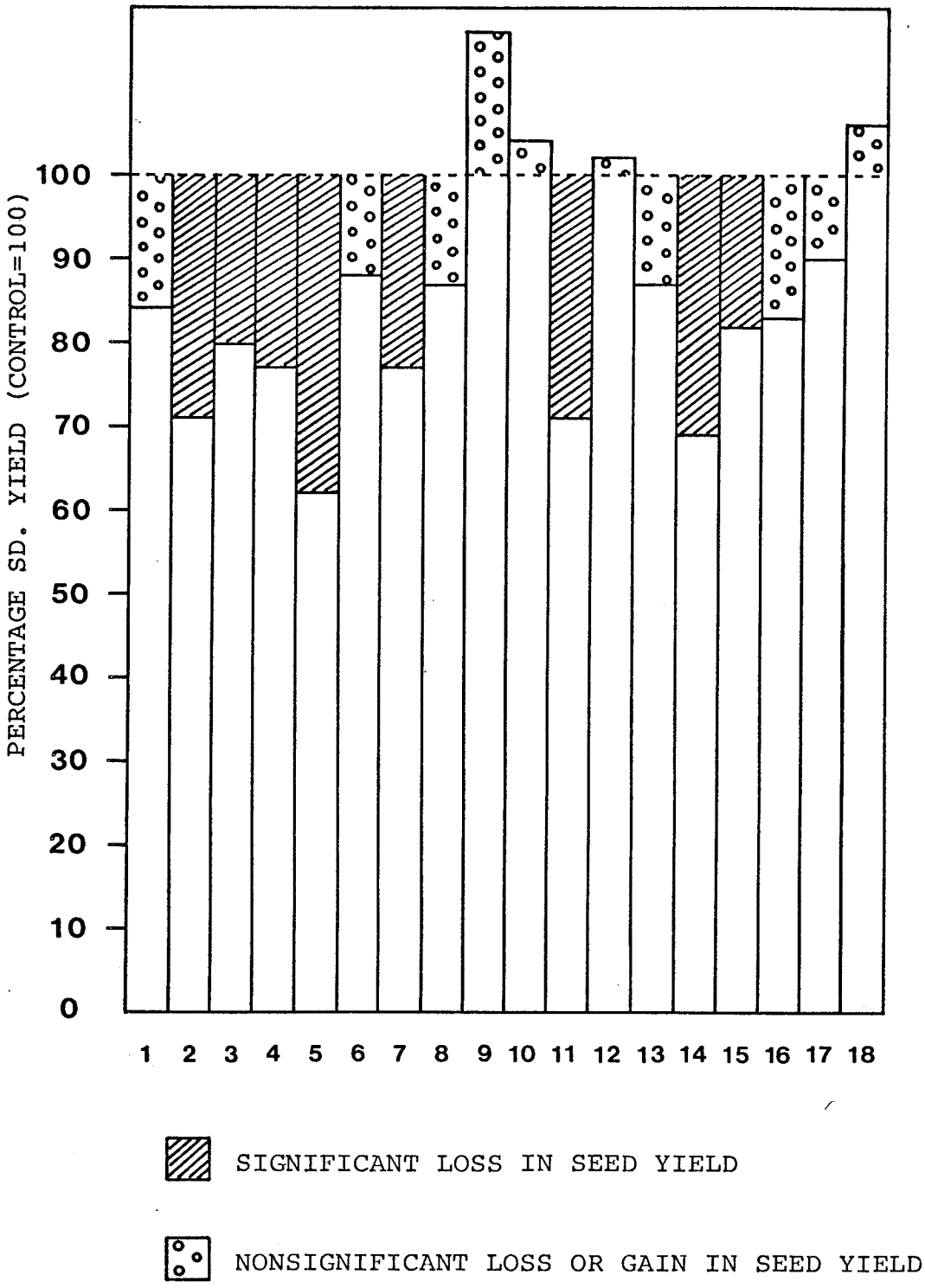
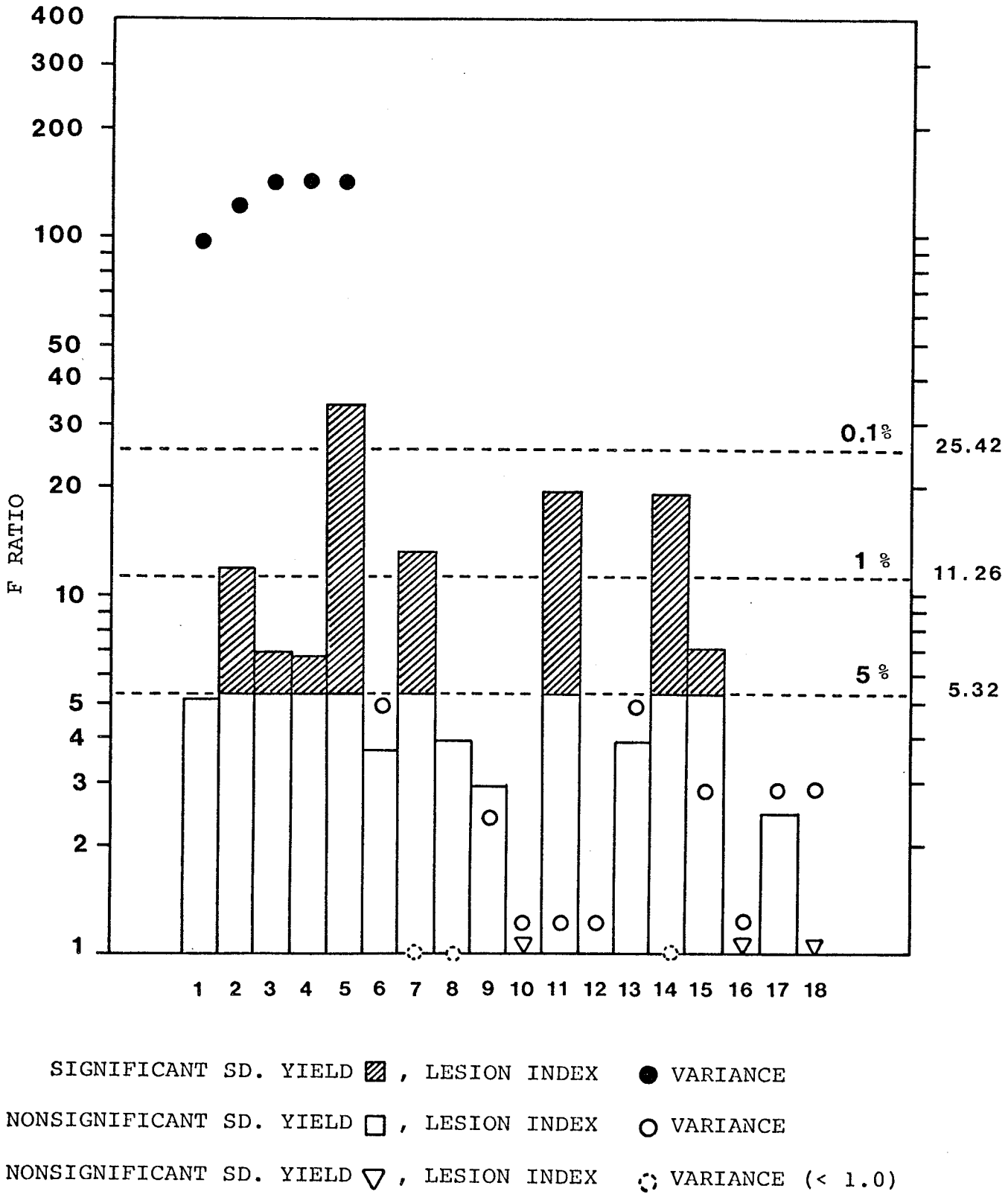

PERCENTAGE SD. YIELD (CONTROL=100)
100
90
80
70
60
50
40
30
20
10
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
SIGNIFICANT LOSS IN SEED YIELD
NONSIGNIFICANT LOSS OR GAIN IN SEED YIELD

FIG. 2.4

The variance (F ratios) relative to the controls, of the seed yields and lesion indices from wild rice plants inoculated with various fungal isolates under field conditions (Cf: Fig. 2.2). The broken lines represent specific significance levels (Degrees of Freedom $n_1 = 1$; $n_2 = 8$). For Treatments 1-18, see Table 4, p.54.

FIG. 2.4


400
300
200
100
50
40
30
20
10
5
4
3
2
1
F RATIO
0.1%
25.42
1 %
11.26
5 %
5.32
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
SIGNIFICANT SD. YIELD , LESION INDEX VARIANCE
NONSIGNIFICANT SD. YIELD , LESION INDEX VARIANCE
NONSIGNIFICANT SD. YIELD , LESION INDEX VARIANCE (< 1.0)

E. lineatum failed to cause a significant reduction in yield by aerial spraying as with spore injection into the base of the culm. However, a significant variance was recorded from plants injected in the inflorescence stalks. Here the yield was reduced by 29 per cent and the variance was almost half that of D. bicolor. Two Fusarium isolates which caused significant losses in yield were Fusarium 2 and Fusarium 3. The latter was not previously tested in the greenhouse owing to its limited sporulation. When inoculated as a suspension of mycelial fragments it caused a loss in yield to the extent of 18 per cent. The remaining isolates all failed to produce effects upon yield which were significant at the five per cent level.

Lesion Index

Of all the isolates inoculated, only five gave lesion indices with F ratios which were significant at the five per cent level (Fig. 2.4). These were the five Drechslera isolates, all of which had very considerable F ratios. Their lesion indices were indicative of between 15 and 25 per cent lesion coverage (Plate 3#1, 3#2 and 3#3). The remaining lesion indices fell between three and five representing lesion coverages of between 0.2 and 1.0 per cent (Fig. 2.1).

At this point it should be mentioned that a positive correlation existed between yield and lesion index. This was determined by computing a simple linear regression analysis for both parameters (Fig. 2.5). The zero order correlation coefficient was 0.48171, significant at the five per cent level (Appendix 2.8).

Seed Germinability

Fig. 2.2 shows that none of the treatments resulted in significant increases or decreases in germinability relative to all the controls. Standard deviations for individual isolates varied enormously and help to explain the inconclusive results (Appendix 2.4). However a few generalised observations can be made. The germinabilities of seeds from plots inoculated with the more pathogenic isolates such as those of Drechslera spp. were all high. These were between 75 and over 90 per cent. Seed from plants inoculated with Alternaria 2 also had over 80 per cent germinability. The seed from plants inoculated with the remaining isolates showed germinabilities between 30 and over 80 per cent with no apparent trend related in any way to disease. The controls also varied.

Although there was no significance at five per cent, the computation of a correlation coefficient between lesion indices and seed germinabilities revealed that there was

FIG. 2.5

The correlation (and linear regression analysis) between lesion indices and the seed yields of parental plants inoculated with various fungal isolates under field conditions. Bars represent the standard deviations of the means.

Slope of Regression Line	-1.93682
Intercept on y	11.51875
Zero Order Correlation Coefficient	0.48171
S y.x, Standard Error of Estimate	1.85770

For Treatments 1-22, see Table 4, p. 54.

FIG. 2.5

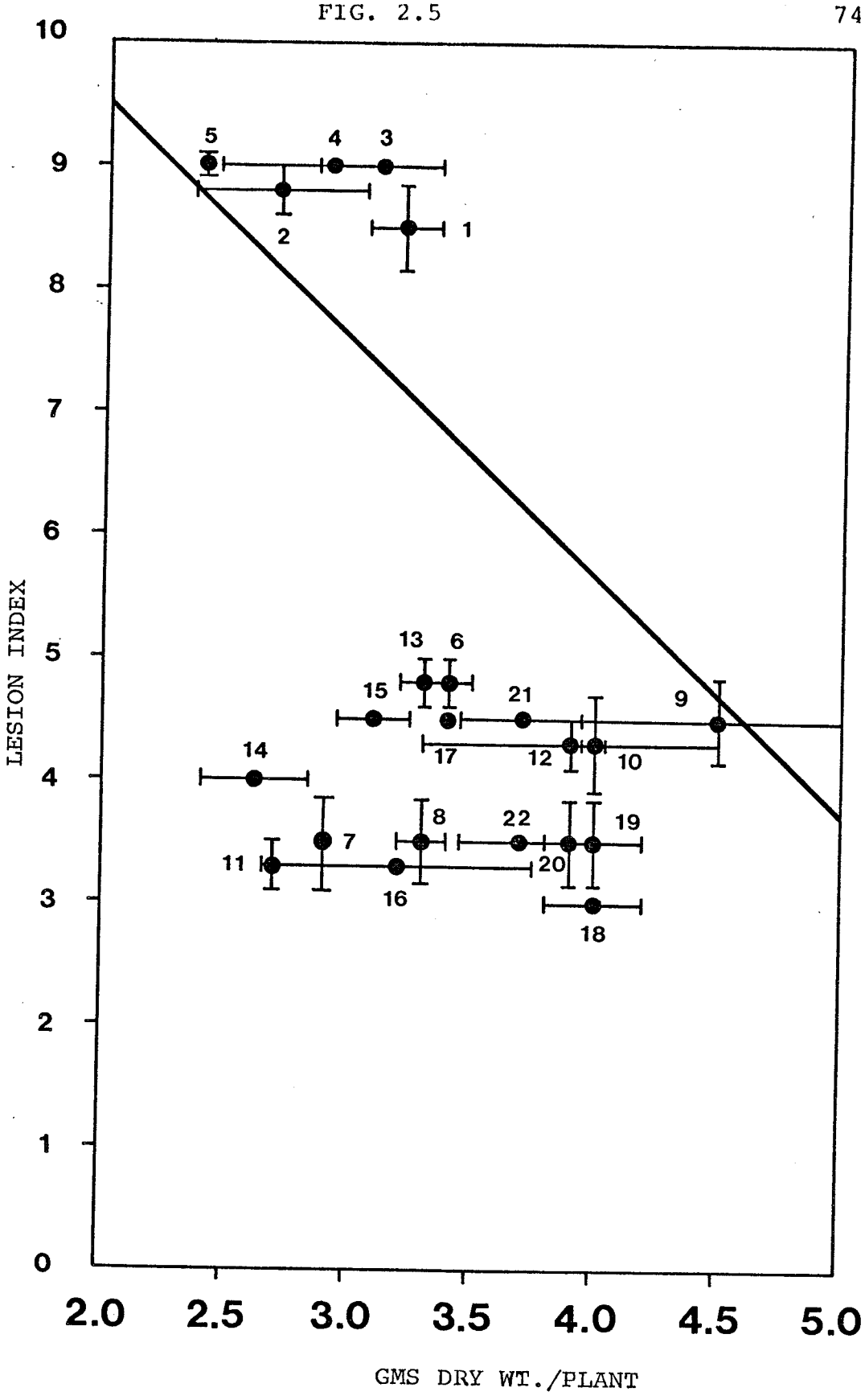

LESION INDEX
GMS DRY WT./PLANT
0
1
2
3
4
5
6
7
8
9
10
2.0
2.5
3.0
3.5
4.0
4.5
5.0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22

FIG. 2.6

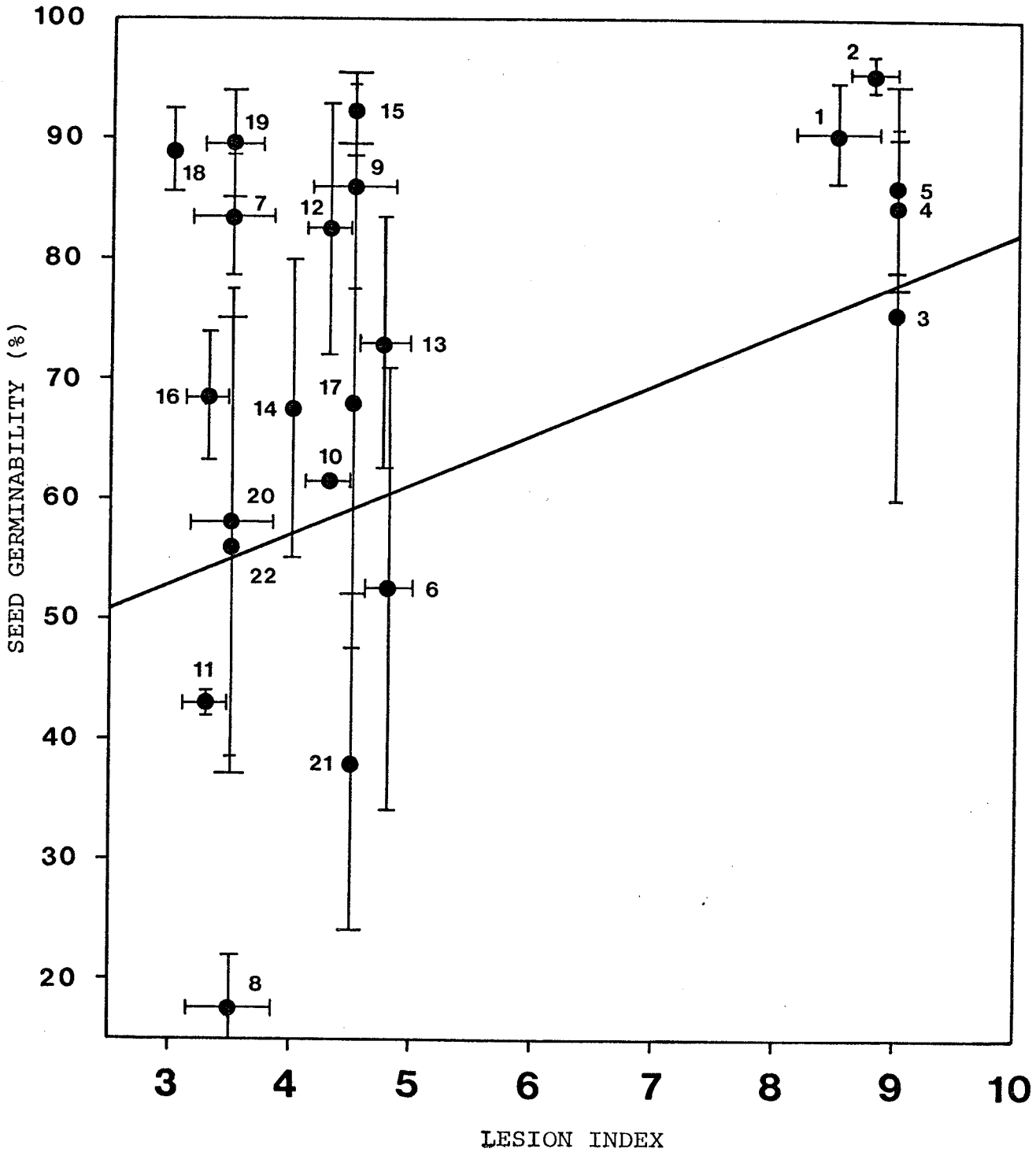
The correlation (and linear regression analysis) between wild rice seed germinability and the lesion index of field inoculated parental plants. Bars represent standard deviations of the means.

Slope of Regression Line	3.93004
Intercept on y	50.96477
Zero Order Correlation Coefficient*	0.42029
S y.x, Standard Error of Estimate (biased)	17.98464

*Significant at the 10 per cent level. (See Appendix 2.8,p.222).

For treatments 1-22, see Table 4, p.54.

FIG. 2.6


100
90
80
70
60
50
40
30
20
SEED GERMINABILITY (%)
3
4
5
6
7
8
9
10
LESION INDEX
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22

some significance at the 10 per cent level (Fig. 2.6, p.76). The zero order correlation coefficient was only marginally non-significant at five per cent (Appendix 2.8). In relation to yield, however, the zero order correlation coefficient was totally non-significant.

Further correlations for the seed germinability were later carried out with respect to the ability of fungi to persist in or on the seed. Results of these are presented in Chapter Four.

DISCUSSION

Yield

The yields of the control plots were sufficiently uniform to allow the detection of yield losses in the inoculated plots with the use of the Analysis of Variance. The need for proper uniform control plots cannot be overemphasised, particularly here where the number of replicates had been reduced to two.

It was found that of the five Drechslera isolates tested in the field, four significantly reduced the yield of wild rice. The differential losses in yield induced by these isolates may probably be attributed to their differing degrees of virulence. The greatest loss in yield

incurred by D. bicolor (38 per cent) suggests that it is the most virulent and important member of this group of pathogens. Drechslera 1, which failed to significantly reduce the yield (16 per cent), may be a less virulent foliar pathogen owing to its adaptation to culm attack. Point inoculation clarified this (Experiment III).Alternatively, being a fall isolate, it may be better adapted to growth on senescent tissue, though D. bicolor was also isolated in fall. The remaining three Drechslera isolates were made in summer.

The reduction in yield of plots inoculated with Alternaria 2 is hard to reconcile with the low lesion index. Lack of visible symptoms suggests low pathogenicity. It may be significant, however, that Alternaria was a very common contaminant of freshly stored wild rice seed. Alternatively, reduction in yield may be due to an internal effect. Fungal toxin production occurs in certain species within this genus; for example A. kikuchiana and A. citri (Oku, 1967).

E. lineatum reduced the yield significantly only when injected into the inflorescence stalks. Although this was a substantial reduction (29 per cent), no specific controls had been established to indicate the effect on the plant of the hypodermic syringe alone. This and other inoculations were repeated in the greenhouse the following winter to

clarify the nature of this loss in yield (Experiment III & IV).

Fusarium 2 and Fusarium 3 also reduced the yield significantly but with very low lesion indices of 4.0 and 4.5 indicating only about 0.5-1.0 per cent lesion cover (Fig. 2.1).

Those isolates which failed to cause a loss in yield or significant lesion index in both greenhouse and field inoculations, were assessed as non-pathogenic to wild rice. These include Alternaria 1, Alternaria 3 (parasitized by Gonatobotrys simplex) and unidentified fungi #1 & #2. Other isolates not tested in the field for reasons of space and time but found non-infective in the greenhouse, can also be added to this list and these include E. nigrum, the Basidiomycete and the member of the Mucorales.

Lesion Index

Lesion indices for Drechslera 1, 2, 3, 4 and D. bicolor were the only significantly variant indices of all the isolates and corresponded to a significant reduction in yield in all cases except for Drechslera 1 where nonsignificance was only marginal. In these cases a loss in yield was presumably a direct result of reduced photosynthetic functions of the aerial parts. It is probable that

the 10 to 25 per cent lesion coverage of the leaves led to a significant reduction in the amount of material translocated to the inflorescence at the time of seed development. This is borne out by the strong (inverse) correlation found between lesion index and yield (Fig. 2.5). D. bicolor was outstanding in this respect with a lesion index of 9.0 and a yield of only 2.4 g. per plant (dry weight) (Discussion, p.160).

Despite the overall correlation, other isolates which caused a significant reduction in yield gave low lesion indices. These include Alternaria 2, E. lineatum, Fusarium 2, and Fusarium 3 with lesion indices from 3.2 to 4.5 or from 0.25 to 0.75 per cent lesion cover. Any of these fungi may have affected the plant internally without the external manifestation of severe symptoms.
E. lineatum was considered a possible systemic invader. Fusarium 2 and Fusarium 3 may have produced toxins (Discussion p.161). The effect of the fungus on the plant could be magnified many times if each lesion were a site of toxin production, at least temporarily. The necrotic spreading nature of the Fusarium lesions obtained later in Experiment III (e.g. Plate 5#1 and 5#7) as well as the blotch-like character of the lesions of Fusarium 2 (Plate 2#3) would not be in conflict with this conjecture. Whatever the cause of the loss in yield, it can hardly be explained by the reduction in photosynthetic material associated with a few lesions.

The results of the Fusarium inoculations confirmed the importance of environmental factors (particularly the R.H.) in infection in the field, already inferred from the greenhouse tests described in Chapter One. The "shot-hole" lesions produced by Fusarium spp. in the greenhouse under conditions of low humidity were more or less absent in the field trials (Plate 3#4) and this was attributed largely to the humid conditions prevalent in the field at the times of inoculation.

Seed Germinability

Germinabilities of seed from inoculated plants ranged from 17 to 96 per cent and in no case varied significantly from the controls, which themselves ranged from 37 to 89 per cent (Appendix 2.4 & 2.7). An overall general correlation between seed germinability and lesion index, however, was significant at the 10 per cent level and as Fig. 2.6 indicates, high germinabilities more commonly occurred in those seed samples which came from inoculations producing a high lesion index. This was particularly noticeable for the Drechslera isolates which all corresponded with germinabilities above 75 per cent. The zero order correlation coefficient for germinability and yield however, was altogether nonsignificant (Appendix 2.8). As yield and lesion index showed such strong correlation (Fig. 2.5), the fact that only one, the lesion index, correlated with

the seed germinability to any level of significance suggests that the pathogen may in some way affect the seed germinability by a factor or factors other than, and separate from the factors such as chlorophyll destruction by which it affects the dry seed weight (yield). Secondly, it is interesting that the effect of the pathogen is not entirely adverse, for a high lesion index is correlated at the 10 per cent level (Fig. 2.6) with apparent stimulation rather than depression of seed germinability at a level of significance closely approaching 5 per cent (Appendix 2.8).

For the remaining isolates, germinabilities were generally good and above 50 per cent with one exception. This was that of Alternaria 3 parasitized by G. simplex where the germinability was 17 per cent. Why this occurred is not known, but it may simply be a reflection of the variability in the germination rates of the seed. This feature of wild rice is not without reason; a low germinability does not necessarily indicate a lack of seed viability, but may merely indicate high dormancy. A tetrazolium test for viability may have been useful here though it was reported that it was not very reliable for wild rice seed (J. Dean, personal communication). In nature it is not uncommon for there to be early spring freshets which "drown out" whole stands of wild rice in the early floating leaf stage. However, the following spring

a new stand of wild rice invariably appears owing to the fact that there was plenty of dormant seed which remained and did not germinate in the previous spring. Thus a relatively low seed germinability (high dormancy) is advantageous to the survival of the species. It can be argued therefore, that the apparent stimulation of seed germinability by Drechslera spp. may in the long run be unfavourable to the plant by diminishing the pool of dormant seed (p.247).

Lastly, the seed was stored under conditions which were very different from the natural milieu of the lake or river floor. It is not known to what extent the germination was modified by the artificial storage conditions. This could be ascertained by a comparison with naturally stored inoculated seed.

CHAPTER THREE

EXPERIMENT III

GREENHOUSE SPRAY AND POINT INOCULATIONS

Most of the isolates which had shown any pathogenic tendencies in the preliminary greenhouse and field inoculation trials were subjected to more detailed study in the greenhouse. In addition to Drechslera, Fusarium and Entyloma isolates from Manitoba, isolates from Minnesota paddies of Drechslera (designated 5L and 6C, from leaf and culm respectively) and Fusarium (designated 4, from leaf, culm and inflorescence stalks) were included in these studies for comparative purposes. Spray and point inoculations were aimed at determining the pathogenic status of each isolate with respect to leaf and/or culm invasion.

MATERIALS AND METHODS

Design

Fifty-six tanks, prepared as previously described (Chapter Two), were arranged to accommodate a randomised block design consisting of 12 treatments and two controls in each of four blocks. The isolates and treatments are

listed in Table 5, p.86. Planting was carried out in four or eight tanks at a time as were the subsequent inoculations.

Plants

Plants of the partially non-shattering 'Algot Johnson' type were grown from seed (16 per tank for spray and 25 per tank for point inoculations) at a water and air temperature of 17-21^{o}C and a relative humidity of approximately 60 per cent. More plants (25) were required per tank for point inoculations because approximately half in each tank were used as controls. For spray inoculations, controls consisted of separate tanks of plants (16) per block. A thermohygrograph was used to provide a continuous record of temperature and humidity; this data is presented later in Figs. 3.1 to 3.8 for the infection periods. During the period of the inoculations the ambient temperature was raised to 28^{o}C in order to facilitate infection.

The plants were prepared for inoculation by the removal of all moribund or dead submersed and floating leaves as well as any aerial leaves which bore lesions.

Inoculum

The inoculum of all isolates was produced in the manner previously described (Chapter Two). However, this time

Table 5

REISOLATION (FROM LESIONS) OF INOCULATED FUNGI

	FUNGI INOCULATED ON WILD RICE	METHOD OF INOCULATION	SUCCESSFUL REISOLATION (r)	OTHER FUNGI ALSO ISOLATED
1	DRECHSLERA BICOLOR	spray	r	-
2	DRECHSLERA BICOLOR	toothpick	r	-
3	DRECHSLERA 1	spray	r	-
4	DRECHSLERA 1	toothpick	r	ALTERNARIA SP.
5	DRECHSLERA 5L	spray	r	-
6	DRECHSLERA 5L	toothpick	r	-
7	DRECHSLERA 6C	spray	r	-
8	DRECHSLERA 6C	toothpick	r	-
9	FUSARIUM 4	spray	r	-
10	FUSARIUM 4	spray (dry)	r	-
11	FUSARIUM 1	spray (dry)	r	-
12	E. LINEATUM	spray	-	-
13	E. LINEATUM	spray(+inject)	-	-
14	CONTROL	spray	-	ALTERNARIA SP.
15	CONTROL	spray(+inject)	-	-

further steps were taken to determine the number of propagules per ml as well as the composition and the germinability of the propagules in each inoculum. The use of a Coulter Counter made it possible to obtain counts from suspensions of all isolates regardless of propagule size. For the large-spored Drechslera isolates, 25 ml of spore suspension was made up to 200 ml (needed to fill the apparatus) with the standard electrolyte solution (Isoton). Six 2 ml samples were passed through the 400 μ aperture. The mean of 6 counts corrected for dilution and sample factors gave the value for the number of propagules per ml. In the case of E. lineatum or Fusarium spp., five ml of spore suspension was diluted to 100 ml with Isoton. Each count was based on a 20 λ sample passed through the 100 μ aperture.

The composition of the inoculum was determined by counting the relative numbers of conidial and mycelial fragments on a haemocytometer grid cell. To determine spore germinability the relative number of germinated and ungerminated spores was counted after spreading the suspension on PYG plates and incubating for four to five hours at 25°C. The mean values of both inoculum composition and germinability were derived from four counts, each of a minimum total of 25 and 100 units, respectively.

Dikaryon formation in E. lineatum was assessed by counting bridge cells with a haemocytometer grid cell 24 hours after mixing monokaryon cultures of opposite mating type. A further check was made by counting mycelial dikaryon colonies which developed when a mixed spore suspension (24 hrs) was spread on PYG plates and incubated at 25°C overnight.

Inoculation

Three methods of inoculation were used for the respective isolates. These included (a) spray inoculation, (b) point inoculation by toothpick and thirdly point inoculation by hypodermic syringe.

a Spray Inoculation

Spore suspensions of D. bicolor, Drechslera 1, D. 5L, D. 6C, Fusarium 1 and Fusarium 4 were sprayed on the aerial parts under the temperature and humidity conditions shown graphically in Figs. 3.1 - 3.8. For 18 hours after inoculation, artificial humidification was provided as described in Chapter Two except for Fusarium 1 and a single treatment of Fusarium 4. Initially plants were inoculated in the early aerial leaf stage (eight to nine weeks). These spray inoculations were repeated when more aerial leaves had emerged (10-11 weeks). The plants in control tanks were

sprayed with sterile distilled water containing a few drops of the surfactant Tween 80.

b Point Inoculation

Toothpick

The Drechslera isolates were also inoculated by this second method. Sterilized wooden toothpicks were scraped along the surface of densely sporulating cultures and then inserted, just above water level, into the culms of 12 randomly selected plants per tank. This left 12 to 13 plants per tank to act as controls which were treated in the same way except their toothpicks lacked any inoculum. All toothpicks were removed after 24 hours.

Hypodermic Syringe

In addition to spray inoculation alone second inoculations of E. lineatum consisted of injecting 0.5 ml of spore suspension into the inflorescence stalks at the early flowering stage of previously spray-inoculated plants. Ten to twelve randomly selected plants were inoculated in this way. Plants in control tanks were inoculated in the same manner, but with sterile distilled water. Essentially the same method had been used for some inoculations in the field (Chapter Two).

Assessment of Pathogenicity

a Lesion Development

In the case of the spray inoculations, observations of disease development commenced 10 to 12 hours from the time of inoculation. Lesion indices were again determined using the card series described in Chapter Two. This time the actual progress of disease development was followed by examining infected plants on a periodic basis for at least six days after inoculation. Notes were made and photographs taken of the various disease symptoms.

In order to verify that infection was directly attributable to the inoculated fungus, reisolations were attempted from one replicate of each treatment (Table 5, p.86).

b Yield

The yield of the experimental plants was also determined in the same manner as described in Chapter Two, though fewer plants were involved. No bags were necessary to retain the seed here, as the plants were of the largely non-shattering 'Algot Johnson' type. Tillers were excluded from yield determination as some may have escaped inoculation owing to their later development.

RESULTS

The results of the inoculation studies in Experiment III are presented under three main headings: 1. Symptom development 2. The influence of treatment on seed yield 3. Correlation between lesion index and seed yield.

1. SYMPTOM DEVELOPMENT

Detailed records of symptom development following both first and second inoculations of each pathogen were obtained; however in the interest of brevity these are presented only in summary form.

Spray Inoculations

For each treatment involving spray inoculations a lesion index progress curve was plotted along with values for temperature and relative humidity (Figs. 3.1 - 3.8; pp. 95 - 117). Data relating to the density, germinability and composition of the inocula are also summarised in these figures. Illustrations of lesions are shown in Plates 4#1 - 4#9 (p. 123) and 5#1 - 5#10 (p. 125). To compare these with natural field symptoms, see Plate 1, p. 39.

The lesion types encountered in Experiment III are classified and listed in Table 6 for ease of reference. Minor variations within the basic types are indicated by secondary numerals (e.g. 1.1, 2.1). Plates are cited in Table 6 to illustrate these symptoms. Invariably, more than one lesion type is found in each plate. Some citations are also made to earlier plates to illustrate symptoms which were not photographed in Experiment III.

1. Controls

Alternaria sp., weakly parasitic in this instance, was isolated from occasional very localised lesions (Table 5, p.86). Leaves and culms were otherwise completely free of infection.

2. Drechslera bicolor (Fig. 3.1)

After four days many shrivelled and dead leaves were apparent in all four replicates, though in general, stem lesions were less abundant than with Drechslera 1, except at the junction of lamina and sheath in the area of the ligule where conidia had accumulated. It was noticeable that necrotisation of localised lesions had lead to some shot-hole effect (lesion type 1.0, Plate 4#1) at first attributed solely to Fusarium spp. (Plate 2#1 and 2#2, Chapter One). In some replicates a brown discoloration

Table 6

Lesion Types

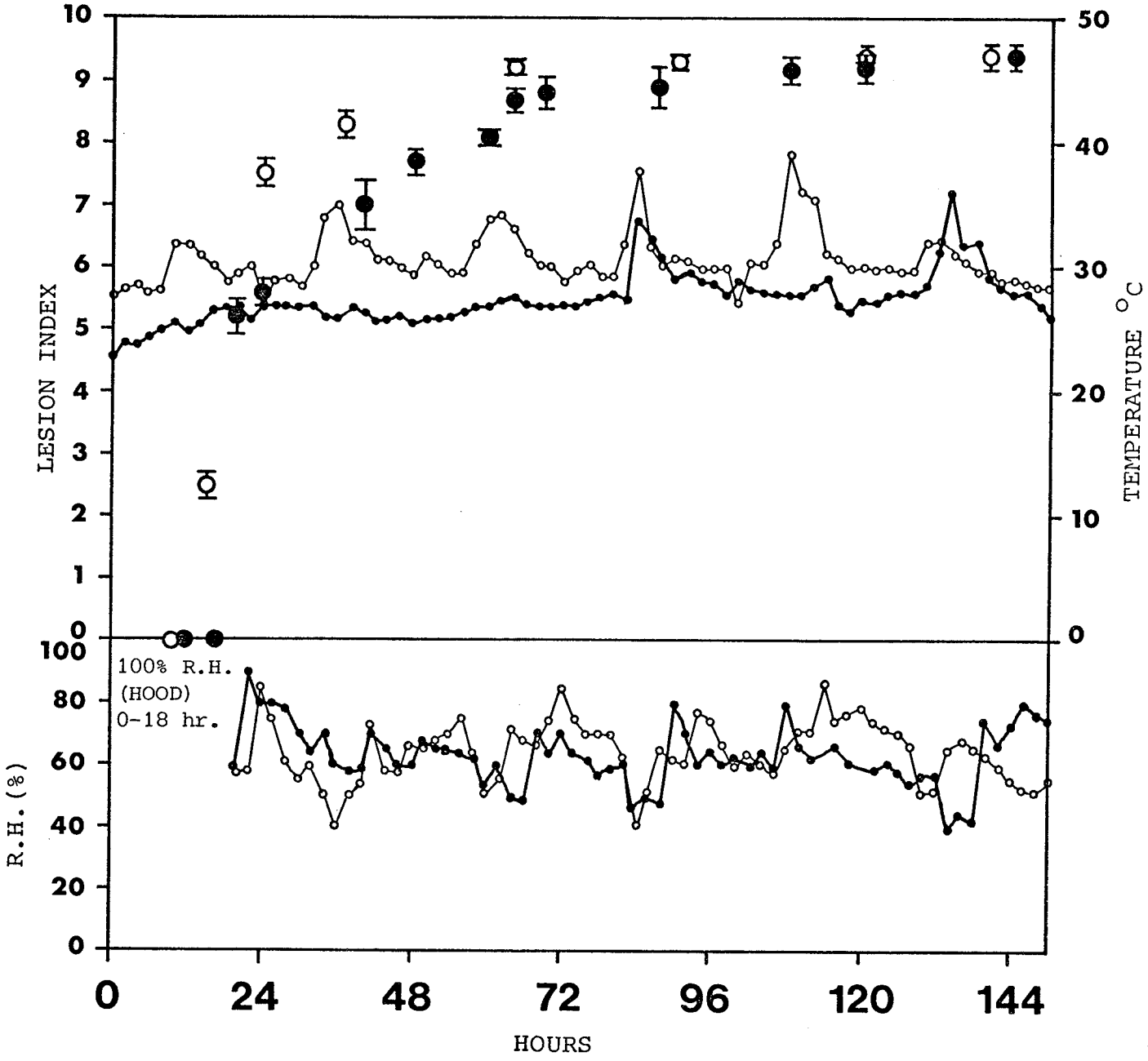
PLANT PART	CODE	DESCRIPTION
Leaf	1.0	Localised necrosis (shot hole) 0.1 X 0.1 cm (Plate 4#1 - 4#4, 4#6 and 4#9)
Leaf	1.1	Larger necrotic spots 0.2 X 0.2 cm (Plate 4#5)
Leaf	2.0	Necrotic areas, often a later stage of 1.0, but also of 4.0 0.5-1.0 X 1.0-10.0 cm (Plate 1#3, 4#2, 4#7 and 4#9)
Leaf	2.1	Necrotic areas with extensive bleached tissue 0.5-1.0 X 1.0-10.0 cm (Plate 4#8, 5#1, 5#2 and 5#10)
Leaf/Culm	3.0	Brown discoloration 0.5-1.5 X 1.0-10.0 cm (Plate 4#8, 4#9, 5#1, 5#3 - 5#7, 5#10 and 6#1 - 6#9)
Leaf/Culm	3.1	Similar to 3.0 except in the distinctive red-brown colour (Plate 1#6)
Leaf/Culm	4.0	Localised brown spot 0.1-0.4 X 0.1-0.5 cm (Plate 1#1, 1#2, 1#4, 4#1 - 4#3, 4#7 - 4#9, 5#5, 5#7 and 5#8)
Leaf/Culm	4.1	Larger brown spot; centre often necrotic 0.5-1.0 X 0.5-2.0 cm (Plate 1#3, 1#5, 5#4, 5#7 and 5#8)
Leaf	6.0	Elongate black-brown spot 0.05-0.4 X 0.2-0.7 cm (Plate 2#9)
Inflorescence and Stalk	6.1	Black to lead-grey sori 0.05-0.4 X 0.2-0.7 cm (Plate 3#5 and 3#6)

Lesion types 5.0 and 7.0 (Table 2, p.35)
did not occur in Experiment III

FIG. 3.1

The development of lesions from first (closed circles) and second (open circles) inoculations with Drechslera bicolor, under greenhouse conditions with initial artifical humidification. Large circles represent the lesion indices.

FIG. 3.1



SPORE COUNT (PER ML)		SPORE GERMINABILITY (%)		INOCULUM COMPOSITION (%)	
				CONIDIA	MYCEL. FRAG.
1st	2nd	1st	2nd	2nd	
5.4×10^4	5.1×10^4	98.4	96.7	71.1	28.9

(lesion type 3.0, Plate 5#3) developed towards the tip of the sheath of the flag leaf. This resulted in a withering of the main upper culm region and browning of the partially emerged female flowers which had been attacked while enclosed within the infected inflorescence sheath.

A summary of lesion types includes the following:

Lesion type 1.0: local in extent (Plate 4#1)

Lesion type 2.0: abundant (Plate 4#7)

Lesion type 3.0: abundant (Plate 5#3)

Lesion type 4.0 and 4.1: present but generally not persistent; short-lived (Plate 4#1 and 4#7).

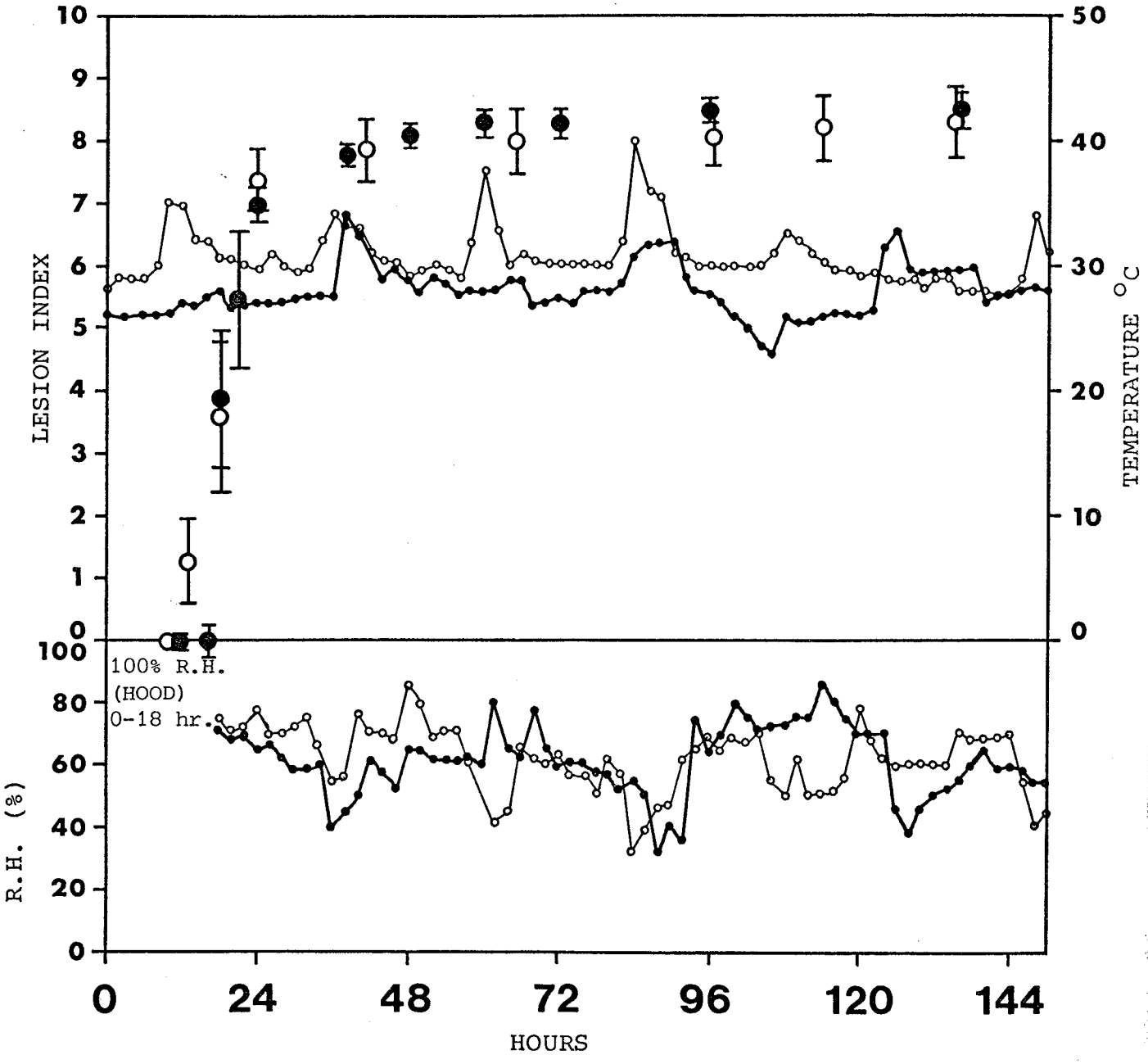
3. Drechslera 1 (Fig. 3.2)

Spreading lesions were found particularly on leaf mid-ribs and leaf bases where clumps of conidia occurred. This led to leaf collapse in a few isolated plants as a result of infection in the lower mid-rib regions. Stem lesions were more apparent than in D. bicolor. The spread of some stem lesions gave rise to heavily blackened areas. Similarly spreading leaf lesions of types 2.0 and 3.0 (Plate 4#2 and 4#8) were progressing towards type 2.1 (Plate 4#8) with the development of white to yellow necrotic leaf areas. Extensive leaf damage of this type, however, was confined to a few leaves of a relatively small number of plants.

FIG. 3.2

The development of lesions from first (closed circles) and second (open circles) inoculations with Drechslera 1, under greenhouse conditions with initial artificial humidification. Large circles represent the lesion indices.

FIG. 3.2



SPORE COUNT (PER ML)		SPORE GERMINABILITY (%)		INOCULUM COMPOSITION (%)	
				CONIDIA	MYCEL. FRAG.
1st	2nd	1st	2nd	2nd	
3.2×10^4	2.6×10^4	100	100	71.3	28.7

Accompanying these, but usually on different plants, were the localised necrotic shot holes (lesion type 1.0, Plate 4#2). More or less total infection of the main culm of one plant in one replicate resulted in the abortion of its browned female flowers.

Once again five lesion types were outstanding:

Lesion type 1.0: moderately localised (Plate 4#2)

Lesion type 2.0 and 2.1: moderately localised (Plate 4#2 and 4#8)

Lesion type 3.1: moderate (as in Plate 1#6; natural infection)

Lesion type 4.0: extensive (Plate 4#2, 4#8 and 5#8)

Lesion type 4.1: moderate (Plate 5#4).

For this isolate lesion type 3.1 was less severe and lesion type 4.1 (culm) slightly more severe than for the corresponding lesion types (3.0 and 4.1) caused by D. bicolor.

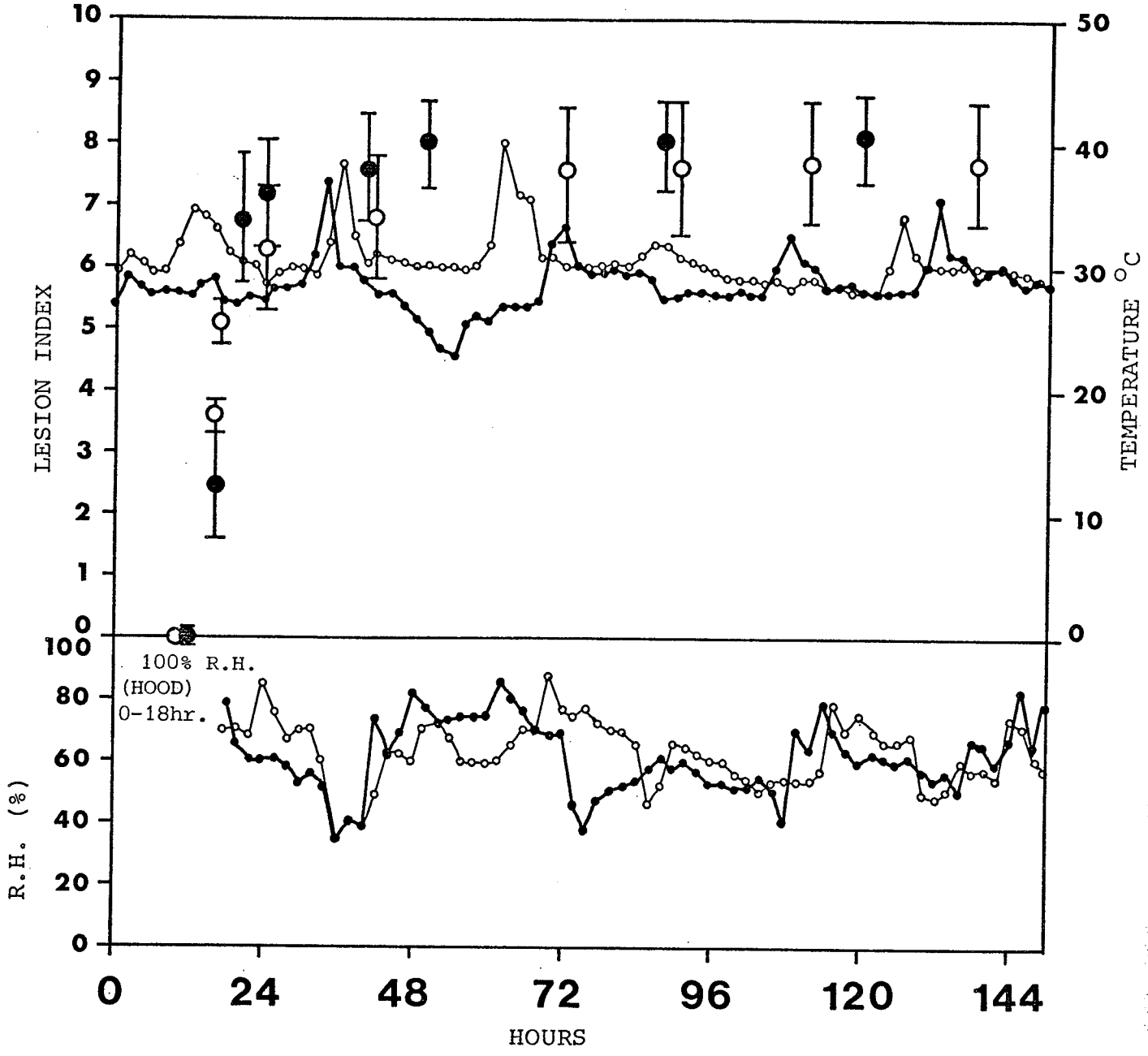
4. Drechslera 5L (Fig. 3.3)

In one replicate lesion development was limited compared to the other three replicates and this difference resulted in moderately high standard deviations (Fig. 3.3).

FIG. 3.3

The development of lesions from first (closed circles) and second (open circles) inoculations with Drechslera 5L, under greenhouse conditions with initial artificial humidification. Large circles represent the lesion indices.

FIG. 3.3



SPORE COUNT (PER ML)		SPORE GERMINABILITY (%)		INOCULUM COMPOSITION (%)	
				CONIDIA	MYCEL. FRAG.
1st	2nd	1st	2nd	2nd	
3.0×10^4	3.4×10^4	100	100	79.6	20.4

A few large leaves collapsed although the number of lesions borne by them was not very great. Lesion type 1.0 (Plate 4#3, 4#4 and 4#9) spread, forming necrotic zones (lesion type 2.0, Plate 4#9).

Moderately extensive damage to the inflorescence was noted in one replicate. Browned and withered male and female flowers were found. Some male flowers were infected after the emergence of the inflorescence at the pre-opening pollen maturation phase; these did not open and consequently failed to release pollen. Others suffered similar damage but prior to the emergence of the inflorescence.

A summary of lesion types includes the following:

Lesion type 1.0: relatively scarce compared to D. bicolor and Drechslera 1 (Plate 4#3 and 4#4)

Lesion type 2.0: present, but less frequently associated with the confluence of lesion type 1.0 than in D. bicolor and Drechslera 1 (Plate 4#3, 4#4 and 4#9)

Lesion type 3.0: moderately abundant (Plate 5#5 and 4#9)

Lesion type 4.0: moderately abundant (Plate 5#5)

Lesion type 4.1: moderately abundant (as in Plate 1#3 and 1#5; natural infection).

5. Drechslera 6C (Fig. 3.4)

Dark brown to black discolorations were apparent on inflorescence stalks after three days. These were very typical of the original source material collected in Minnesota, where they could be confused with smut sori, which occurred in abundance with them. Considerable damage to the male flowers was also found and browning of the female flowers in one replicate. These eventually aborted. In another replicate severe shot-hole blasting of the leaf was recorded (lesion type 1.0, as in Plate 4#4 and 4#9) and spreading necrotic lesions caused considerable damage. Both localised and spreading stem lesions occurred particularly in one replicate (lesion type 4.1 and 3.0, respectively, compare Plate 5#9 and 6#1).

A summary of lesion types includes the following:

Lesion type 1.0: limited in extent (as in Plate 4#2)

Lesion type 2.0: also limited; more or less confined to the areas where there had been a higher density of lesion type 1.0 (as in Plate 4#9)

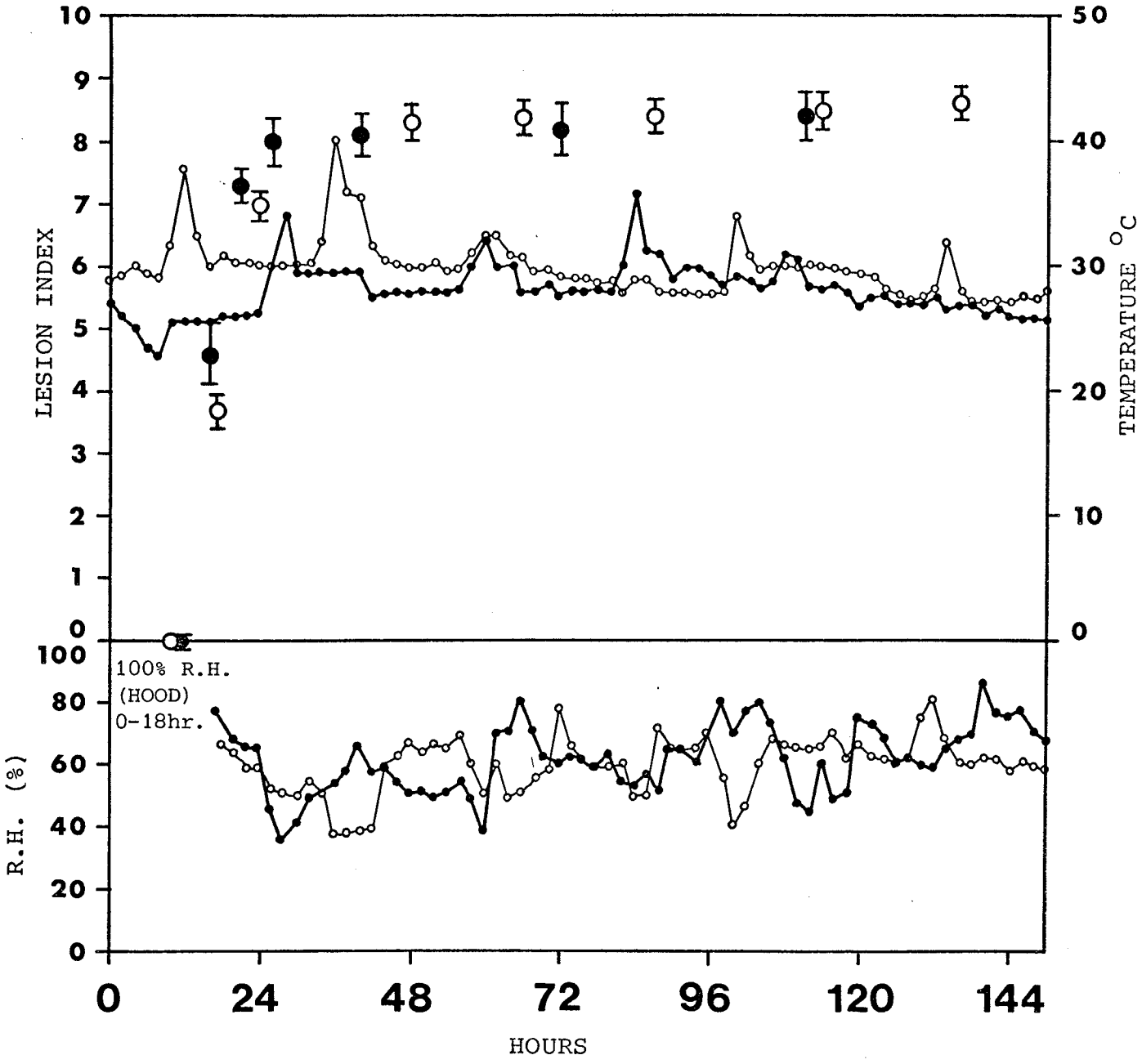
Lesion type 3.0: local on leaves; moderately widespread on culms (as in Plate 6#1)

Lesion type 4.0: moderate abundance (as in Plate 1#1, 1#2 and 1#4; natural infection).

FIG. 3.4

The development of lesions from first (closed circles) and second (open circles) inoculations with Drechslera 6C, under greenhouse conditions with initial artificial humidification. Large circles represent the lesion indices.

FIG. 3.4



SPORE COUNT (PER ML)		SPORE GERMINABILITY (%)		INOCULUM COMPOSITION (%)	
				CONIDIA	MYCEL. FRAG.
1st	2nd	1st	2nd	2nd	
2.7×10^4	2.9×10^4	99.6	100	82.1	17.9

6. Fusarium 1 WITHOUT HUMIDIFICATION (Fig. 3.5)

The growth and maturation of lesions was slower than the Drechslera isolates so that a more gently sloping lesion index curve resulted. This is a distinguishing feature of the Fusarium isolates tested.

After 16 hours shot-hole lesions were not much in evidence. More typically, a light brown spotting and blotching of the leaves occurred (lesion type 1.1, Plate 4#5). A number of plants in two replicates were still almost devoid of lesions after 24 hours. A few plants bore uniformly reddish brown spreading lesions (lesion type 3.1) which continued to spread after four days (lesion type 3.1, as in Plate 1#6; natural infection).

A summary of lesion types includes:

Lesion type 1.0: generally present; usually only abundant on plants which bore fewer lesions of type 2.0 and 3.1 (as in Plate 4#3)

Lesion type 1.1: moderately abundant on some plants (Plate 4#5)

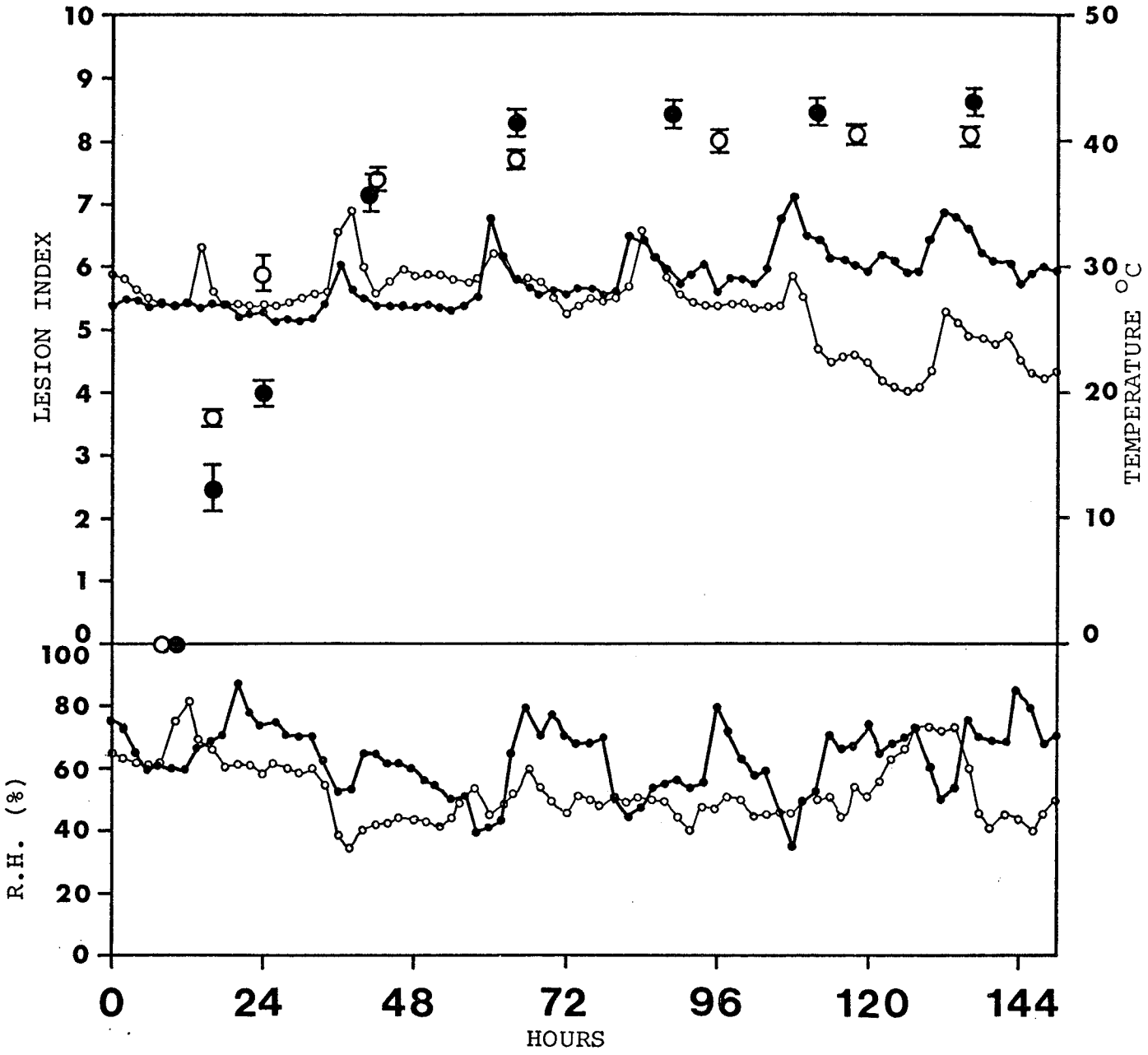
Lesion type 2.0 and 2.1: present, but more common on plants exhibiting fewer lesions of type 1.0 (Plate 5#1)

Lesion type 3.0: moderate abundance (Plate 5#6)

FIG. 3.5

The development of lesions from first (closed circles) and second (open circles) inoculations with Fusarium 1, under greenhouse conditions without initial artificial humidification. Large circles represent the lesion indices.

FIG. 3.5



SPORE COUNT (PER ML)		SPORE GERMINABILITY (%)		INOCULUM COMPOSITION (%)					
				MICROSPORES (0- to 1-SEPTATE)		MACROSPORES (>1-SEPTATE)		MYCEL.FRAG.	
1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
4.6×10^6	4.3×10^6	98.5	98.5	91.8	67.7	6.1	32.3	2.1	0

Lesion type 3.1: confined to a few plants which usually lacked lesions of type 1.0 (as in Plate 1#6; natural infection).

7. Fusarium 4 WITHOUT HUMIDIFICATION (Fig. 3.6)

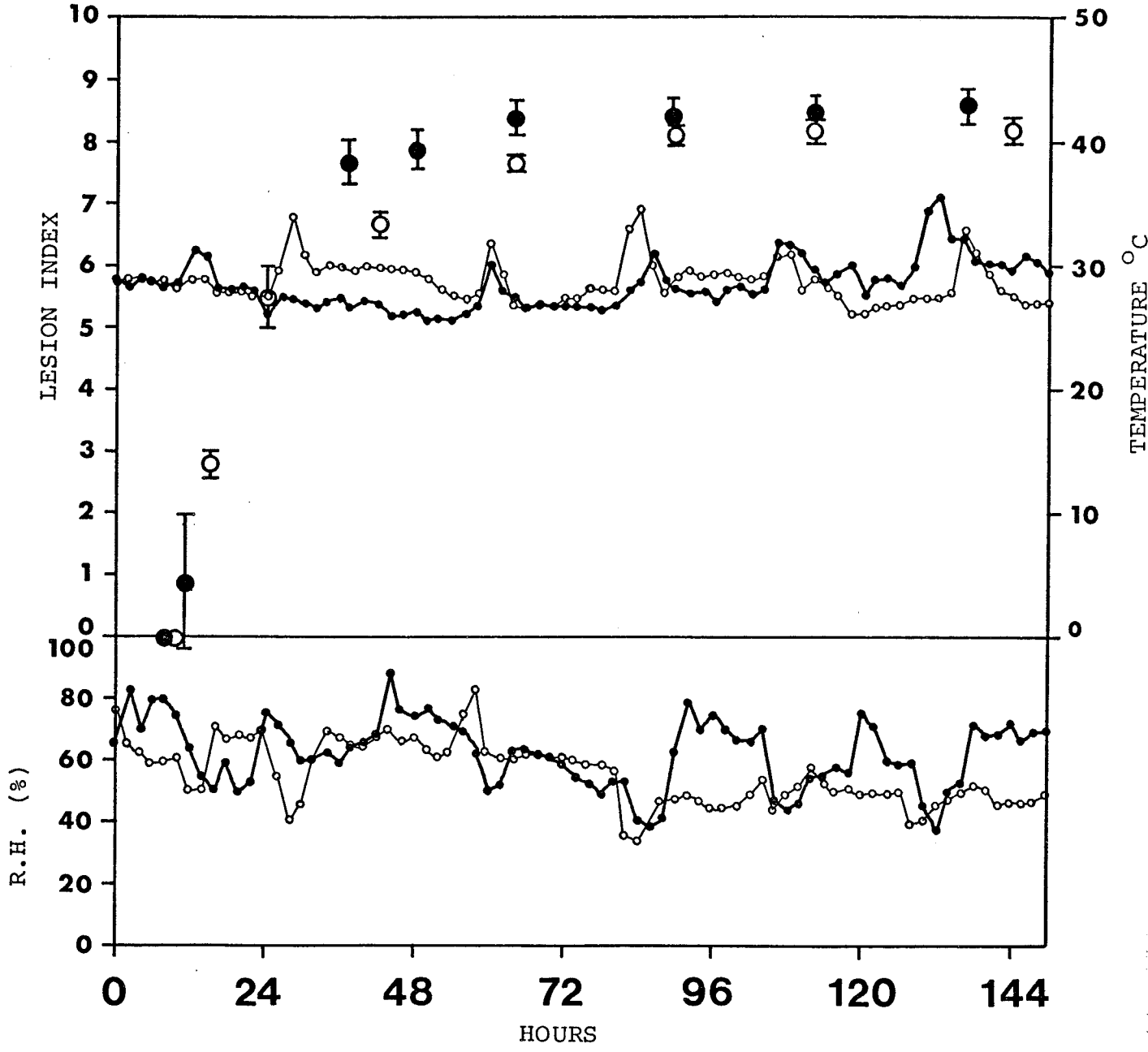
As this isolate had not been inoculated before, it was tested both with and without artificial humidification. The slope of the lesion index curve although slightly greater than that of Fusarium 1 was less than that of the Drechslera isolates.

After 12 hours definite lesions were apparent in all replicates. Leaves were seen to be developing a variegated and blotch-like pale green to brown pattern, so that comparison with the lesion index card series was very difficult. Lesions were sufficiently defined to enable estimation of the lesion index after 36 hours. Even after 48 hours blotched zones retained greenish areas and had not turned completely brown (lesion type 2.1, Plate 5#2). A partial chlorosis was seen in certain leaves. Longitudinal light brown lesions formed flecks on the culms of a few plants. The emerging male flowers frequently turned brown, though the already emerged female flowers appeared uninfected.

FIG. 3.6

The development of lesions from first (closed circles) and second (open circles) inoculations with Fusarium 4, under greenhouse conditions without initial artificial humidification. Large circles represent the lesion indices.

FIG. 3.6



SPORE COUNT (PER ML)		SPORE GERMINABILITY (%)		INOCULUM COMPOSITION (%)					
				MICROSPORES (0- to 1-SEPTATE)		MACROSPORES (>1-SEPTATE)		MYCEL. FRAG.	
1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
3.0×10^6	2.3×10^6	95.9	96.3	99.6	70.5	0	27.5	0.4	2.0

A summary of lesion types includes the following:

Lesion type 1.0: mainly on those plants which lacked the spreading lesions (Plate 4#6)

Lesion type 1.1: numerous on most plants (as in Plate 4#5)

Lesion type 2.0 and 2.1: present mostly on those plants which had fewer lesions of type 1.0 at the end of lesion maturation (Plate 5#2)

Lesion type 3.0: abundant on a number of plants most of which lacked lesions of type 1.0 (as in Plate 5#7 and 5#10).

In general this isolate appeared to cause more drastic symptoms than Fusarium 1 under the conditions specified.

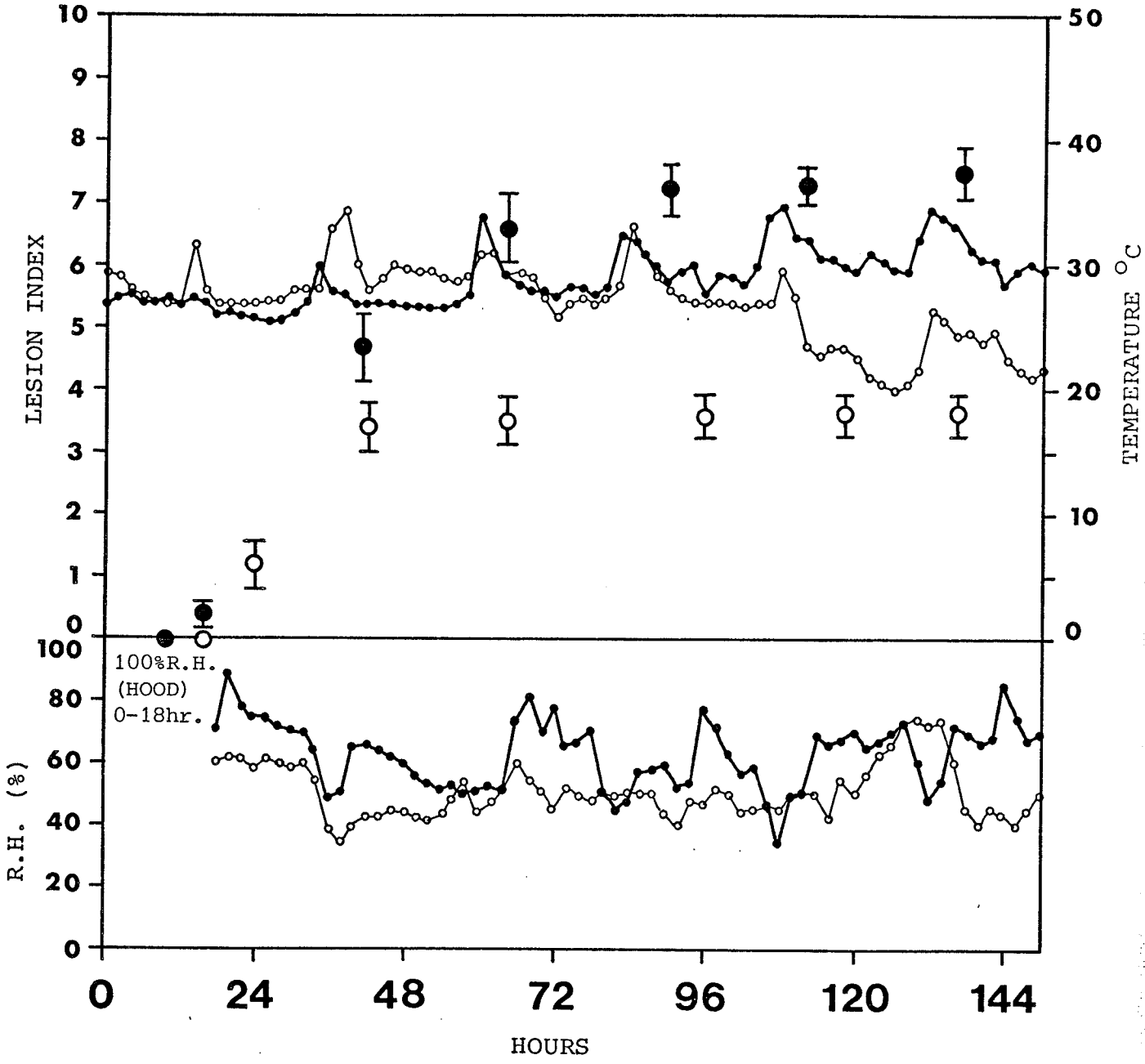
8. Fusarium 4 WITH HUMIDIFICATION (Fig. 3.7)

After 4 days, it was clear that symptom development qualitatively paralleled the corresponding inoculations without humidification, but differed quantitatively. A complete necrosis of the leaf tissue, 'bypassing' the initial browning process, resulted in the whitening of central and marginal leaf areas on some plants (lesion type 2.1, Plate 5#10). Second inoculations resulted in less infection than first inoculations as humidification was more complete (Discussion p. 161, second paragraph).

FIG. 3.7

The development of lesions from first (closed circles) and second (open circles) inoculations with Fusarium 4, under greenhouse conditions with initial artificial humidification. Large circles represent the lesion indices.

FIG. 3.7



SPORE COUNT (PER ML)		SPORE GERMINABILITY (%)		INOCULUM COMPOSITION (%)					
				MICROSPORES (0- to 1-SEPTATE)		MACROSPORES (>1-SEPTATE)		MYCEL. FRAG.	
1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
5.4×10^6	6.5×10^6	82.5	89.3	89.9	74.9	5.2	23.3	4.9	1.8

A summary of lesion types would include the same lesion types as those for the inoculations without humidification. Although they were as severe as the latter locally (Plate 5#10) they were less extensive.

9. Entyloma lineatum LEAF LESIONS (Fig. 3.8)

In the first inoculation lesion development had commenced in three replicates after 36 hours. Typically the lesions were confined to the base of the lamina (as in Plate 2#9). Infection was equally apparent on young and old leaves. The lesion index was very low at 2.9 and unless examined closely, the plants appeared as healthy as the uninoculated controls. The lesions were very dark grey to black in color, 0.5-1.0 X 1.0-2.0 mm.

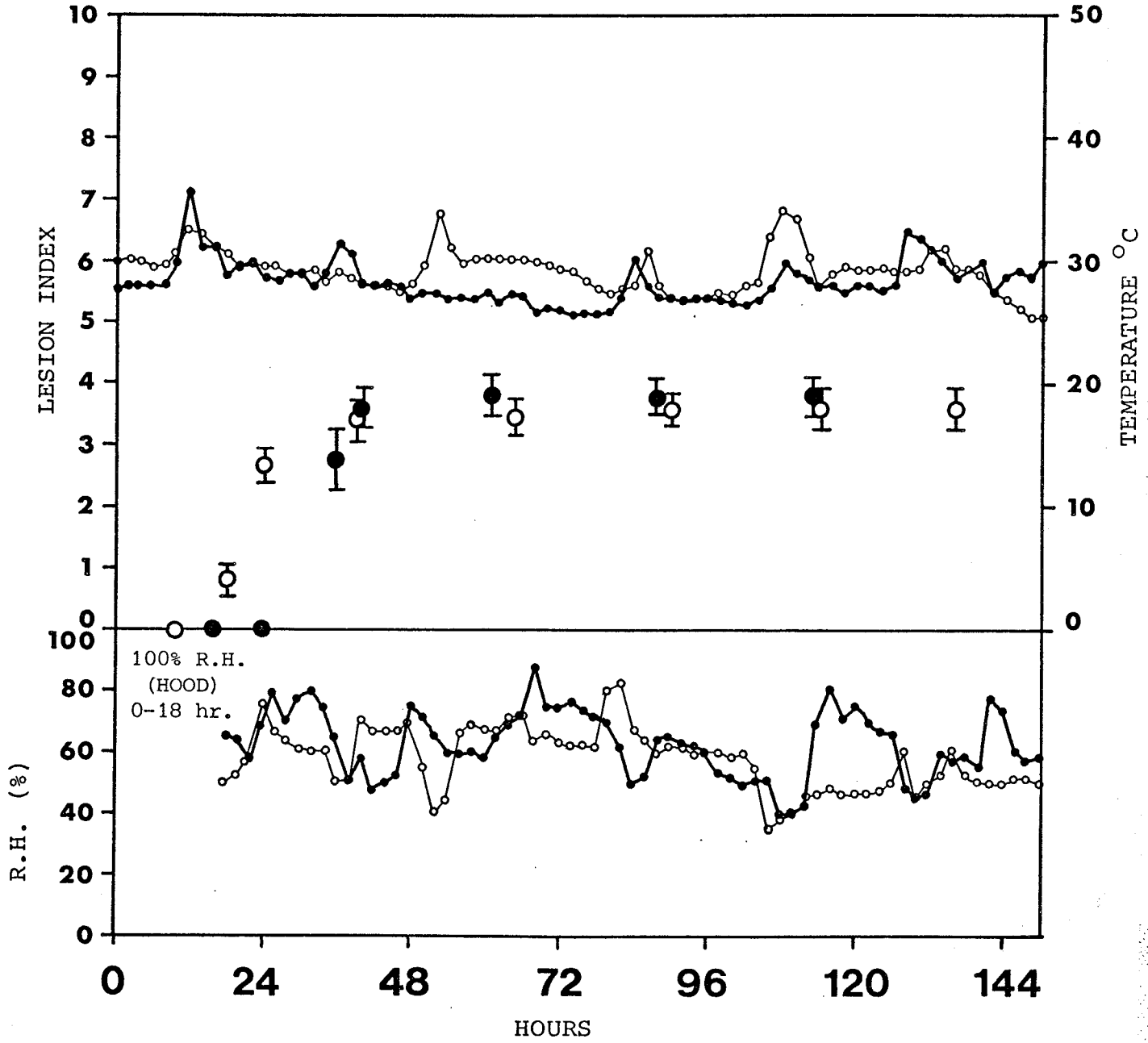
For the second inoculation, the initial stages of lesion development were apparent after 18 hours in all four replicates. After 24 hours browning was sufficient to discern the elongate shape of the lesions and by 39 hours lesions had darkened to grey-black and were again approximately 0.5-1.0 X 1.0-2.0 mm.

A noticeable infection of the male and female flowers was observed in two replicates after the second inoculation. Male flowers were browned slightly and withered. Browning

FIG. 3.8

The development of lesions from first (closed circles) and second (open circles) inoculations with Entyloma lineatum, under greenhouse conditions with initial artificial humidification. Large circles represent the lesion indices.

FIG. 3.8



SPORE COUNT (% PER ML)		DIKARYOTISATION (%)	
		BRIDGED CELLS (HAEM. CELL COUNT)	MYCEL. COLONIES (AGAR PLATES)
1st	2nd	2nd	
9.1×10^6	6.4×10^6	18.50	25.93

of the female flowers was generally confined to the plumose stigmas.

A summary of lesion types includes only the typical smut lesion:

Lesion type 6.0: localised, but present in all replicates. Usually approximately 0.5-1.0 X 1.0-2.0 mm (as in Plate 2#9).

10. Entyloma lineatum SORI (Table 7)

Sori were first observed 22 days from the first inoculation and were confined to a few plants of two replicates. The sori appeared light brown at first and were always located on the inflorescence stalk, usually just below the male flowers. They darkened and increased in size by 23 days. By 26 days these sori were black and had fully expanded to approximately 0.75-1.0 X 1.0-2.0 mm (as in Plate 3#5); still small in size compared to sori commonly found in the field (Plate 1#10 and 3#6). At 36 days new sori were still appearing.

Table 7

THE OCCURRENCE OF SORI FROM
THE INOCULATIONS OF *E. LINEATUM*

INOCULATION TREATMENT	MEAN NO. OF PLANTS PER REPLICATE WITH SORI	MEAN NO. OF PLANTS PER REPLICATE WITHOUT SORI	MEAN PERCENT PRESENCE OF SORI PER REPLICATE
SPRAY ONLY	2.5	11.5	17.9
SPRAY & INJECT	1.0	6.3	13.7

Point Inoculations

Toothpick

1 Controls

These showed extremely limited browning in the vicinity of the inserted toothpick.

2 Isolates

Lesions were not in sufficient abundance to warrant the estimation of lesion indices. The symptoms which resulted when D. bicolor, Drechslera 1, 5L and 6C were inoculated were similar for each isolate, except in their extent. Illustrations of symptoms are shown in Plates 6#1-6#9, p.127.

After 36 hours spreading brown culm lesions were noticeable at the point of toothpick insertion, for all the isolates tested (e.g. Plate 6#7). With D. bicolor a few basal leaves later withered and died as a result of some lesion spread into proximal vascular tissue (early stages in Plate 6#4) and partial damage to inflorescence stalks occurred (Plate 6#3).

Drechslera 1 showed some lesion development in upper leaf zones, but this was confined to the vicinity of the holes punctured through the leaves by the toothpick at the time when they were still within the culm. Similarly, damage to culm and inflorescence sheath regions occurred (Plate 6#1) as well as browning of male and female flowers (Plate 6#2, 6#5 and 6#6). Drechslera 6C also resulted in infection which was moderately drastic to culm and inflorescence (Plate 6#8 and 6#9).

Hypodermic Syringe

1 Controls

Very localised pale browning was apparent, confined to the immediate vicinity of the point of inoculation. In some replicates no browning was visible.

2 Entyloma lineatum

Injection of the inoculum of E. lineatum caused no additional development of lesions or sori over and above those which had resulted from spray inoculation (Table 7, p.119).

Plate 4

Greenhouse Spray Inoculations

Isolates are listed in the approximate order of the predominant lesion type (1.0-2.0) present in each sub-plate.

1 Drechslera bicolor

Lesion type 1.0: occasional
4.0

2 Drechslera 1

Lesion type 1.0: occasional
2.0: early stages
4.0

3 Drechslera 5L

Lesion type 1.0
2.0: early stages
4.0

4 Drechslera 5L

Lesion type 1.0
2.0: early stages

5 Fusarium 1 (without initial humidification)

Lesion type 1.1

6 Fusarium 4 (without initial humidification)

Lesion type 1.0
4.0 (faint brown spot)

7 Drechslera bicolor

Lesion type 2.0
4.0

8 Drechslera 1

Lesion type 2.1
3.0: limited
4.0: occasional

9 Drechslera 5L

Lesion type 1.0
2.0
3.0: some
4.0: limited

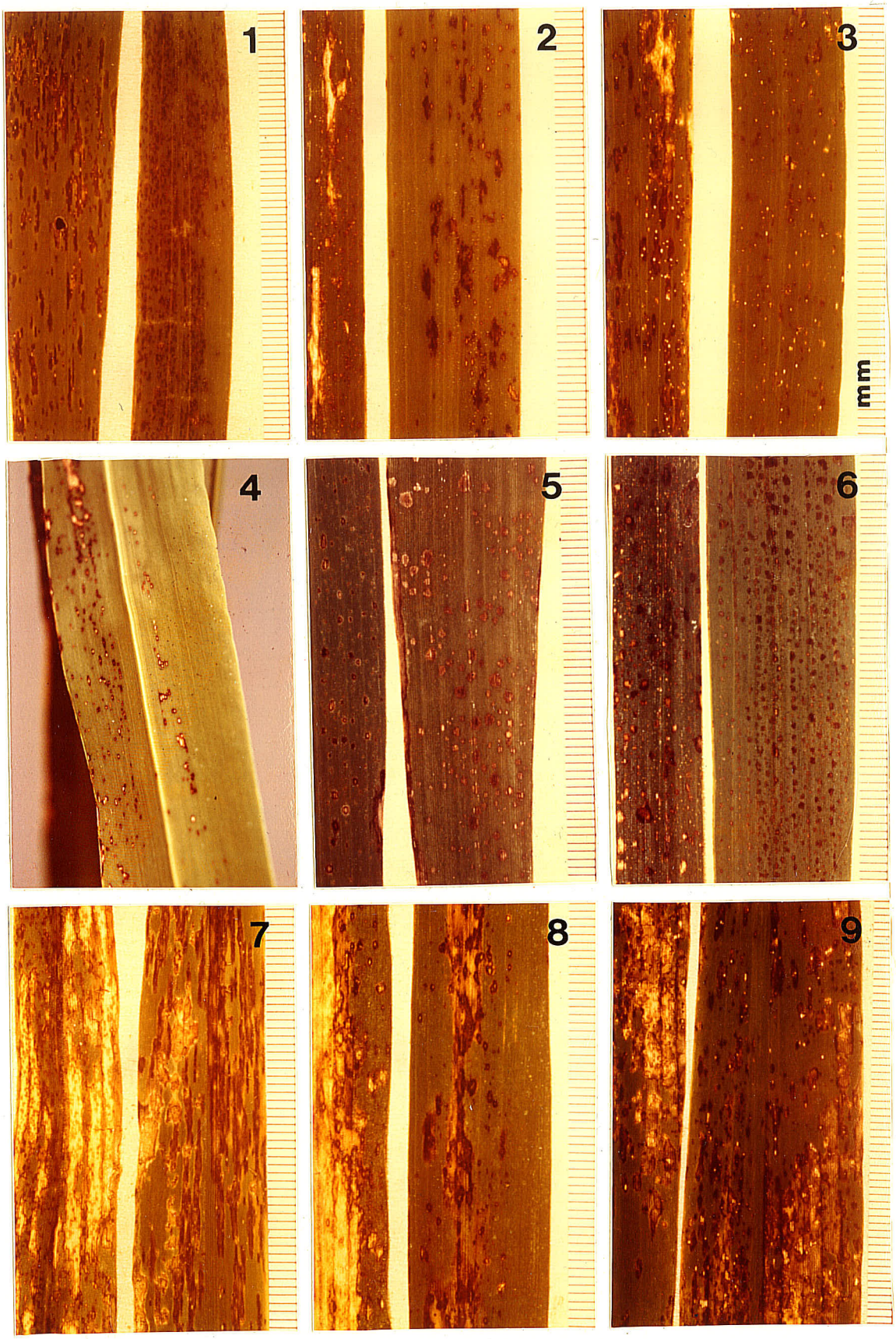

1
2
3
mm
4
5
6
7
8
9

Plate 5

Greenhouse Spray Inoculations

Isolates are listed in the approximate order of the predominant lesion type (3.0-4.0) present in each sub-plate.

1 Fusarium 1 (without initial humidification)
Lesion type 2.1
3.0

2 Fusarium 4 (without initial humidification)
Lesion type 2.1

3 Drechslera bicolor
Lesion type 3.0

4 Drechslera 1
Lesion type 3.0
4.1

5 Drechslera 5L
Lesion type 2.1
3.0
4.0

6 Fusarium 1 (without initial humidification)
Lesion type 3.0

7 Fusarium 4 (WITH initial humidification*)
Lesion type 3.0
4.0
4.1

8 Drechslera 1
Lesion type 4.0
4.1: early stages

9 Drechslera 5L
Lesion type 4.1: spreading

10 Fusarium 4 (WITH initial humidification*)
Lesion type 2.1
3.0

* Initial humidity was less than 100%.
See Discussion pp. 161, 162.

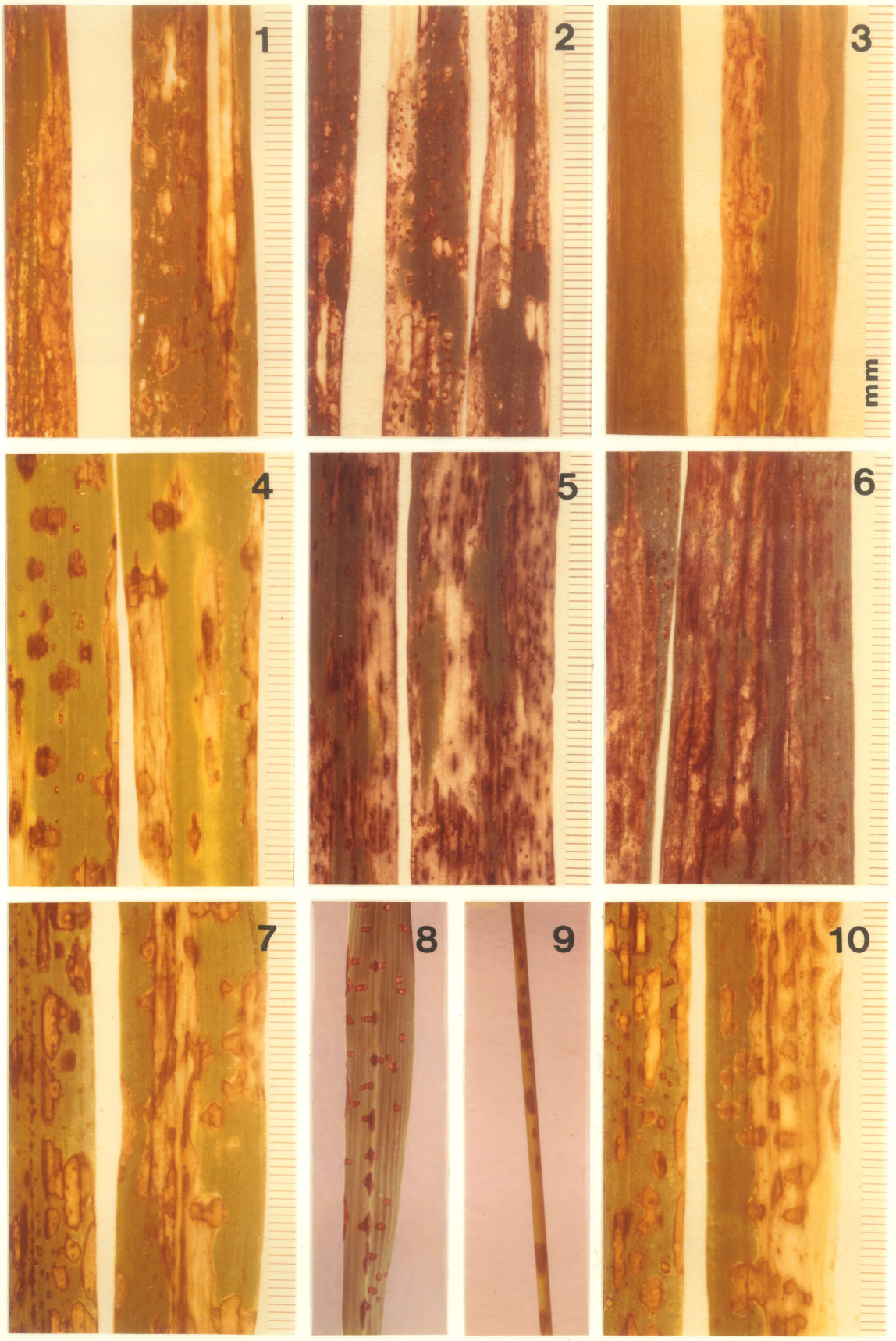

1
2
3
mm
4
5
6
7
8
9
10

Plate 6

Greenhouse Point Inoculations (toothpick)

1 Drechslera 1

Lesion type 3.0: flag leaf sheath

2 Drechslera 1

Lesion type 3.0: inflorescence sheath
(same plant as in Plate 6#1).

3 Drechslera bicolor

Lesion type 3.0: inflorescence stalk and male flowers

4 Drechslera bicolor

Lesion type 3.0: base of culm
(same plant as in Plate 6#3).

5 Drechslera 1

Lesion type 3.0: inflorescence stalk with browned and "adhering" male flowers

6 Drechslera 1

Lesion type 3.0: inflorescence tip
(continuation of Plate 6#5).

7 Drechslera 5L

Lesion type 3.0: point of inoculation

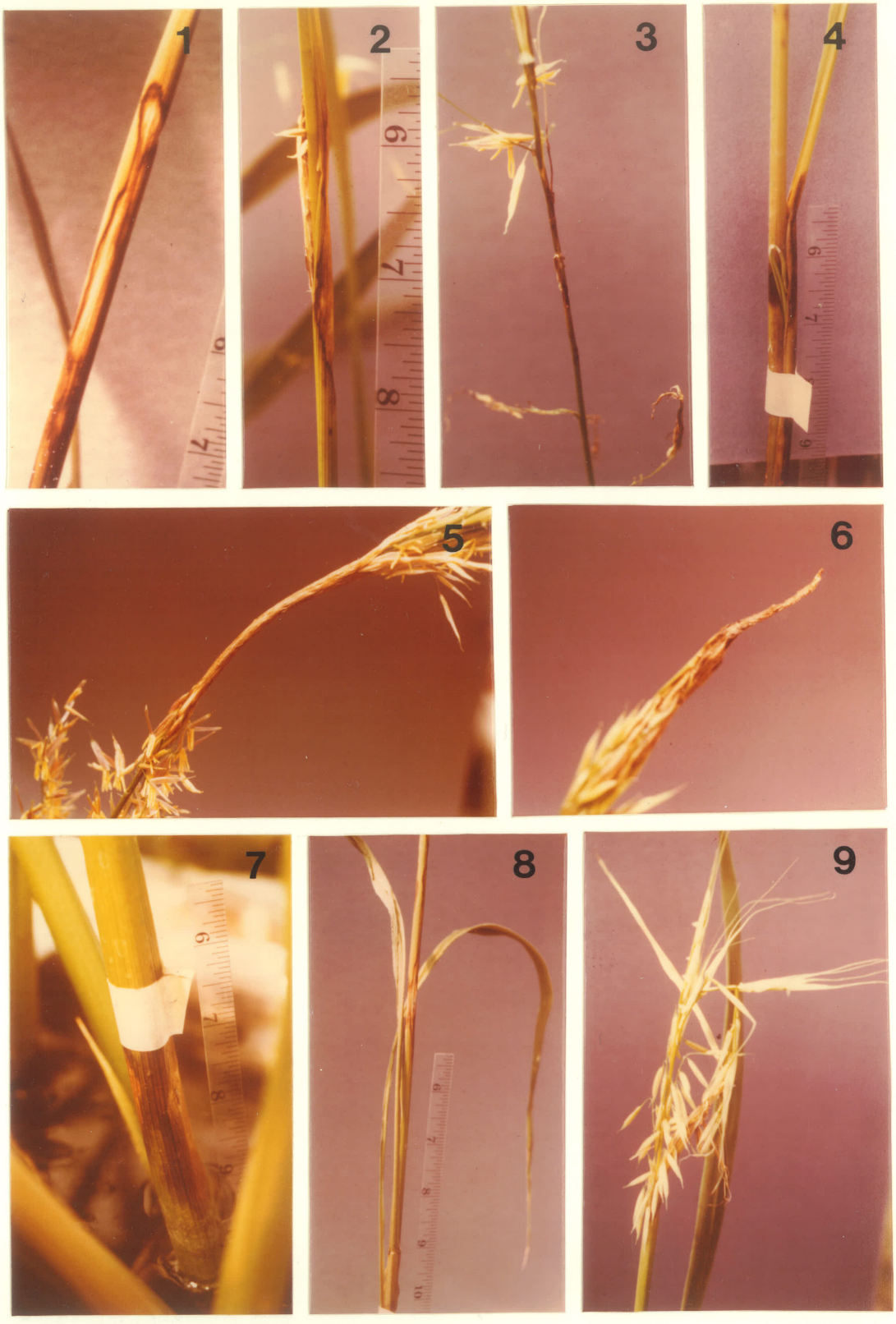
8 Drechslera 6C

Lesion type 3.0: mid culm region
Plant showing leaf and partial culm desiccation.

9 Drechslera 6C

Lesion type 3.0

Damaged inflorescence (note browned female flowers).


1
2
3
4
5
6
7
8
9

2. THE INFLUENCE OF TREATMENT ON SEED YIELD

Spray Inoculations

The mean seed yields of spray inoculated plants are shown in Fig. 3.9 together with the lesion index values. Three isolates reduced the yield of wild rice at the 5 per cent level of significance. These were D. bicolor, Drechslera 5L and Fusarium 4 (the latter inoculated without humidification). D. bicolor reduced the yield most drastically to the extent of over 70 per cent with an F ratio of 14.17. Drechslera 5L and Fusarium 4 resulted in more or less comparable losses of between 50 and 60 per cent. Their F ratios were also similar at about 7.50. The loss in yield from Drechslera 6C was significant only at the 10 per cent level. Although other isolates did not reduce yield sufficiently to be significant even at the 10 per cent level they were not entirely without effect upon yield, which they reduced in the range of 15 to 45 per cent.

The F ratios of these data are provided in Fig. 3.10.

FIG. 3.9

The seed yields and lesion indices of wild rice plants inoculated with various fungal isolates under greenhouse conditions. Unhatched histograms and open circles represent controls. Bars represent standard deviations of the means. Circles represent lesion indices (final).

SPRAY INOCULATIONS

1. Drechslera bicolor
2. Drechslera 1
3. Drechslera 5L
4. Drechslera 6C
5. Fusarium 4
6. Fusarium 4 (without initial humidification)
7. Fusarium 1 (without initial humidification)
8. Entyloma lineatum
9. Control
10. Control

POINT INOCULATIONS: toothpicks

11. Drechslera bicolor
12. Drechslera 1
13. Drechslera 5L
14. Drechslera 6C
15. Entyloma lineatum (hypodermic syringe)

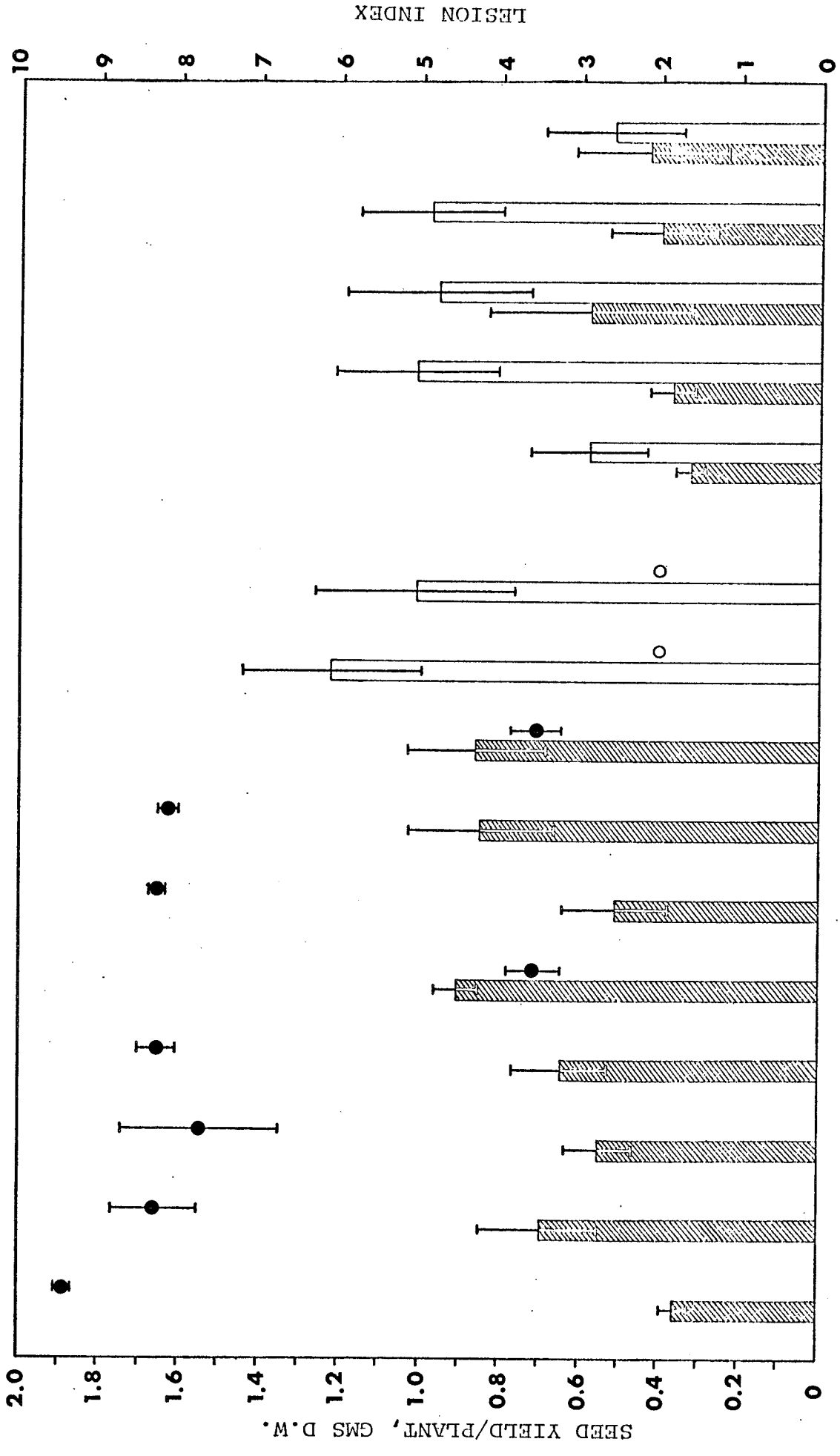

LESION INDEX
10
9
8
7
6
5
4
3
2
1
0
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
SEED YIELD/PLANT, GMS D.W.
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0

FIG. 3.9

FIG. 3.10

The variance (F ratios), relative to the controls, of the seed yields and lesion indices of wild rice plants inoculated with various fungal isolates under greenhouse conditions (Cf: Fig. 3.9). The broken lines represent specific significance levels. (Degrees of Freedom, $n_1 = 1$; $n_2 = 6$).

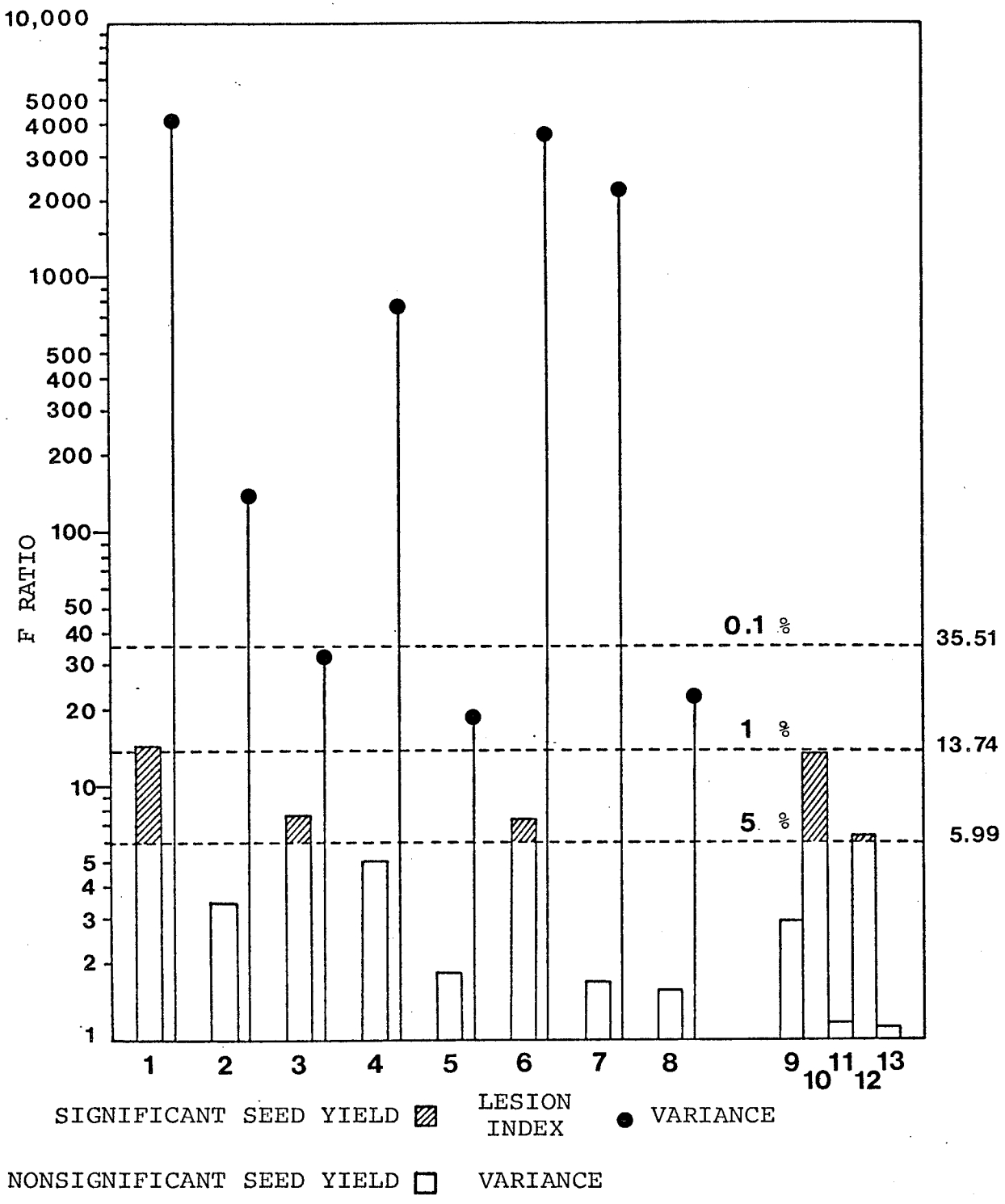
SPRAY INOCULATIONS

1. Drechslera bicolor
2. Drechslera 1
3. Drechslera 5L
4. Drechslera 6C
5. Fusarium 4
6. Fusarium 4 (without initial humidification)
7. Fusarium 1 (without initial humidification)
8. Entyloma lineatum

POINT INOCULATIONS: toothpicks

9. Drechslera bicolor
10. Drechslera 1
11. Drechslera 5L
12. Drechslera 6C
13. Entyloma lineatum (hypodermic syringe)

FIG. 3.10


10,000
5000
4000
3000
2000
1000
500
400
300
200
100
50
40
30
20
10
5
4
3
2
1
F RATIO
0.1 %
35.51
1 %
13.74
5 %
5.99
1
2
3
4
5
6
7
8
9
10
11
12
13
LESION INDEX
SIGNIFICANT SEED YIELD
VARIANCE
NONSIGNIFICANT SEED YIELD
VARIANCE

Point Inoculations

Toothpick

Only two of the isolates tested significantly reduced the yield at the 5 per cent level. These were Drechslera 1 and Drechslera 6C, which caused losses of approximately 70 and 50 per cent, respectively. Neither of these isolates had produced a significant loss in yield by the spray inoculation method. D. bicolor caused a loss greater than 40 per cent but this was only significant at the 20 per cent level (Fig. 3.10).

Hypodermic Syringe

This method of inoculation only involved E. lineatum. Injecting the inflorescence stalks of previously spray-inoculated plants only resulted in approximately 8 per cent reduction in yield relative to the infected (and previously sprayed) controls; a non-significant loss in yield.

3. CORRELATION BETWEEN LESION INDEX AND SEED YIELD

Fig. 3.11 represents a linear regression plot of lesion index vs. seed yield data. The zero order correlation coefficient is 0.88732 which is significant at the 0.1 per cent significance level: a very positive correlation.

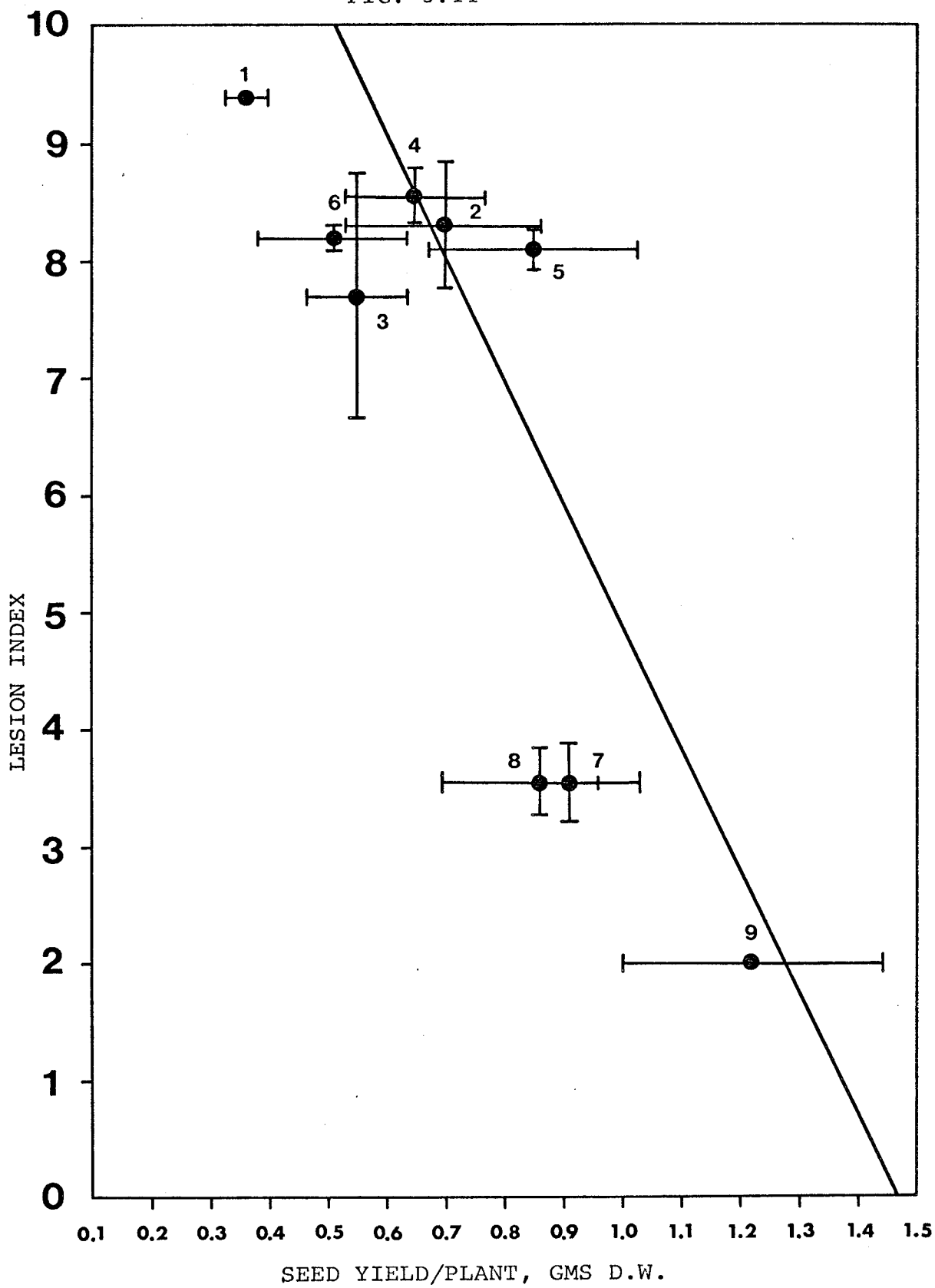
FIG. 3.11

The correlation (and linear regression analysis) between seed yield and the final lesion indices of greenhouse spray-inoculated wild rice plants. Bars represent standard deviations of the means.

Slope of Regression Line	-10.61257
Intercept on y	14.40690
Zero Order Correlation Coefficient	0.88732
S y.x, Standard Error of Estimate (biased)	1.41209

1. Drechslera bicolor
2. Drechslera 1
3. Drechslera 5L
4. Drechslera 6C
5. Fusarium 1 (without initial humidification)
6. Fusarium 4 (without initial humidification)
7. Fusarium 4
8. Entyloma lineatum
9. Control (pooled data)

FIG. 3.11


10
9
8
7
6
5
4
3
2
1
0
LESION INDEX
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
1.1
1.2
1.3
1.4
1.5
SEED YIELD/PLANT, GMS D.W.
1
2
3
4
5
6
7
8
9

EXPERIMENT IV

POINT INOCULATIONS BY HYPODERMIC SYRINGE

To help clarify the behaviour of each isolate used in Experiment III with respect to foliar or culm tissue, the fungi were further tested by point inoculation (hypodermic syringe). In order to avoid repetition, the discussion of Experiment III is deferred until after the presentation of data relating to Experiment IV (p.149).

MATERIALS AND METHODS

Wild rice of the 'Algot Johnson' seed source was germinated in May 1972 and the resulting seedlings grown to the early aerial leaf stage in bulk in sterilised soil. A 10 cm layer of Sphagnum peat was placed in a large experimental tank (7 X 1.5 m) and flooded to a depth of 10 cm. This was divided by string into 4 blocks each of which contained 9 randomised plots (7 treatments plus 2 controls). Uniform, vigorous plants in the early aerial leaf stage, were transplanted into the experimental tank, 21 per plot. Because of the open soil and peat media, transplantation shock and root damage were minimal. Inoculation was carried out 2 weeks after transplanting by which time original vigour was completely restored.

With the exception of E. lineatum for which fresh inoculum was prepared, the inocula consisted of the spore suspensions prepared for the second inoculations of Experiment III. These had been stored at 2°C for 11 to 17 days. Drechslera 1, 5L and 6C maintained their high germinabilities even following cold storage, though germinabilities of D. bicolor and the Fusarium spp. were reduced as indicated in Table 8, p.138.

Half (0.5) ml inoculum was injected into the basal culm region of each plant, just above the water level. A hypodermic syringe was used for this as well as the control inoculations of sterile distilled water. Inoculations commenced May 11 and were completed in four days on May 15.

After the inoculations had been completed, the plants began to show some nutrient deficiency. One hundred gm ammonium nitrate phosphate fertilizer (25.25.0) was added to the tank (equivalent to 112 kg/hectare). In less than a week a marked response to the fertilizer was evident; the plants looked considerably greener and new leaves were being produced.

RESULTS

In conformity with the system adopted for Experiment III, data are presented under the main headings of Symptom

Table 8

The spore germinabilities of isolates used for point inoculation in Expt. IV

	STORAGE (days) AT 2°C	GERMINABILITY %
DRECHSLERA BICOLOR	17	8.16
DRECHSLERA 1	16	94.38
DRECHSLERA 5L	16	87.26
DRECHSLERA 6C	16	96.80
FUSARIUM 1	11	36.53
FUSARIUM 4	13	15.45

Development and the Influence of Treatment on Seed Yield. Symptom development following each treatment is described in the form of brief notes. Seed yields are recorded in Fig. 3.12 (dry weights per plant) and variance ratios of the yields in Fig. 3.13. (pp. 146 and 148, respectively).

1. SYMPTOM DEVELOPMENT

1. Control: Uninoculated

The plants were normal and healthy without any disease symptoms.

2. Control: 0.5 ml sterile distilled water

The plants were generally free of lesions. In many cases it was difficult to find the point of injection and damage was almost imperceptible. A few plants showed slight browning at the point of inoculation but this did not spread. Holes with white margins were apparent in some expanded leaves. These were caused by the needle at the time of inoculation when these leaves were still within the culm.

3. Drechslera bicolor

In 48 hours, local browning was evident at the point of inoculation. Upward spread of the infection occurred within

7 days. By 11 days, some plants in each replicate were seen to have lodged over at the stem base more or less coincident with the point of inoculation. This was also accompanied by evidence of more distal infection in the form of a browning and drying up of the emerging inflorescence which resulted in non-functional female flowers.

4. Drechslera 1

Within 48 hours, spreading lesions had begun to develop from the point of inoculation and in 7-11 days, lodging of plants occurred as with D. bicolor. Some plants were almost entirely brown and emerging male and female flowers were withered. In one replicate, complete plants appeared to be dying but began to recover between 11 and 12 days after inoculation when some new shoots were produced.

5. Drechslera 5L

Lesion development was similar to that noted with Drechslera 1. Some browning occurred but this was less frequently in the inflorescence tip region. Rejuvenation by the production of new shoots was moderately vigorous in one replicate.

6. Drechslera 6C

Culm lesions were more localised and darker than those caused by the other Drechslera isolates; in other respects symptoms were similar. Internal culm damage was as great and reproductive organs were similarly browned while still within the culm.

7. Fusarium 1

Symptoms produced by this isolate and Fusarium 4 were qualitatively similar to those of the Drechslera isolates, but much more severe. In four days, many plants appeared to be dying because of the extensive development of culm lesions and the desiccation of all larger leaves. After nine days some of the most vigorous plants which were about to flower were also completely withered and lodged. Longitudinal brown lesions seen in mid-rib regions were most likely the result of spread of the inoculum within the internal spaces at the time of inoculation. In 10 days distinct white and pink areas of sporulating mycelium appeared on moribund tissue browned by spreading lesions. Such areas of sporulation occurred on both dead and surviving plants and were more or less confined to the lower culm area close to the water line.

8. Fusarium 4

The symptoms described above for Fusarium 1 were incited by this isolate with even greater severity. In seven days a complete lodging of formerly vigorous plants occurred. Many others were more or less totally withered, but had not as yet died. In two plants the damage was confined to the main axis including the inflorescence stalk, while the surrounding sheaths remained green. Central browning of some large leaves was also observed. As in the case of Fusarium 1, sporulation occurred on moribund and dead tissues close to the water level. In this case it was detected slightly earlier at approximately 8 days from the time of inoculation.

9. Entyloma lineatum

No leaf or stem symptoms were found up to 11 days after inoculation although most plants showed a very localised browning not more than 1.5-2.0 mm in diameter at the point of inoculation. This was also noted in control plants. Slight browning also occurred in some plants around the holes formed in the developing leaves as a consequence of puncture by the needle of the syringe at the time of inoculation.

By 18 days from inoculation, sori were observed to be almost mature in all four replicates on from two to four plants in each. They occurred on the inflorescence stalks, generally between the flag leaf and the male flowers, but in some cases extending well into the upper head region within the area of the developing kernels. A black flecking of the male flowers was noted, giving a 'smutty' appearance to the glumes. Some heads affected in this way showed subnormal male flower development. Table 9, p. 144, shows that over 20 per cent of the plants in each replicate developed smut symptoms in the form of sori or the black flecking of the male glumes.

2. THE INFLUENCE OF TREATMENT ON SEED YIELD

Fusarium 1 and Fusarium 4 reduced the seed yield by over 95 and 90 per cent, respectively (Fig. 3.12 and 3.13). The early death of 74 per cent of the plants in each case accounted for much of this loss and meant that seed could only be collected from the few plants which remained.

The three Drechslera isolates also greatly reduced the yield, especially Drechslera 1. The losses in yield were between 75 and 86 per cent, and the number of plants killed by infection, between 56.5 and 68.2 per cent. D. bicolor also resulted in a significant loss in yield but it was considerably less, at 42 per cent, killing

Table 9

THE INCIDENCE OF INFECTION
FROM INOCULATIONS OF *E. LINEATUM*
Expt. IV

INOCULATION TREATMENT	MEAN NO. OF PLANTS WITH INFECTION*	MEAN NO. OF PLANTS WITHOUT INFECTION*	MEAN PERCENT INFECTION*/ REPLICATE
INJECT 0.5 ML INOC. INTO BASE OF CULM	5.0	17.0	22.7

* Infection in this instance (leaf lesions were absent) refers to the presence of sori and/or flecking of the flower parts.

FIG. 3.12

The seed yields of wild rice plants point-inoculated (hypodermic syringe) with various fungal isolates under greenhouse conditions. Unhatched histograms represent controls. Bars represent standard deviations of the means.

1. Drechslera bicolor
2. Drechslera 1
3. Drechslera 5L
4. Drechslera 6C
5. Fusarium 1
6. Fusarium 4
7. Entyloma lineatum
8. Control (inject sterile distilled water)
9. Control (untreated)

FIG. 3.12

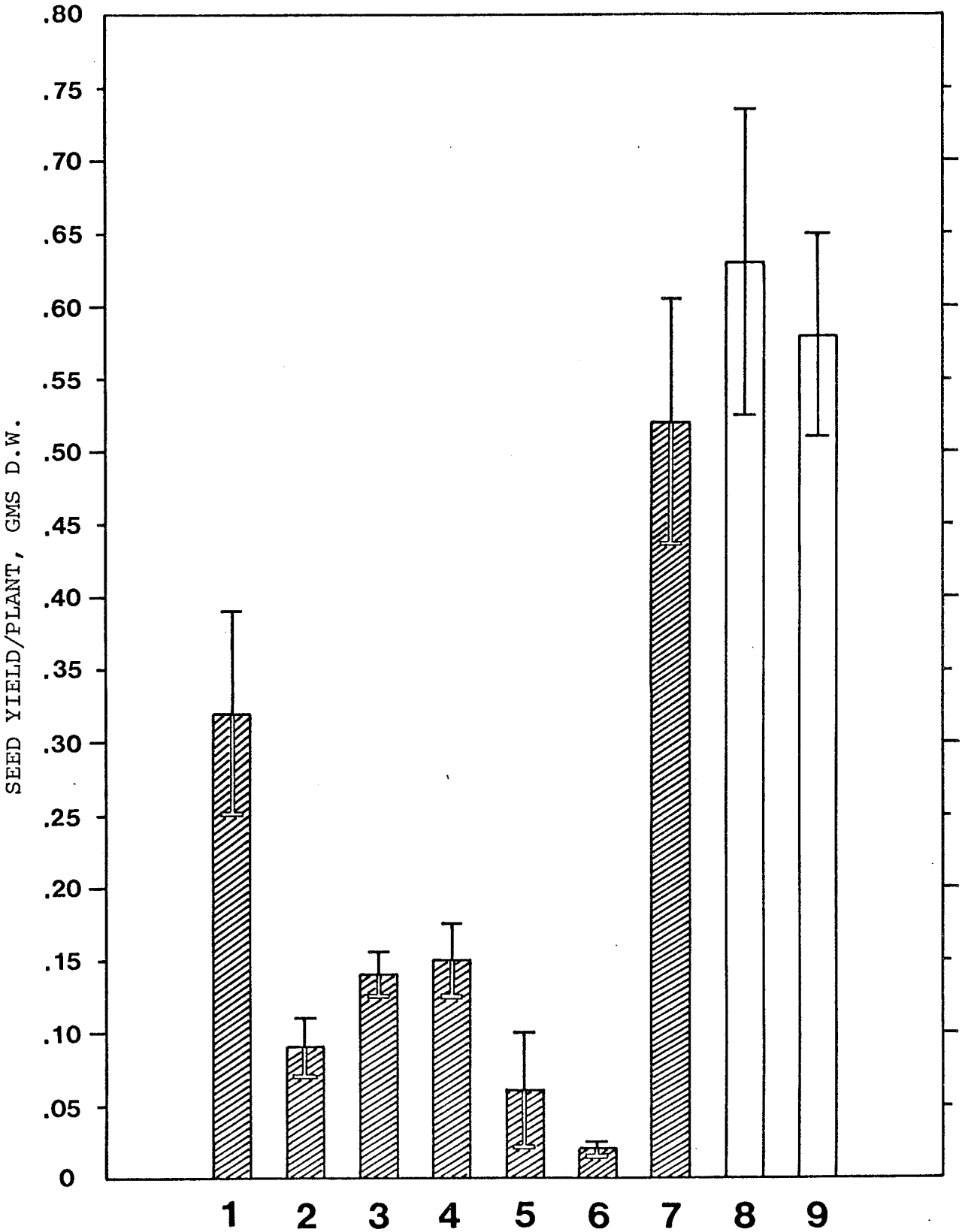
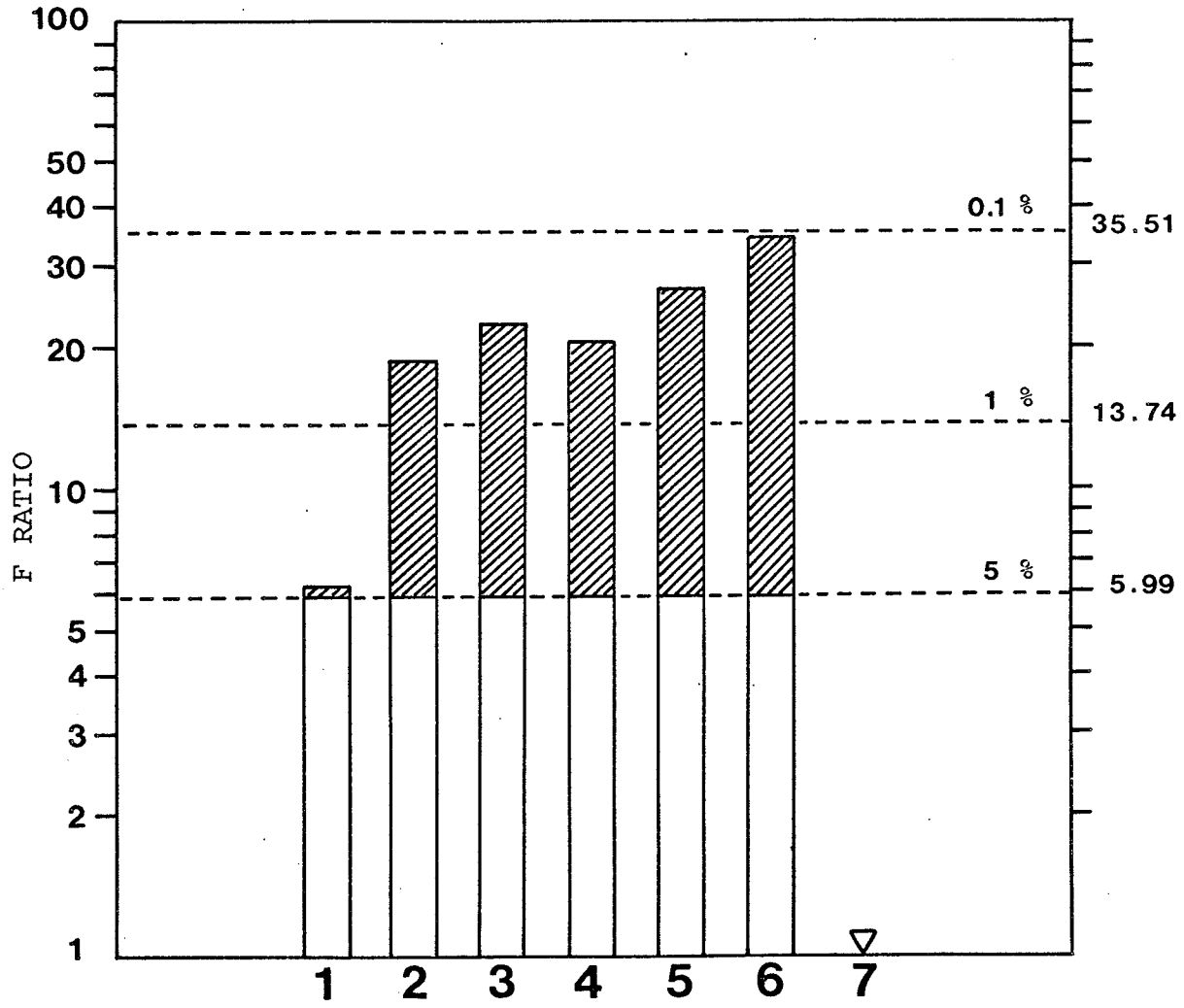

SEED YIELD/PLANT, GMS D.W.
.80
.75
.70
.65
.60
.55
.50
.45
.40
.35
.30
.25
.20
.15
.10
.05
0
1
2
3
4
5
6
7
8
9

FIG. 3.13

The variance (F ratios),relative to the controls, of the seed yields of wild rice plants point-inoculated by hypodermic syringe with various fungal isolates under greenhouse conditions (Cf: Fig. 3.12). The broken lines represent specific significance levels (Degrees of Freedom $n_1 = 1$; $n_2 = 6$).

1. Drechslera bicolor
2. Drechslera 1
3. Drechslera 5L
4. Drechslera 6C
5. Fusarium 1
6. Fusarium 4
7. Entyloma lineatum

FIG. 3.13



SIGNIFICANT SD. YIELD ▨ VARIANCE

NONSIGNIFICANT SD. YIELD ▽ VARIANCE (< 1)

30.9 per cent of the plants.

The reductions in yield due to Drechslera 1, 5L and 6C and Fusarium 1 and 4 were so great as to be significant at the 1 per cent level with F ratios between 19.0 and 34.0 (Fig. 3.13). D. bicolor had an F ratio of 6.2 which was only just significant at 5 per cent. E. lineatum was the only isolate tested which failed to cause a significant loss in yield.

DISCUSSION

In Experiments III and IV, a variety of inoculation techniques led to findings which suggested that there were several distinct types of pathogens involved. These results are discussed in the light of overall, as well as specific, experimental methods used.

The results suggested a differentiation between foliar pathogens, generally more successful by spray inoculation (Drechslera bicolor, Drechslera 5L and Fusarium 4) and culm pathogens which were more successful when introduced into the host by point inoculation (Drechslera 1 and Drechslera 6C). Evidence for this may be seen in Fig. 3.14 where the per cent significant losses in yield incurred by spray inoculation of D. bicolor, Drechslera 5L and

Fusarium 4, compare closely with the per cent significant losses in yield incurred by point inoculation (toothpick) of Drechslera 1 and Drechslera 6C.

The distinction between foliar and culm pathogens was masked in Experiment IV as overall infection was too severe owing to factors which rendered the plants more susceptible. However, the total pattern of results suggested that there were etiological dissimilarities between isolates which were related to the conditions presented them at the time of infection, in particular the occurrence of mechanical injury (toothpick and hypodermic syringe) or not (spray).

Although fungal infection of uninjured tissues may often induce initial responses and metabolic alterations qualitatively similar to mechanical injury (Uritani, 1971) one may expect at least quantitative differences when a pathogen is inoculated in a way that causes concurrent mechanical damage. The presence of a continuing stimulus (the pathogen) may be expected to initiate secondary responses at a later stage regardless of the initial conditions of infection.

The response of injured tissues to a single artificial and mechanical stimulus was represented by the controls for the inoculations by toothpick and hypodermic

FIG. 3.14

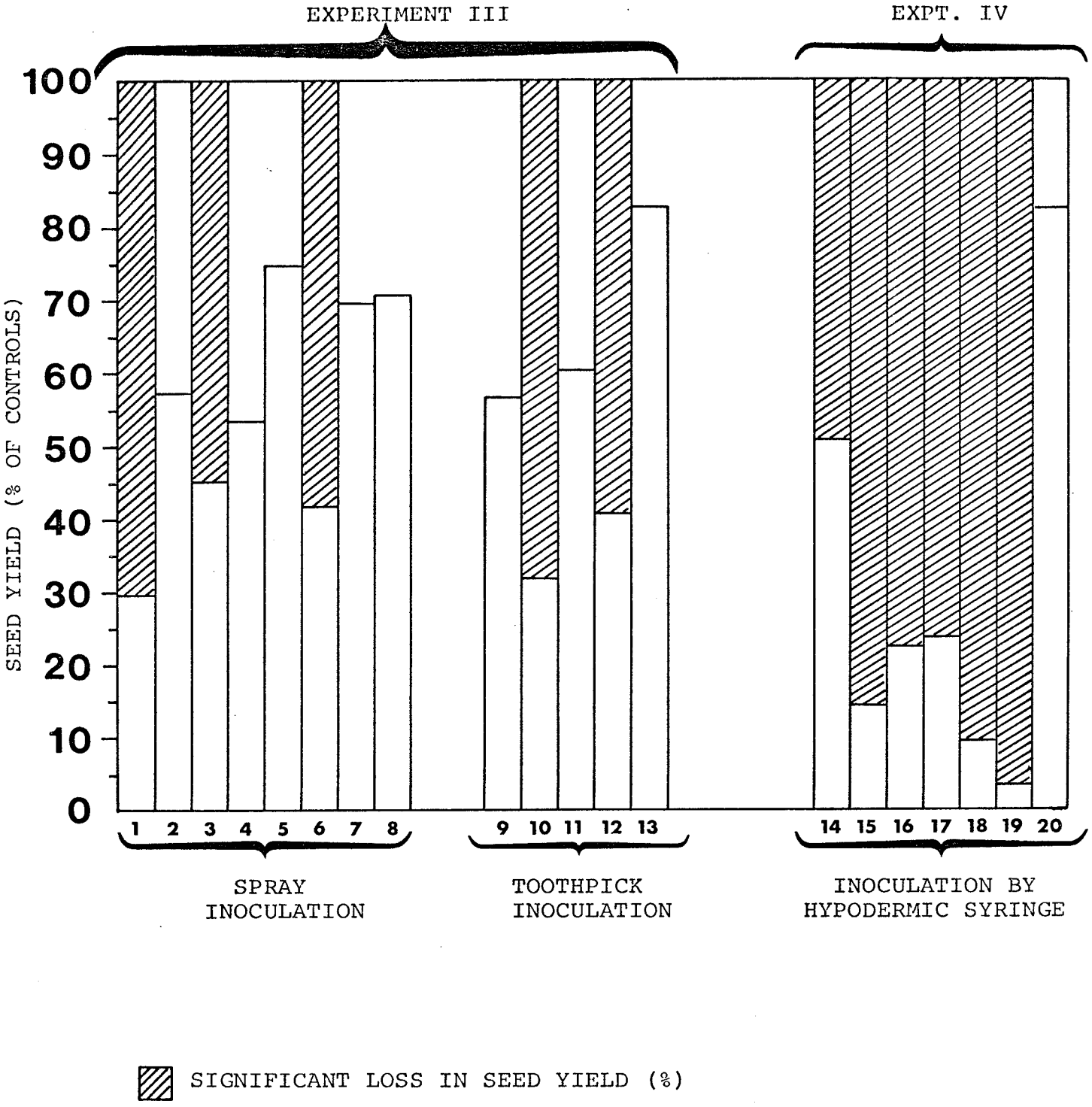
The percentage seed yields of wild rice plants inoculated with various fungal isolates under greenhouse conditions.

1. Drechslera bicolor
2. Drechslera 1
3. Drechslera 5L
4. Drechslera 6C
5. Fusarium 4
6. Fusarium 4 (without initial humidification)
7. Fusarium 1 (without initial humidification)
8. Entyloma lineatum

9. Drechslera bicolor
10. Drechslera 1
11. Drechslera 5L
12. Drechslera 6C
13. Entyloma lineatum

14. Drechslera bicolor
15. Drechslera 1
16. Drechslera 5L
17. Drechslera 6C
18. Fusarium 1
19. Fusarium 4
20. Entyloma lineatum

FIG. 3.14


EXPERIMENT III
EXPT. IV
SEED YIELD (% OF CONTROLS)
100
90
80
70
60
50
40
30
20
10
0
1 2 3 4 5 6 7 8
9 10 11 12 13
14 15 16 17 18 19 20
SPRAY INOCULATION
TOOTHPICK INOCULATION
INOCULATION BY HYPODERMIC SYRINGE
SIGNIFICANT LOSS IN SEED YIELD (%)

syringe. Similarly, normal healthy plants were represented by totally uninjured controls in both spray and point inoculations (hypodermic syringe).

The determination of the lesion index (in correlation with seed yield) was a key factor for assessing disease following spray inoculation (p.133). For point inoculations, yield determination alone was used to assess disease as leaf lesions were of little or no significance.

1. INFECTION OF UNINJURED TISSUES (Spray Inoculations)

The lesion index curve provided a good indication of the progress of lesion development following spray inoculation. Lesion development can be divided into a lag phase (up to the 11th to 16th hour from inoculation), an exponential phase (up to the 24th to 34th hour) and a plateau where lesion development is more or less complete (van der Plank, 1963). The precise length of the lag and exponential phases varied with each isolate (Fig. 3.1-3.8 and Fig. 3.15).

Strong lesion development (a high final lesion index, Fig. 3.9) was associated with some significant reductions in yield. The isolate producing the highest lesion index, D. bicolor, resulted in the greatest loss in yield. This

relationship between lesion index and yield is borne out by their correlation coefficient, significant at 1 per cent (Fig. 3.11), and suggests that the photosynthetic area was a major factor in reducing yield.

However, such a correlation was not found in all cases. Fusarium 1, spray inoculated without initial humidification, had a lesion index comparable to the Drechslera group but without a significant loss in yield. Nor did the comparably high final lesion indices within the Drechslera group result in equal reductions in yield; the losses varied individually (Fig. 3.9). Apparently other factors related directly or indirectly to chlorophyll loss were also operative in lowering the yield (p. 160).

Primary Responses

Numerous initial metabolic changes, thought to generally parallel responses to pathogenic infection, have been recorded in plant tissues in response to mechanical injury by cutting (single stimulus) (Uritani, 1971). Many of these were recorded after 12 to 24 hours. It is probable that changes in infected wild rice occurred at a similar stage in accordance with the development of 'first instance' lesions indicated by the lesion index curves (Fig. 3.1-3.8).

The moderately large standard deviations in the lesion indices for Drechslera 5L (Fig. 3.3) were likely caused by different levels of resistance which were controlled by dissimilar rates of abnormal metabolite synthesis. It is the rate of protein synthesis related to 'abnormal metabolism' which distinguishes resistant and susceptible plants. The degree of defence action is sometimes in parallel to the amount of abnormal metabolites produced (Cruikshank, 1963; Kuc, 1964; Uritani, 1961 and 1971).

The types of lesions as well as their extent may have been determined by these metabolic phenomena. It was noticeable that lesion types 1.0 and 4.0 (localised shothole and localised brown spot) were usually confined to plants which exhibited fewer of the spreading lesions. Localised lesions were indicative of a certain degree of successful defence action by relatively resistant plants. The spreading lesions, however, led to plant deterioration (see secondary responses) in relatively susceptible plants. Where both lesion types occurred on the same plant, deterioration resulted when the pathogen overcame the plant's initial resistance (e.g. D. bicolor).

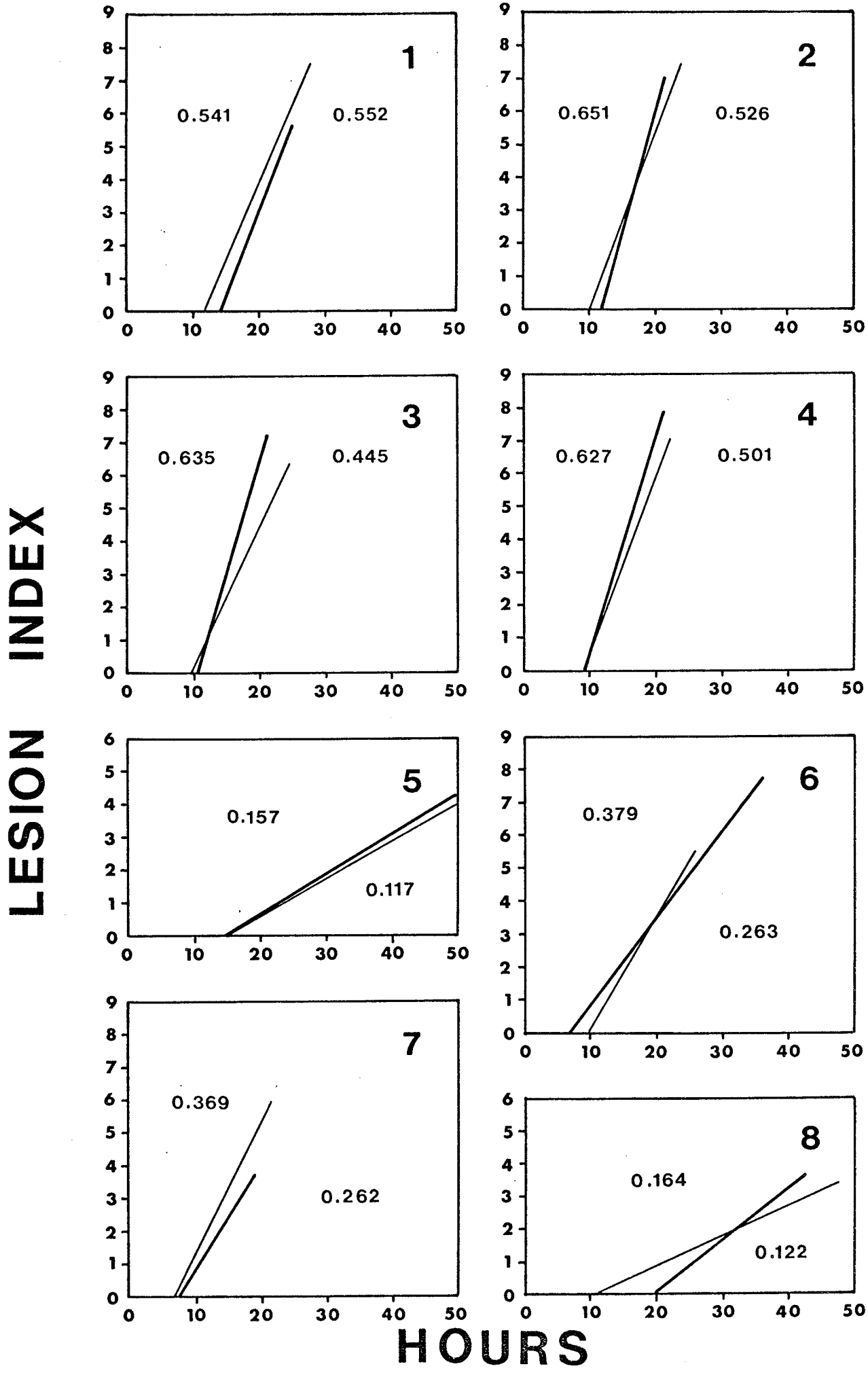
Drechslera 1, which failed to significantly reduce the yield by greenhouse and field spray inoculations, is perhaps a weak facultative saprophyte. Its higher exponential rate of lesion development in the first

FIG. 3.15

The exponential rates of lesion development for various pathogens (Cf: Fig. 3.1-3.8). Figures given represent the slopes of the lines. Thick lines represent first, and thin lines second inoculations, respectively.

1. Drechslera bicolor
2. Drechslera 1
3. Drechslera 5L
4. Drechslera 6C
5. Fusarium 4
6. Fusarium 4 (without initial humidification)
7. Fusarium 1 (without initial humidification)
8. Entyloma lineatum

FIG. 3.15


LESION INDEX
HOURS
1
0.541
0.552
2
0.651
0.526
3
0.635
0.445
4
0.627
0.501
5
0.157
0.117
6
0.379
0.263
7
0.369
0.262
8
0.164
0.122

inoculation (Fig. 3.15) may be indicative of enzyme build-up (somewhat typical of facultative saprophytes) to a threshold (Uritani, 1971) following which symptom development was rapid but not severe.

D. bicolor, which so vigorously infected wild rice, may have acted as a facultative parasite producing abundant cell wall penetrating enzymes fast, or alternatively, infecting with the aid of a few very specific enzymes or with the aid of some other factor(s). Facultative parasites often produce some enzymes that decompose several cell wall and membrane constituents such as cellulose, pectin (Albersheim, 1969; Liese, 1970) and phospholipid (Lumsden, 1968). These are enough to penetrate, but are not furnished in so large a measure as in some facultative saprophytes (Uritani, 1971).

Obligate parasites, such as Entyloma lineatum, may not produce such penetrating enzymes, because of maintenance of an equilibrium between the pathogen and the plant. In the case of obligate parasites that maintain a fine balance with host plants, the injury to adjacent noninfected tissues is not severe, compared to cut injury or penetration of facultative parasites (Uritani, 1971).

Secondary Responses

Following the primary response (usually after 1 day), a secondary response is revealed in both infected and adjacent noninfected tissues which suffer continuous stimulation attributable to pathogenic penetration. That is, the secondary response is related to increase in the mass of the pathogen and to continuous interaction of plant cytoplasmic macromolecule-parasite macromolecule or plant cytoplasmic macromolecule-parasite secretion. Some stimulating agents (e.g. ethylene) for the secondary response may be formed in the pathogen-containing living cells by host-parasite interactions (Uritani, 1971).

The lesion index curve for the first inoculation of D. bicolor indicated that lesion development temporarily slowed down at 24 hours (Fig. 3.1). This may be related to the time-course curve for the transition from primary to secondary activities which is rather biphasic showing a shoulder at about 1 day (Uritani, 1971).

For the other isolates of Drechslera, lesion development was largely complete in 24 hours, indicating that it was mainly a primary response. Fusarium 1 and 4, on the other hand, showed considerable lesion development after 24 hours (secondary response). This was particularly noticeable for Fusarium 1 (Fig. 3.5). E. lineatum showed

virtually no lesion development after 24 hours though sori appeared in 18 to 22 days. This fungus caused little or no damage to the plant and may have elicited only limited primary responses specific to obligate parasitism.

The similar high lesion indices incited by Drechslera isolates may have resulted from the induction of equal complements of polyphenol oxidising factors controlling lesion development. But resultant dissimilar losses in seed yield incurred by such isolates may have been related to dissimilar levels of effectors or suppressors which control host glycolytic enzymes, resulting in a decrease in translocatable materials such as sugars reaching the developing kernels of infected plants (Akai et al., 1958-1966, I-VI. Macdonald, 1970). Thus the correlation between high lesion index and reduced seed yield is dependent upon the operation of both sets of factors within the host.

Fusarium isolates in the present study produced moderately drastic damage by both shot-holes (Fusarium 1) and localised blotches (Fusarium 4). Damage of this kind and the slower (Fig. 3.5) though steady lesion development shown by their lesion index curves (Fig. 3.5, 3.6, 3.7 and 3.15) are suggestive of sustained cellulolytic activity or toxin production within the tissues.

It is not known to what extent the present isolates of Drechslera spp. and Fusarium spp. exhibited toxic effects on wild rice. However, it is suspected that toxins were operative in infection by Fusarium 4 and possibly Fusarium 1 (see p.166). D. bicolor may have also produced toxic compounds, in view of its severe infection by spray inoculation. Some kinds of pathogens bring death of the invaded cells as well as the surrounding cells by production of toxic compounds which initiate reactions similar to those resulting in coagulated precipitates from oxidised polyphenols (Durbin, 1971; Hess, 1970; Johnson, 1969; Scheffer, 1967). These toxins are related to either specific or nonspecific pathogenicity. Specific forms include victorin (Scheffer, 1967; Wheeler, 1963) produced by Helminthosporium victoriae and ophiobolin (Oku, 1967) by Drechslera oryzae (Ophiobolus miyabeanus Ito) the causal organism of brown spot of rice.

Fusarium 4 inoculated at 100 per cent R.H. failed to cause a significant loss in yield and also had a low lesion index (Fig. 3.7 ; second inoculation). This merely indicates that it has a moderately low optimal relative humidity for successful infection; inoculations without initial humidification resulted in substantial infection and significant losses in seed yield (Fig. 3.6 and 3.14). The high level of infection by Fusarium 4 at first inoculation is explained by the partially unsuccessful

humdification procedure for that time. With partial humidification, lesion development was as advanced on susceptible plants as in parallel inoculations without humidification (Fig. 3.7, Plate 5#7 and 5#10). These overall findings indicate that Fusarium 4 tolerates a moderately wide range in relative humidity (say, 35-75% R.H.), whereas Fusarium 1 is more limited to low humidity regimes (say, 10-35% R.H.).

E. lineatum is considered obligately parasitic as it did not appear to significantly harm the plant in field and greenhouse inoculations. However, it is postulated that the equilibrium with the host may be considerably unsettled by a concomitant infection of, for example, Drechslera or Fusarium species. This may lead to synergism and enhanced parasitism. Field observations showed that smut sori were often abundant on plants with heavy leaf and stem spotting, particularly in more southerly paddies (Minnesota) from which Drechslera 5L and 6C were isolated.

2. INFECTION OF INJURED TISSUES

(Point Inoculations)

In Experiment III, Drechslera 1 and Drechslera 6C caused their greatest damage when inoculated on toothpicks, while Drechslera 5L and D. bicolor were more damaging after spray inoculation. This tendency for the former group to

develop more successfully on injured (culm) tissue in contrast to the latter group which was more effective on uninjured (leaf) tissue was expected to be duplicated in Experiment IV. However, little or no distinction between the groups was evident from the results of Experiment IV. Drechslera 1 and Drechslera 6C again became established successfully in injured culm tissue but this was also true for most other isolates including Fusarium 1 and Fusarium 4 which were not point inoculated in Experiment III owing to lack of space. At first sight it might appear that inoculation by hypodermic syringe was much more successful than that by using toothpicks, e.g. Drechslera 1 and Drechslera 6C caused much greater reductions in yield in Experiment IV than in Experiment III (Fig. 3.14). Other complicating factors must be taken into account, in particular the difference in host vigour between these experiments.

It is highly probable that lower host vigour in plants of Experiment IV contributed to the greater severity of disease. The reason for the lower vigour was that the plants were grown in a Sphagnum peat substrate low in nutrients. Although peat is known to support good growth of wild rice -- natural stands occur on Sphagnum peat at Waskish, Minnesota and paddies have been established on sedge peat at Stead, Manitoba (J.M. Stewart, personal communication) -- the plants in Experiment IV began to

show nutrient deficiency symptoms in the early aerial leaf stage. The application of nitrogen and phosphorus (p.137) had an obviously beneficial effect upon the vigour of the plants. When applied to rapidly growing plants close to the time of inoculation it may well have increased their susceptibility to fungal invasion. Similar increases in susceptibility of rice (Oryza) to stem rot caused by nitrogen and perhaps phosphorus applications have been observed on several occasions (Nakata and Kawamuru, 1939; Cralley, 1939; Keim and Webster, 1974).

Although the same spore suspensions were used in each experiment, storage at 2°C between experiments caused general but variable reduction in germinability (Table 8, p.138). It seems hardly reasonable to expect a reduction in spore viability to promote infection. Perhaps more remarkable is the lack of evidence, except in the case of D. bicolor, that lowered germinability hindered infection. It is possible that storage may have modified the spore suspensions. Accumulation of excretory or secretory products of the spores in storage might have enhanced the development of symptoms.

The only isolate which did not show severe culm attack in Experiment IV, and which showed a comparable loss in yield (approximately 16 per cent) for both experiments, was E. lineatum.

Although the results of point inoculations in Experiments III and IV do not correspond as fully as might have been expected, they cannot be regarded as seriously inconsistent in the light of the modifying factors.

Primary Responses

Point inoculations gave rise to primary responses similar to those outlined in the previous section. More specific to point inoculation is the occurrence of wound healing. Cork, lignin and callus layers have been observed in some tissues (Asada, 1969; Hyoda et al., 1968), often in response to the production of inducer substances by the fungus. It is possible that similar wound healing inducer agents were operative in the present study, though no attempt was made to detect them. A callus layer as such was not observed in response to injury. However, definite browning was noted within 36 hours from the time of inoculation of all isolates by the toothpick method (p.120). This was also observed in some of the controls, but it did not spread and was very localised, indicating response to mechanical injury alone, from toothpick and hypodermic syringe. Browning appeared to be delayed in the inoculations by hypodermic syringe and was not apparent before 48 hours (p.140). This may be explained by assuming that inoculum was confined to more or less central culm regions

so that it simply took time for the response to become evident in epidermal and subepidermal tissue.

Fusarium 1 and Fusarium 4, inoculated by the injection method, caused the greatest seed yield losses of all the isolates (Fig. 3.12 and 3.14). Since the inoculum had been stored before use, it is possible that toxic factors had accumulated to damaging levels. Toxin production is common within the genus Fusarium (Wood, 1967; Wood et al., 1972). The presence of such a toxicity factor could be tested by comparison of inoculations of centrifuged supernatant media (without spores) from inocula which had been stored for various periods, with those of fresh inocula. The low spore germinability (Table 8, p.138) suggests that such a toxic factor may be detrimental to the pathogen itself, if accumulated in sufficient quantities. It may therefore be a 'non-specific' metabolite which was accumulated to harmful concentrations by host cytoplasm.

After an initial 48 hour lag, culm lesions of Fusarium in Experiment IV developed more rapidly than corresponding leaf lesions in Experiment I and III. This may be linked to the fact that the germinability of the fresh inoculum (Experiment III) was about 100 per cent; the slow lesion development may have been a function of the postulated 'time-dependent' toxicity factor, released on active spore germination and growth. If a toxicity factor is operative

in Fusarium isolates, it may be greater in Fusarium 4 as this resulted in the severest symptoms inciting a greater loss in yield than Fusarium 1. This is in agreement with Experiment III (spray inoculations) where Fusarium 4 induced a significant loss in yield though Fusarium 1 did not.

Primary responses among Drechslera isolates were confined to localised tissue browning at the point of inoculation (p.120). Toxins, perhaps, were not primarily involved in infection by Drechslera isolates, as early symptoms by toothpick inoculations lacked dramatic effects. A toxic product may be involved in Drechslera 6C (see below). The principal agents of disease are thought to be germinating and infecting spores, without toxic secretions of the type postulated for Fusarium 4 and Fusarium 1. Whereas the Fusarium isolates produced dramatic effects upon yield despite low spore germinabilities, all Drechslera isolates caused yield losses which were directly 'correlated' with their spore germinabilities. The overall findings suggested that yield losses associated with the point inoculations of Drechslera isolates were more related to secondary responses involving host-parasite interactions dependent on growth of the pathogen.

Primary responses to infection by E. lineatum in the form of leaf and culm lesions, were not apparent.

Secondary Responses

In certain combinations of plant and pathogen, infection extends well into adjacent noninfected tissues through pathogenic penetration or toxin and enzyme secretions of pathogenic origin, or both (Scheffer, 1967; Wheeler, 1963). This leads to host deterioration whereby the plant is incapable of regulating its own metabolism. Furthermore some toxic compounds of plant origin, normally produced under plant control and effective against pathogenic penetration, secreted toxins and/or secreted enzymes, are produced without regulation and themselves accelerate irregular metabolism in the plant (Uritani, 1971).

Changes of this type leading to host deterioration took place following inoculation of wild rice plants by hypodermic syringe (Experiment IV) with Fusarium 4 and Fusarium 1. Plants were more or less completely overcome by infection (pp.141,142). Fungal sporulation was confined to moribund or dead basal regions proximal to points of inoculation, though symptom development was more widespread (p.141). This localisation suggests that internal spread of the mycelium itself was limited, but penetration by toxic and/or enzymatic factors was not. This also suggests that Fusarium isolates were facultative saprophytes with only limited parasitic ability. Similarly, in this respect, earlier findings of less rapid lesion development (pp.106,109).

by spray inoculation, suggest dependency on a toxicity factor to first kill penetrated cells. Also shot-hole lesions may have represented complete necrosis by localised saprophytic activity following death of groups of cells by toxic factors.

The Drechslera isolates when inoculated by the injection technique (Experiment IV) caused somewhat less dramatic symptoms than the Fusarium isolates. There was a general lack of foliar symptoms. Leaf lesions were absent in both treatments involving Drechslera 1 and Drechslera 6C as in the other isolates. It is possible that they reduced seed yield by utilisation of host metabolites involved in translocation to developing kernels, or by enzymatic secretions involving breakdown of host tissue or abnormal metabolism. The foliar wilt, resulting in more or less complete host desiccation, incited by Drechslera 6C, suggests moderately dramatic internal parasitic interaction, possibly related to toxin production. Absence of severe symptoms in toothpick inoculations with D. bicolor and Drechslera 5L was taken as evidence of their greater adaptation as foliar pathogens.

CHAPTER FOUR

Two experiments were conducted to investigate the ability of pathogens to overwinter in association with the seed and to reinfect the resultant seedling. The first involved the examination of plants grown in the greenhouse from the seed of inoculated and infected plants of the previous summer. The second involved examination of the seed itself to detect systemic fungal infection. Germinability counts made in conjunction with the first experiment have been presented in Chapter Two in relation to the yield and lesion indices of field inoculated plants. They are also discussed in this chapter.

EXPERIMENT V

SEED SURVIVAL OF PATHOGENS - 1

MATERIALS AND METHODS

Seed from wild rice plants inoculated in the field in summer 1971 was stored under water at $2^{o}C$ in sealed polyethylene bags. The storage period was from December to May (6 months). On June 1st 1972, seed was removed from storage

and a randomised program of germination and planting commenced, according to the method outlined in Chapter Two. Seedlings of uniform age were again selected and grown under standardised conditions of soil depth, water depth and plant density. Plants were grown for 7 weeks by which time inflorescences were beginning to appear. During this time examination was made every two days for any lesion development. Those lesions which occurred were damp-chambered by the method described in Chapter One, in an attempt to recover the fungus which had been inoculated onto the parent plant. In the case of E. lineatum, recovery was attempted by plating the tissue containing lesions on agar (PYG). In this experiment the typical smut lesions failed to appear.

RESULTS

The fungi recovered from leaf lesions are recorded in Table 10. Their relative importance is indicated by means of a scale of abundance: sparse, moderately abundant, abundant. Of the fungi which had been inoculated in the field the previous summer, only two were recovered by re-isolation from the greenhouse-grown plants. These were Alternaria and Cladosporium. Both were re-isolated from nearly all treatments as were sundry other fungi not previously inoculated. The only organisms to attain an 'abundant' rating were Cladosporium on plants (rep. I)

TABLE 10

THE RESULTS OF REISOLATION FROM WILD RICE PLANTS

	ISOLATES	FUNGUS RECOVERED					
		REP I			REP II		
	Inoculated on parental plants (spray inoc., unless otherwise stated)	ALTERNARIA	CLADOSPORIUM	OTHER FUNGI	ALTERNARIA	CLADOSPORIUM	OTHER FUNGI
1	DRECHSLERA 1	+	+	++			
2	DRECHSLERA 2				+	+	
3	DRECHSLERA 3	+	++	++			+
4	DRECHSLERA 4		+		++		+
5	DRECHSLERA BICOLOR		+	+	+	+	++
6	ALTERNARIA 1	++	++			+	+
7	ALTERNARIA 2	++	++	+			+
8	ALTERNARIA 3 +GONATOBOTRYS SIMPLEX					++	++
9	ENTYLOMA LINEATUM inject culm + spray	+	+	+	+	+	
10	ENTYLOMA LINEATUM inject culm	+	++		++		
11	ENTYLOMA LINEATUM inject inflorescence stalk	+	++		+		+++
12	PEYRONELLEAE SP.					++	++
13	FUSARIUM 1	++	++	+	+	+	
14	FUSARIUM 2	+	++				+
15	FUSARIUM 3						
16	unidentified fungus #1	++	++				+
17	unidentified fungus #2	++	++				++
18	unidentified fungus #2 inject culm		+++	+		+	++
19	CONTROL 1		++				++
20	CONTROL 2						
21	CONTROL 3	+	+				
22	CONTROL 4						

+++ ABUNDANT; ++ MODERATELY ABUNDANT; + SPARSE

derived from parents injected with unidentified fungus #2, and 'other fungi' on those (rep. II) derived from parents injected with E. lineatum (Table 10). Seed from the control plots produced plants with noticeably fewer lesions than seed from parentally inoculated plants and correspondingly fewer fungi were isolated.

DISCUSSION

It should be noted that Cladosporium is not listed among the isolates inoculated onto parental plants in Table 10 as insufficient seed of this treatment had been available from the field-inoculated plots the previous summer. No attempt was made to identify to species those which were recovered in this experiment nor to demonstrate any inter-relationships. This was in view of the fact that, first, Alternaria was only recovered ('moderately abundant') from one replicate of the material whose parental stock had been inoculated with Alternaria 2. Secondly, there was poor agreement between replicates I and II for the frequency of re-isolated fungi, with the exception of the controls from which fungi were rarely recovered.

The complete lack of re-isolated field-inoculated pathogens suggests an absence of disease carry-over in the living plant (seed) from one year to the next. The living plant is specified because it is accepted that certain fungi

such as Nakataea sigmoidea Hara (Helminthosporium sigmoideum) and Sclerotium sp. can survive and overwinter in dead wild rice tissue (King, 1970)(Oryza: Ou, 1972). It is thought that this is one of the reasons for the occurrence of a time-related build-up of these diseases in artificial wild rice paddies (Discussion, p.194).

In light of their relative absence from controls, occurrence of Alternaria, Cladosporium and other fungi may be linked in some way to disease incidence on parentally inoculated stock. Although direct carry-over seems unlikely, infection of parental stock might have altered exudation from developing seed so that nonpathogenic organisms could build up in or on the glumes and be carried over.

Alternatively, lesions may reflect the relative vigor or resistance levels of the plants. The controls, which lacked any form of parental inoculation, may have been more resistant to general infection than the inoculated treatments. The inference is that plant vigor had been reduced by the disease inoculations the previous summer, and that this had affected the seed and resultant seedling. Some support for this comes from the observation that re-isolation was most successful in cases where the parental generation had been point-inoculated. Mechanical damage might well have had an especially dramatic effect upon plant vigor. However, there appeared to be no correlation

between the number of fungi re-isolated and the level of disease of parental plots.

Finally it should be pointed out that the conditions of seed storage (in water at $2^{o}C$ in polyethylene bags) did not in any way duplicate overwintering conditions in the field. In natural conditions there is a scatter of seed from shallow lake edge zones to deeper water, so that some is frozen solid in mud while the rest escapes total freeze-up and remains in water just above freezing point. Just how much these conditions alone differentially control viability and survival of fungal mycelium and sclerotial bodies in diseased plant debris as well as, possibly, within the seed itself, have yet to be ascertained. More work is needed here and also in the overwintering of pathogens in the plant debris of commercial wild rice paddies where natural conditions would not pertain owing to certain cultural practices such as draining of the paddies in fall and burning of the plant straw. Present studies indicated that wild rice seed will not tolerate freezing under laboratory conditions (Appendix 4.7), despite a report to the contrary (Edman, 1969). It is not known how much the ambient mud and "fermenting ooze" common on wild rice lake floors, may act as a buffer against this threat to viability of seed, as well as fungal material.

EXPERIMENT VI

SEED SURVIVAL OF PATHOGENS - 2

MATERIALS AND METHODS

Seed collected from the field plots which had been inoculated in the summer of 1971 was stored for 8 months at 2°C and then plated out on agar. Sixteen seeds were removed at random from each sealed polyethylene bag. Each seed sample was surface sterilised by agitating for 3 minutes in a 250 ml Erhlenmeyer flask containing a mixture (1:2) of 95 per cent ethanol and undiluted commercial Javex (4% sodium hypochlorite). It was then washed in four changes of sterile distilled water. Finally the water was decanted and the seed spread out on an aluminum bench top. Each seed was held in forceps and bisected longitudinally by means of a scalpel blade. The half seeds were then transferred, cut surface down, on to PYG agar (p.210). Aseptic conditions were maintained throughout.

In order to minimise interference by bacteria in the growth of seedborne fungi, the agar medium was supplemented with the antibiotics chloramphenicol and streptomycin sulphate each at a final concentration of 50 mg per litre. The antibiotics, being heat labile, were passed through

a sterile micropore filter (0.2 μ) and added to the agar medium as it cooled.

The inoculated agar plates were incubated at 22°C and a 12 hour day of white light supplemented with near-ultraviolet was used to encourage sporulation of seed-borne fungi. The plates were observed daily during incubation. Those plates which contained fast-growing fungi, were segregated and held at 2°C to prevent overgrowth of neighbouring colonies on the same plate. After 4 to 5 days all plates were examined microscopically and scored for the presence or absence of fungal and/or bacterial colonies on each half seed. Mucorales, which were relatively abundant and easily identified, were scored as a separate class. Few other fungi sporulated during the short period of incubation before overgrowth became imminent. Therefore, after the initial scoring of the plates, fungi were subcultured to allow for proper identification. A total of more than 640 cultures was made in this way and incubated in the manner described for the half seeds. Isolates were examined and separate species stocked on slants of V8 agar.

RESULTS

While good sporulation occurred in nearly all the subcultured isolates, none of the pathogenic fungi inoculated onto parental plants the previous summer were recovered.

A list of the fungi, identified to genera, is given below.

Graphium

Crysosporium

Phialophora

Penicillium

A substantial amount of bacterial growth occurred from the half seeds, but growth of the colonies was restricted and localised. Bacillus sp. was identified. (M. Johnson, personal communication).

The frequencies of Mucorales, other fungi, and bacteria were recorded for each original inoculation treatment (Appendix 4.1-4.3). The mean frequency counts from eight plates, each bearing eight half seeds, together with their standard deviations, are plotted in Figs. 4.1, 4.2 and 4.3 against the percentage germination of corresponding seed samples (Fig. 2.2, Appendix 2.4). The results of linear regression analyses are also presented in these figures.

Fig. 4.1 shows that the seed germinability drops off as the frequency of bacteria increases beyond approximately 60 per cent, indicating an inverse relationship between bacterial frequency and seed germinability and also a moderately high tolerance of the seed to bacterial contamination. This is confirmed by the zero order correlation

FIG. 4.1

The correlation (and linear regression analysis) between mean bacterial frequency and seed germinability. Bars represent standard deviations of the means.

Slope of Regression Line	-6.57
Intercept on y	107.32
Zero Order Correlation Coefficient	0.42941
S y.x, Standard Error of Estimate (biased)	17.90

For Treatments 1-22, see Table 4, p. 54.

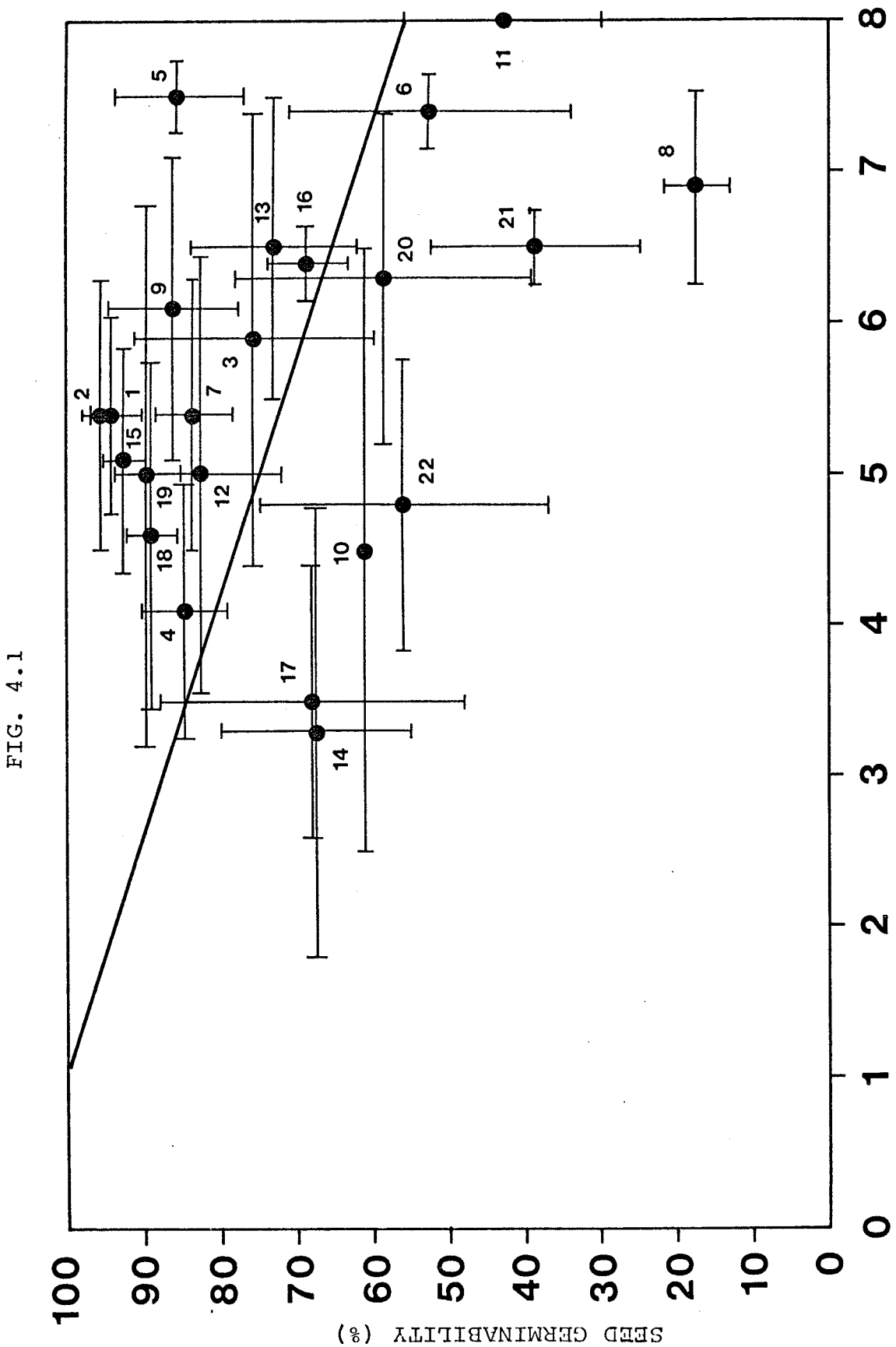

SEED GERMINABILITY (%)
100
90
80
70
60
50
40
30
20
10
0
0
1
2
3
4
5
6
7
8
MEAN BACTERIAL FREQUENCY
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22

FIG. 4.1

FIG. 4.2

The correlation (and linear regression analysis) between mean Mucoralean frequency and seed germinability. Bars represent standard deviations of the means.

Slope of regression line	-1.58
Intercept on y	72.19
Zero Order Correlation Coefficient	0.0648
S y.x, Standard Error of Estimate (biased)	19.78

(The regression line is broken as the correlation was non-significant at the 5 per cent limit).

For Treatments 1-22, see Table 4, p. 54.

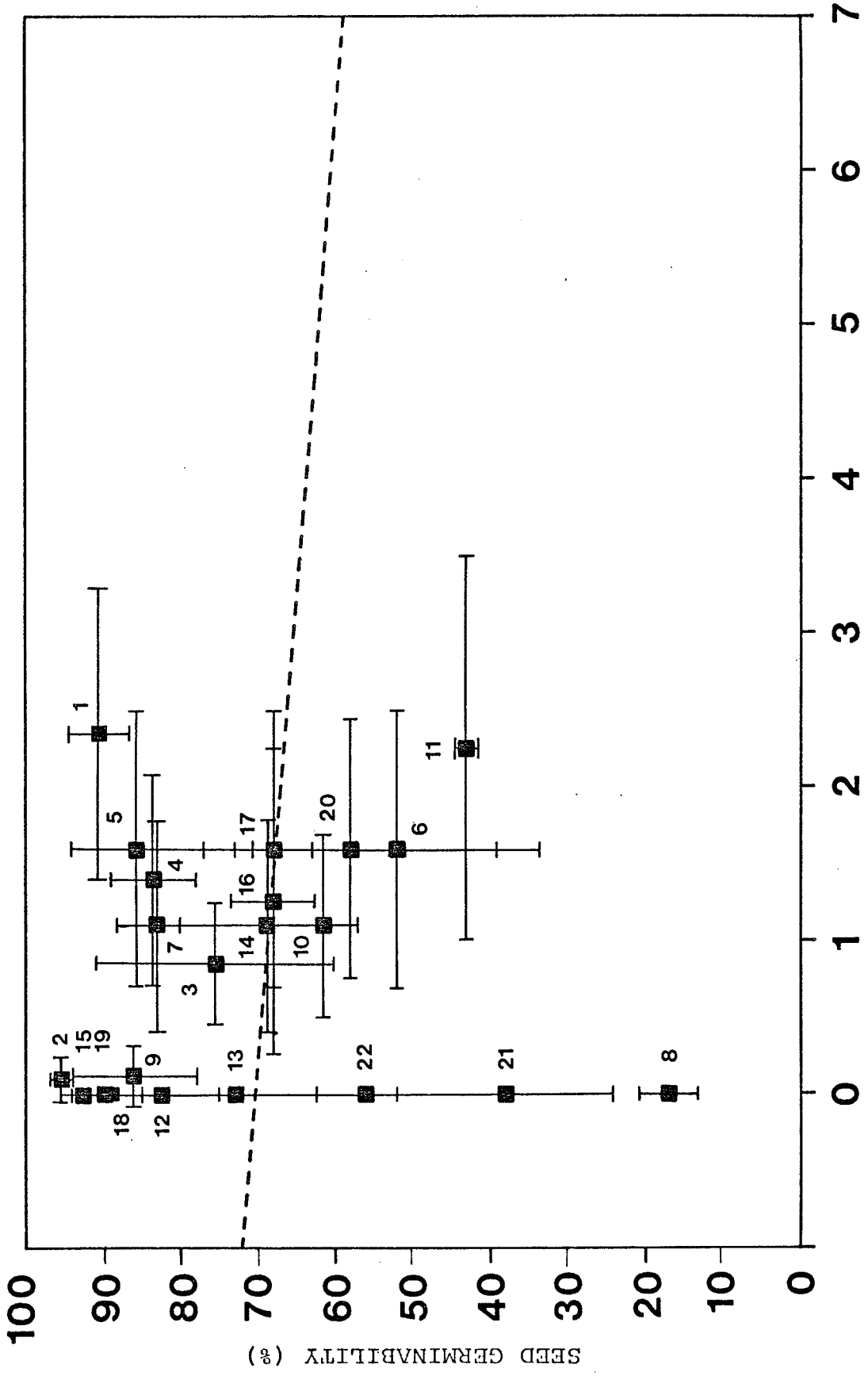

SEED GERMINABILITY (%)
100
90
80
70
60
50
40
30
20
10
0
0
1
2
3
4
5
6
7
MEAN MUCORALEAN FREQUENCY
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22

FIG. 4.2

FIG. 4.3

The correlation (and linear regression analysis) between mean fungal frequency (excluding Mucorales) and seed germinability. Bars represent standard deviations of the means.

Slope of Regression Line	0.97
Intercept on y	68.28
Zero Order Correlation Coefficient	0.0866
S y.x, Standard Error of Estimate (biased)	19.75

(The regression line is broken as the correlation was non-significant at the 5.0 per cent limit).

For Treatments 1-22, see Table 4, p.54.

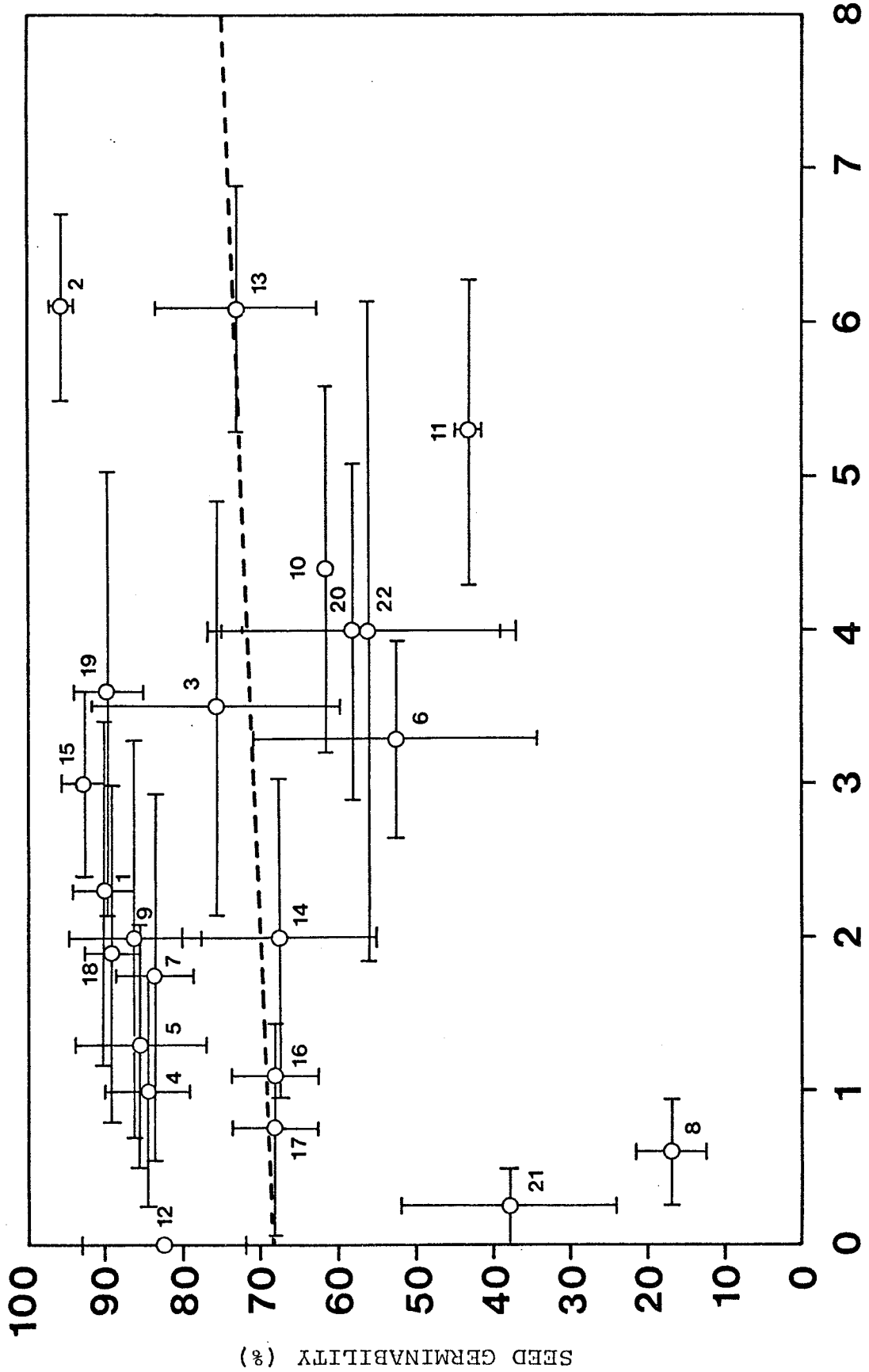

SEED GERMINABILITY (%)
100
90
80
70
60
50
40
30
20
10
0
MEAN FUNGAL FREQUENCY
0
1
2
3
4
5
6
7
8
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22

FIG. 4.3

FIG. 4.4

The correlation (and linear regression analysis) between seed yield (Expt. II) and the mean Mucoralean frequency (Expt. VI). Bars represent standard deviations of the means.

Slope of Regression Line	-0.27089
Intercept on y	3.5660
Zero Order Correlation Coefficient	0.42039
S y.x, Standard Error of Estimate (biased)	0.47839

(The regression line is broken as the correlation was marginally non-significant at the 5 per cent level. Significance at the 10 per cent level occurred. See Appendix 4.5, p. 244).

For Treatments 1-22, see Table 4, p. 54.

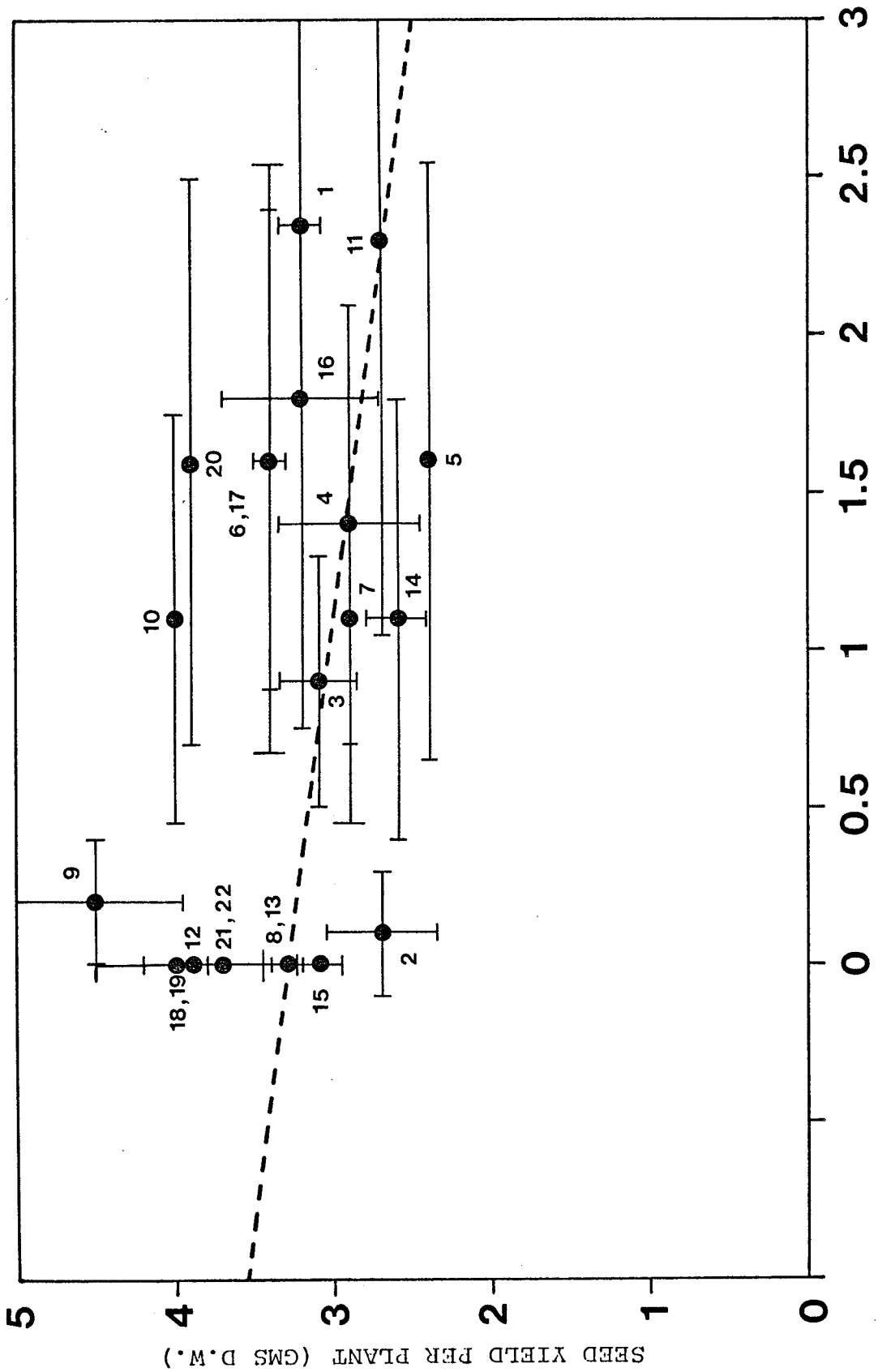

SEED YIELD PER PLANT (GMS D.W.)
MEAN MUCORALEAN FREQUENCY
0
1
2
3
4
5
0
0.5
1
1.5
2
2.5
3
1
11
16
5
20
6,17
4
10
7
14
3
9
12
21, 22
8,13
2
18,19
15

FIG. 4.4

coefficient of 0.42941, significant at the 5 per cent significance level (Appendix 4.4).

A similar though much less marked relationship is observed in the case of the Mucorales (Fig. 4.2). However, the very low correlation coefficient (0.0648) indicated that the interaction was statistically non-significant.

A direct relationship appeared to exist between seed germinability and frequency of occurrence of other fungi, but once again, the zero order correlation coefficient (0.0866) was nonsignificant (Fig. 4.3).

Correlation coefficients were calculated for interactions between the presence of these three groups of organisms and the seed yields and lesion indices of the parental plants (Appendix 4.5 and 4.6). Fig. 4.4 is the regression plot of the Mucorales frequency against parental seed yield, the only one of these interactions which showed any significant correlation (10 per cent level). The correlation coefficient was marginally nonsignificant at the 5 per cent level (Appendix 4.5, p. 244).

DISCUSSION

The results provide evidence that the fungi which were considered pathogenic on the basis of earlier inoculations do not survive laboratory storage under water at $2^{o}C$ on or in the seed. This does not rule out the possibility

that other fungi associated with wild rice may be commonly seedborne or that pathogenic fungi may be seedborne under natural overwintering conditions.

With respect to the correlation between bacterial contamination and seed germinability it is worth noting that although bacteria occurred at moderately high frequency on all seed samples they never caused complete suppression of germination. Secondly, a low level of germinability does not necessarily indicate low viability. It is well known (Steeves, 1952) that in a sample of wild rice seed, there will be a wide range in dormancy so that not all the seed will germinate in the next growing season, but some will remain dormant for a further eight to 12 months. It may be postulated that the dormancy period is increased by high levels of bacterial contamination and that this is reflected in the lowered germinabilities. If the high bacterial content does indeed increase dormancy without causing a loss in viability, it may well be beneficial to the species, in which case the relationship would appear to be symbiotic rather than parasitic or saprophytic.

Alternatively, however, bacterial colonisation may be harmful to the seed by reducing viability as well as germinability. Data was not obtained to indicate this. There was some doubt as to the reliability of the tetrazolium test for testing seed viability of wild rice.

With reference to Fig. 4.1, the wide range of seed germinability for approximately the same bacterial frequency and vice versa suggests that individual seed samples responded to bacterial colonisation in different ways. This poses the question as to the nature of the seed-bacteria interaction. The position of the bacteria within the seeds is unknown. It is possible that in seeds which germinated they were confined to the outer tissues (flower parts, pericarp/testa) while in seeds which did not germinate they invaded the endosperm and embryo thus reducing viability. Finally, it should be stated that if wild rice seed was not able to tolerate or resist bacteria in nature, it would be at a severe ecological disadvantage as most seed is deposited in lake floors where bacteria are important decomposers of organic matter.

The correlation between fungal growth in the seed and seed germinability, though statistically nonsignificant bears some comment. As stated in Chapter Two (Fig. 2.6) there is a direct correlation between seed germinability and the parental lesion index, at the 10 per cent level of significance, suggesting that to some degree, fungal infection of the leaves may promote rather than inhibit germinability of the seed. The inference here (Experiment VI) is that the fungal content of the seed, with respect to the mean fungal frequency, may have some indirect relationship with the disease incidence on the parental plants. The

overall tendency, in the present experiment, is similar in nature. Higher frequencies of fungi in the seed were associated with increased rather than decreased seed germinability, suggesting that in some way the activities of pathogenic fungi in parental plants is related to those of nonpathogenic ones in the seed. Evidence for this is also supported by some interaction between the presence of Mucorales in the seed and reduced parental seed yield (significant at the 10 per cent level, Fig. 4.4).

In conclusion, the evidence suggests that, by and large, the effects of disease on the seed would appear to be no more detrimental than they are beneficial, as indicated by the high tolerance levels to bacterial and fungal contamination on the one hand, and the apparently increased seed germinability on the other. However, on the long term basis, detrimental influences may become more visible, owing to the importance of reserve dormant seed. It should be added that despite the correlations evidenced, no direct correlations with pathogenic fungi could be made.

CHAPTER FIVE

GENERAL DISCUSSION

Earlier reports of diseases on the aerial parts of wild rice have focussed attention on 'Helminthosporium' species as the main causal agents. Bean and Schwartz (1961) mention Helminthosporium oryzae B. de Haan causing an epidemic of brown spot disease in Minnesota and Morrison and King (1971) also attribute heavy losses in Minnesota to H. sigmoideum Cav. and other Helminthosporium spp. The results of the present study indicate that of the fungal isolates initially screened for pathogenicity, species of two genera, Drechslera and Fusarium, are the principal agents of damage to the aerial parts of wild rice in Manitoba. With the exception of the Nakatea state of Leptosphaeria salvinii Catt. (≡Helminthosporium sigmoideum B. de Haan) which has not been isolated from Manitoba material to date, all the 'Helminthosporium' isolates examined so far, both from Minnesota and Manitoba, are referable to Drechslera Ito (J. Reid, personal communication). It therefore seems probable that those unidentified 'Helminthosporium spp.' mentioned by Morrison and King (1971) are of similar affinity. Up to now Bipolaris zizaniae(Nisikado) comb. nov. Shoemaker (≡Helminthosporium zizaniae Nisikado) has not been reported on Zizania aquatica in North America.

Drechslera bicolor (Mitra) Subram. and Jain(≡H. bicolor Mitra) which proved to be the most highly pathogenic of the Manitoba isolates, has not been cited before on wild rice, nor has it been recognised on cereals in North America. However, the isolate was only induced to sporulate in culture after considerable experimentation with various combinations of growth media and light regimes. It is possible that D. bicolor has merely been overlooked on wild rice and other host species in Minnesota although it did not occur on the diseased material from Minnesota which was examined by the author.

On a number of occasions several morphologically distinct Drechslera isolates were obtained from a single leaf or lesion type. It has not yet been possible to determine whether these isolates represent many separate species or merely variability within one or more species. Those tested were generally less pathogenic than D. bicolor, but undoubtedly account for much of the brown spot syndrome in Manitoba as in Minnesota. Fusarium spp. were not implicated in the brown spot disease by Bean and Schwartz (1961) or Morrison and King (1971) but pathogenic isolates have since been obtained from diseased material from Manitoba and Minnesota. The range of lesion type produced by Fusarium spp. corresponds closely to that of Drechslera spp. indicating that the brown spot syndrome is a general response of the host to fungal attack rather than that of

specific host pathogen reactions. More specific responses do occur, however, toward other pathogens such as Entyloma lineatum.

The importance of environmental conditions in determining the success of infection by both Drechslera and Fusarium isolates was clearly indicated by greenhouse inoculations with and without humidification. Higher relative humidity may account in part for the observation of Bean and Schwartz (1961) that brown spot caused by H. oryzae was more severe in denser stands. However, plants tend to mature more rapidly in denser stands and it appears that their physiological age is as important as the microclimate in determining their susceptibility. Plants in densely populated paddy areas began to be lesioned at a particular stage in aerial leaf development while those in sparser areas were not infected until later when they reached the corresponding stage.

Within the group of predominantly foliar pathogens which was studied, some isolates possessed some capacity to invade leaf sheaths and thence induce a culm rot. Among the Drechslera isolates, 1 and 6C and D. bicolor showed culm rotting tendencies. Fusarium isolates 1 and 4 also caused extensive damage when introduced into the culm, an effect which may have been related to toxin production. Although H. sigmoideum and Sclerotium sp, the characteristic

stem rotting organisms described by Morrison and King (1971), were not found in the early stages of this study, a Sclerotium sp has since been reported attacking paddy wild rice at Fort Alexander, Manitoba (D. Punter, 1972: personal communication).

Bean and Schwartz (1961) noted an increase in the severity of brown spot disease with each successive year of wild rice cultivation. A similar trend was observed in the present study and was also reported by Morrison and King (1971) with respect to H. sigmoideum and Sclerotium sp. The latter authors suggest that infested seed may contribute to the spread and build-up of the diseases. However, attempts to demonstrate the carry-over of foliar pathogens in or on seed did not yield any evidence in support of this hypothesis. In view of the wide host range of many Drechslera and Fusarium spp. it is probable that these isolates from wild rice could over-winter and increase on grass or cereal hosts near wild rice stands, as well as wild rice straw.

With reference to Zizania latifolia, Yamamoto et al (1938) report a brown spot caused by Helminthosporium oryzae Breda de Haan (≡Drechslera oryzae Sub. and Jain). These symptoms probably compare with those observed on Z. aquatica in Manitoba and Minnesota due to infection by Drechslera and Fusarium species. Smut symptoms, however, are quite

dissimilar for the two plants with Ustilago esculenta on Z. latifolia inducing dwarfed plants with hypertrophied culms and inflorescences resulting in considerable losses in yield (Introduction, p. 25).

Ergot (Claviceps purpurea) has been a severe problem on occasions in the past (Conners, 1939). However, it was not observed on wild rice in the present study. Conners reported a distinct strain which infected wild rice and not cereal crops and grasses. This strain had sclerotia which floated on water, indicating a certain amount of specialised adaptation to infection of an emergent aquatic grass.

No pathogenic bacteria were observed in the present study (isolation techniques involved fungi only), though bacteria including Bacillus sp did occur on 'over-wintered' seed and their presence correlated with a loss in seed germinability (Experiment VI). No reports were found in the literature with reference to bacteria on wild rice (Z. aquatica) in North America. Zizania latifolia, however, is infected by the pathogenic bacterium Xanthomonas oryzae (Goto et al., 1953) which also attacks Oryza causing a leaf blight. It is possible that bacteria infect the aerial parts of Z. aquatica since the causes of certain lesions were not completely accounted for. The black flecking of flag leaf sheaths (lesion type 6.2) for example is only

tentatively correlated with infection by Entyloma lineatum. Such lesions yielded no microorganisms, and more specific techniques may be necessary for isolation. Alternatively, these may represent a secondary toxic effect of the already known pathogens. Makulova (1970) reported Pseudomonas, Bacterium, Bacillus and Achromobacter from the aquatic root microflora of healthy and vigorous wild rice plants. The host was cited by generic name only, though it was probably Z. latifolia. The bacteria were apparently beneficial to the plant. A similar rhizosphere flora may occur for Z. aquatica in North America.

Although nematodes have been reported on wild rice (Sanwal, 1957) no reports have been made of disease directly related to the presence of root or culm nematodes in wild rice. However, pathogenesis by nematodes is not unlikely in view of the known pathogenicity of species of Radopholus, the genus isolated from Ontario by Sanwal. It is possible that they may also form synergistic relationships with fungal pathogens including root organisms.

Insect species may act as vectors of fungi causing diseases of wild rice or they may predispose the plants to infection. The "rice stem maggot", Eribolus longulus (frit fly) may increase fungal accessibility to inner culm regions through wounds caused by larval attack. The larvae of Hydrellia griseola (shore fly) may aid the attack of

foliar pathogens by providing infection courts at points where it has burrowed into leaf mesophyll.

It is not known if the epicuticular wax on surfaces of aerial leaves of wild rice (Zizania aquatica) (Hawthorn and Stewart, 1970) can limit or modify foliar infection. Surface waxes of certain plants are known to possess anti-fungal properties. Martin et al (1957) showed that waxes and some of their separated fractions extracted from leaves of certain varieties of apple, were able to inhibit germination of conidia of Podosphaera leucotricha. Johnston and Sproston (1965) suggested that the surface wax contributes to the antifungal nature of leaves of Ginkgo biloba.

Pollen deposited on fruits or leaves of anemophilous plants has been shown to stimulate fungal infection (Chu Chou and Preece, 1968; Fokkema, 1971; Warren, 1972). It is possible that such a pollen effect was present in the field. It could help account for the heavy infection by Drechslera isolates particularly in the case of the second inoculations which were carried out at the time of flowering. It is not known how much this 'pollen effect' may have been modified by antagonistic activities of saprophytic microflora (Fokkema, 1973) associated with wild rice.

The highly variable nature of wild rice (Z. aquatica var. angustifolia) and its pathogens, particularly Drechslera isolates, suggests that they are still evolving at a relatively rapid rate, both individually and with respect to each other. That wild rice is relatively new to its growth locale is borne out by the work of McAndrews (1969) who found that the initial increase of wild rice type pollen in borings from a lake floor in Minnesota dated at 1935 ± 100 radiocarbon years B.P. Ivanov and Ivanova (1968) also report Z. latifolia in Russia to be newly establishing itself in lake habitats which it is overgrowing and Pikava (1972) reports Z. aquatica as new in Arizona. In any given natural stand, Z. aquatica is a highly outbreeding (cross-pollinating) heterogeneous population and the acuteness of disease symptoms fluctuates from plant to plant. The corollary suggested here is the existence of a large pool of genes the random mixing of which could allow for the inheritance of a maximal number of beneficial characters within the population. This could possibly account for the absence of epidemics of Claviceps purpurea for example, in recent years. Only a long term program of research with respect to the plant itself and to the individual pathogens would seem able to provide the basis for an understanding of the host-pathogen relationships specific to North American wild rice.

CONCLUDING REMARKS

1. There are a number of foliar pathogens of wild rice in Manitoba including Drechslera and Fusarium species. Of these isolates, some show tendencies for culm rot. The most severe, mainly foliar pathogen so far isolated in Manitoba is Drechslera bicolor (Mitra) Subr. and Jain.

2. Drechslera species (specifically Drechslera 1, 5L, 6C and D. bicolor which were tested in the greenhouse) as well as Entyloma lineatum, produce maximal infection under greenhouse conditions of high humidity and high temperature. Fusarium 1, 2, 3 and 4 produce maximal infection in greenhouse conditions of relatively low humidity and high temperature. It is likely from field observations that this occurs in natural stands also.

3. The brown spot syndrome with respect to lesion types 1.0 to 5.0 is more a response of the host plant than a specific host-pathogen interaction.

4. Foliar infection is from airborne spores of Drechslera and Fusarium species.

5. Drechslera and Fusarium species do not appear to be systemic invaders though there is some evidence for internal spread of D. bicolor and possibly Drechslera 1 and 6C.

6. Entyloma lineatum shows signs of systemic infection but this is still subject to confirmation.

7. None of the foliar pathogens isolated from Manitoba wild rice appear to be seedborne.

8. Entyloma lineatum infecting alone is weakly pathogenic causing little or no reduction in the vigour of wild rice plants. However, in naturally occurring mixed infections with Drechslera and Fusarium species it is possible that some synergism may occur.

9. Alternaria 2 shows some evidence for pathogenicity by field trials. Apart from this isolate, other Alternaria as well as Cladosporium isolates frequently obtained

from wild rice, are probably secondary invaders being weakly parasitic or saprophytic organisms. Within this group of normally nonpathogenic fungi can also be included the isolates of Epicoccum nigrum and Peyronellaea sp.

10. Wild rice plants in denser stands mature more rapidly than those in open stands. The high incidence and severity of disease reported in denser stands probably reflects age-related differences in host susceptibility. Other factors such as humidity may also contribute to the greater development of symptoms.

11. The bacteria such as Bacillus sp. associated with the seed of wild rice may possibly be beneficial to the plant on a long term basis by extending dormancy. They may possibly become secondarily parasitic in association with other pathogens.

The following is a list of specific areas in which the need for further work became apparent during the course of this study.

1. Determination of the chronological changes in the air-borne inoculum of pathogenic fungi in natural and paddy stands by the use of a spore trap. The correlation of

these with environmental parameters such as rainfall, wind, temperature, humidity and sunlight would provide a basis for the prediction of epidemics. This is also of significance in the application of fungicides.

2. Mixed infections by greenhouse and field inoculations combining for example Drechslera and/or Fusarium species with Entyloma lineatum.

3. The continuation of studies with relation to the seed-borne transmission of pathogenic fungi.

4. A study of the overwintering habits of the pathogenic fungi such as Drechslera and Fusarium spp. in relation both to the grasses neighbouring wild rice stands and the wild rice plants themselves. It should also be determined how the survival of sclerotia or other overwintering structures may be effected by cultural practices such as drainage, cultivation and the burning of wild rice straw.

5. Studies of any root-infecting organisms in the water/mud interphase in wild rice habitats.

6. Biochemical and physiological studies to determine the factor(s) controlling disease resistance in wild rice.

7. A breeding program to develop disease resistant cultivars of wild rice.

8. The exact characterisation of bacteria associated with wild rice seed and their relation to the plant.

9. A determination of suitable fungicides in relation to specific pathogens and appropriate times of application.

APPENDIX 1.1

THE ISOLATION AND CULTURE OF ENTYLOMA LINEATUM

Isolation

Fresh inflorescence stalks of wild rice from Fort Alexander, Manitoba, were selected for the presence of the sori of E. lineatum. Pieces, approximately 5 to 6 cm in length, were surface-sterilised for 3 minutes in a 250 ml Erhlenmeyer flask containing a 1:3 dilution with water of 4.5 per cent sodium hypochlorite (commercial Javex). After sterilization the pieces were serially washed three or four times in sterile distilled water. The surface sterilised pieces were treated in one of the following ways.

1. Terminally located sori were gently macerated with a sterile needle into a few drops of sterile distilled water on a clean sterile slide. The suspension was gently agitated and diluted by removal of 2 drops with a Pasteur pipette to tubes containing 10 ml sterile distilled water. After agitation, 3 drops of the dilution were transferred to the surface of water agar or Martins medium (modified) (Tuite, 1969) in Petri dishes and spread with an L-shaped glass rod.

2. Pieces of tissue, with sori facing downwards, were stuck to the lids of plastic Petri plates, containing

one or other of the same two media. This allowed the monokaryon bud spores to be shot down onto the agar surface.

In each case the plates were incubated at 22°C for 48 hours. They were then examined with a microscope (X10 and X40 objectives) for the presence of the monokaryon yeast-like colonies. These were subcultured with a sterile needle to PYG plates which were sealed with masking tape and incubated at 22°C for approximately 48 hours. They were then stored at 2°C until enough monokaryon colonies had been obtained to allow for cross-compatibility inoculation trials between colonies.

Monokaryon smut cultures were obtained on water agar by Method 1. Bacterial contamination was heavy. However, with the use of the selective medium, 3 monokaryon cultures were found, after the close examination of numerous plates. Method 2 yielded 2 monokaryon cultures on water agar and 1 on the selective medium.

These 6 monokaryon isolates were code numbered and individually inoculated against each other on PYG plates. Three pairs were set up per plate by inoculating cells from different colonies 3 to 4 mm apart, with the use of a sterile needle. The plates were incubated at 22°C for 48 hours or more, and examined for the presence of dikaryon

bridge cells and/or dikaryon mycelia along the line of contact between paired cultures. This indicates the existence of opposite mating types which can be used in the preparation of dikaryon spore suspension.

As a further check pairs of monokaryon spore suspensions were tested against each other by spreading 0.5 ml of each thinly on water agar plates, incubating and examining for bridge cells.

None of these 6 yeast-like colonies showed compatibility by the test for mating type. No bridge cells or hyphae were seen. This indicated incompatibility and the absence of opposite mating types.

The number of monokaryon cultures obtained was too few for the isolation of opposite mating types and secondly for the selection of those mating types showing optimal compatibility. It is probable that the sori of E. lineatum were in a state of dormancy at the time of experimentation (late fall). Overwintering of the sori may be necessary for full development of monokaryon bud spores and this could account for the small number of monokaryon cultures obtained.

For the present study compatible monokaryon cultures were thus obtained from Dr. Jens J. Nielsen of the Canada Department of Agriculture, Winnipeg. These stocks were kept on agar slants at 2^{o}C.

Culture

Cells from the monokaryon stock cultures were inoculated by needle into 75 ml aliquots of liquid PYG in 250 ml Erhlenmeyer flasks, 3 with each mating type. After 15 hours incubation at room temperature on a shaker, one flask from each mating type was selected for uniformity in growth, and used to inoculate similar flasks, 6 of each mating type. The flasks were inoculated with a Pasteur pipette, 6 drops per flask, and incubated in the same way. In 22 hours when growth was dense and uniform in all 12 flasks, the mating types were mixed by adding one aliquot to the other in sterile conditions. The flasks were reincubated for 24 hours, when a cell sample was removed and examined microscopically for the presence of bridge cells.

Bridge cells indicating the occurrences of dikaryotisation were apparent within 24 hours of mixing of monokaryon cultures whereupon the dikaryon spore suspension was ready to be used for the inoculation of the wild rice.

APPENDIX 1.2

SINGLE SPORE CULTURES

Method One

The following technique was employed each time a Fusarium isolate was transferred to or from soil culture or prior to the preparation of inoculum. A small portion of the sporulating mycelium was removed with a sterile needle to a few drops of sterile distilled water on a clean sterilised slide. After gentle agitation this suspension was diluted by adding 3 drops to 10 ml sterile distilled water in tubes. Drops of the diluted suspension were transferred to water agar plates, spread with an L-shaped glass rod and incubated at 22^{o}C for 4 to 5 hours. The dilution procedure was adjusted where necessary to give a final count of between 25 and 35 macroconidia per plate. The incubated plates were examined under a binocular dissecting microscope and germinating conidia removed on small agar blocks cut out by means of an inoculating needle. The agar blocks were inverted singly on PDA agar plates which were incubated at 20^{o}C with 12 hours mixed near-UV and white light per day.

Single spore cultures were successfully established in this way for Fusarium 1, 2 and 4. Fusarium 3, however, sporulated too poorly to allow single spore culture.

Single spore cultures of Cladosporium 1, 2 and 3 were also obtained by this method.

Method Two

For larger spored fungi such as Drechslera spp. a cube of agar was cut from a vigorously sporulating culture obtained directly from leaf isolations. This was inverted on a plate of water agar several times serially, in order to dislodge the spores. By the third or fourth time the block had been touched to the agar surface, spores were sufficiently sparse to be picked off singly with an inoculation needle, using a binocular dissecting microscope. These were inoculated onto PYG agar plates or onto slants of V8 or PCA.

Single spore plate cultures as well as slants were established by this method for Epicoccum nigrum, Alternaria spp. and Peyronelleae sp. in addition to Drechslera spp.

APPENDIX 1.3

GROWTH MEDIA

1. PYG: Peptone Yeast Glucose Agar

Peptone	1.0 g
Yeast	1.0 g
Dextrose (d-glucose)	3.0 g
Agar	20.0 g
Distilled Water	1 litre

2. PDA: Potato Dextrose Agar

Potato	200.0 g
Dextrose	20.0 g
Agar	20.0 g
Distilled Water	1 litre

2 cm cubes of potato, without skin, were added to 500 ml distilled water. This was autoclaved in a large foil-covered beaker for 15 minutes at 120^{o}C and 15 p.s.i. The potato water was then passed through 2 layers of cheese cloth and made up to 1 litre after the addition of agar and dextrose.

3. V8: V8 Juice Agar

'V8' Tomato Juice	100 ml
Agar	20 g
Distilled Water	900 ml

4. V8 Modified:

Ingredients as above. Centrifuge 'V8' Tomato Juice at 10,000 r.p.m. for 20 minutes before use.

5. PCA: Potato Carrot Agar

Potato	20 g
Carrot	20 g
Agar	25 g
Distilled Water	1 litre

1 cm cubes of potato and carrot (without skin) were autoclaved under the conditions specified for PDA.

6. CDA: Czapek (Dox) Agar

Sodium nitrate ($NaNO_3$)	2.0 g
Potassium dihydrogen phosphate (KH_2PO_4)	1.0 g
Magnesium sulphate ($MgSO_4 7H_2O$)	0.5 g
Potassium chloride (KCl)	0.5 g
Ferrous sulphate ($FeSO_4 7H_2O$)	0.01g
Sucrose	30.0 g
Agar	20.0 g
Distilled Water (tap)	1 litre

Trace elements were not added as glass distilled water was not used.

7. Tap Water Agar:

Agar	20.0 g
Tap Water	1 litre

8. Selective Medium: Martin Medium RB-M1 (modified by the author) (Tuite, 1969)

KH_2PO_4	0.5 g
K_2HPO_4	0.5 g
$MgSO_4 7H_2O$	0.5 g
Peptone	5.0 g
Dextrose	10.0 g
Yeast Extract	0.5 g
Streptomycin Sulphate	0.03g
Oxgall	5.0 g
Sodium Propinate	1.0 g
Agar	20.0 g
Distilled Water (tap)	1 litre

The oxgall and streptomycin sulphate were dissolved in 50 ml distilled water and added through a micropore filter to the cooled agar medium (approx. $50^{o}C$) after it had been sterilised.

9. Antibacterial Medium (Experiment VI):

Chloremphenicol and streptomycin sulphate were used at a final strength of 50 mg per litre. 250 mg of each were added to 100 ml tap distilled water. This was stirred and heated at approximately 30°C until all had dissolved. The solution was passed through a micropore filter (0.2 μ diam.) under vacuum and 5 ml added with a sterile hypodermic syringe to 245 ml PYG agar aliquots cooled to approximately 50°C in 500 ml Erhlenmeyer flasks.

APPENDIX 1.4

THE PRESERVATION OF FUSARIUM IN SOIL

Clones of Fusarium 1, 2 and 4 were prepared by single-spore subculture on PDA plates incubated at 20°C with a 12 hour day of mixed near-UV and white light. When sporulation was abundant, the conidia were harvested in sterile conditions. Sterile distilled water was added to the plates which were scraped with a wire loop. The resulting suspensions were passed through 2 layers of cheesecloth in a funnel and collected in 250 ml Erhlenmeyer flasks. Two ml aliquots of the suspension were then added to tubes containing a mixture of black earth, sand and peat (2:1:1) which had been sterilised by autoclaving at 120°C for 3 hours on 3 consecutive days. The tubes were agitated to disperse the suspension which moistened the upper soil (2-3 cm)only. After incubation for 5 days at room temperature (17°C) the tubes were stored at 2°C.

The Fusarium isolates retained their viability and pathogenicity well as evidenced by inoculations conducted 18 months later.

APPENDIX 2.1

SEED YIELD DATA

Expt. II FIELD INOCULATION TRIALS (Cf: Fig. 2.2)

	ISOLATE (Spray inoc. unless otherwise stated)	SEED YIELD/PLANT (g.d.wt.) REP.I	REP.II	MEAN	ST. DEVI-ATION
1	DRECHSLERA 1	3.4	3.0	3.20	0.28
2	DRECHSLERA 2	3.2	2.2	2.70	0.71
3	DRECHSLERA 3	2.7	3.4	3.05	0.49
4	DRECHSLERA 4	2.3	3.5	2.90	0.85
5	D. BICOLOR	2.3	2.4	2.35	0.07
6	ALTERNARIA 1	3.5	3.2	3.35	0.21
7	ALTERNARIA 2	2.9	2.9	2.90	0
8	ALTERNARIA 3+GONATOBOTRYS SIMPLEX	3.2	3.4	3.30	0.14
9	E. LINEATUM	5.2	3.7	4.45	1.06
10	E. LINEATUM (inject culm)	4.0	3.9	3.95	0.07
11	E. LINEATUM (inject infl. st.)	2.7	2.7	2.70	0
12	PEYRONELLAEA SP.	3.0	4.7	3.85	1.20
13	FUSARIUM 1	3.2	3.4	3.30	0.14
14	FUSARIUM 2	2.3	2.9	2.60	0.42
15	FUSARIUM 3	2.9	3.3	3.10	0.28
16	unidentified fungus #1	2.4	3.9	3.15	1.06
17	unidentified fungus #2	3.4	3.4	3.40	0
18	unidentified fungus #2 (inject culm)	3.7	4.3	4.00	0.42
19	CONTROL 1	3.7	4.2	3.95	0.35
20	CONTROL 2	3.9	3.9	3.90	0
21	CONTROL 3	4.0	3.3	3.65	0.49
22	CONTROL 4	3.3	4.0	3.65	0.49

APPENDIX 2.2

THE PERCENTAGE LOSSES IN SEED YIELD

Expt. II FIELD INOCULATION TRIALS (Cf: Fig.2.3)

The percentage losses in seed yield are based on the means of 2 replicates for each treatment relative to the overall mean for all the controls. The differences of individual controls 1-4 from the overall mean of the controls are also given.

	ISOLATE (spray inoc. unless otherwise stated)	PERCENTAGE LOSS (gain) IN YIELD (Cf: Controls)
1	DRECHSLERA 1	16
2	DRECHSLERA 2	29
3	DRECHSLERA 3	20
4	DRECHSLERA 4	23
5	D. BICOLOR	38
6	ALTERNARIA 1	12
7	ALTERNARIA 2	23
8	ALTERNARIA 3+GONATOBOTRYS SIMPLEX	13
9	E. LINEATUM	(+17)
10	E. LINEATUM (inject culm)	(+4)
11	E. LINEATUM (inject infl. st.)	29
12	PEYRONELLAEA SP.	(+2)
13	FUSARIUM 1	13
14	FUSARIUM 2	31
15	FUSARIUM 3	18
16	unidentified fungus #1	17
17	unidentified fungus #2	10
18	unidentified fungus #2 (inject culm)	0(+6)
19	CONTROL 1	(+4)
20	CONTROL 2	(+3)
21	CONTROL 3	4
22	CONTROL 4	4

APPENDIX 2.3

THE LESION INDEX

Expt. II FIELD INOCULATION TRIALS (Cf: Fig. 2.2)

	ISOLATE (spray inoc. unless otherwise stated)	LESION INDEX REP.I	REP.II	MEAN	ST. DEVI-ATION
1	DRECHSLERA 1	8.0	9.0	8.50	0.71
2	DRECHSLERA 2	9.0	8.5	8.75	0.35
3	DRECHSLERA 3	9.0	9.0	9.00	0
4	DRECHSLERA 4	9.0	9.0	9.00	0
5	D. BICOLOR	9.0	9.0	9.00	0
6	ALTERNARIA 1	5.0	4.5	4.75	0.35
7	ALTERNARIA 2	4.0	3.0	3.50	0.71
8	ALTERNARIA 3+GONATOBOTRYS SIMPLEX	3.0	4.0	3.50	0.71
9	E. LINEATUM	4.0	5.0	4.50	0.71
10	E. LINEATUM (inject culm)	4.5	4.0	4.25	0.35
11	E. LINEATUM (inject infl. st.)	3.5	3.0	3.25	0.35
12	PEYRONELLAEA SP.	4.0	4.5	4.25	0.35
13	FUSARIUM 1	5.0	4.5	4.75	0.35
14	FUSARIUM 2	4.0	4.0	4.00	0
15	FUSARIUM 3	4.5	4.5	4.50	0
16	unidentified fungus #1	3.5	3.0	3.25	0.35
17	unidentified fungus #2	4.5	4.5	4.50	0
18	unidentified fungus #2 (inject culm)	3.0	3.0	3.00	0
19	CONTROL 1	4.0	3.0	3.50	0.71
20	CONTROL 2	3.0	4.0	3.50	0.71
21	CONTROL 3	4.5	4.5	4.50	0
22	CONTROL 4	3.5	3.5	3.50	0

APPENDIX 2.4

SEED GERMINABILITIES

Expt. II — GERMINABILITY OF STORED SEED FROM FIELD INOCULATED PLANTS — (Cf: Fig. 2.2)

	ISOLATE (spray inoc. unless otherwise stated)	SEED GERMINABILITY REP.I	REP.II	MEAN	ST. DEVIATION
1	DRECHSLERA 1	84.50	96.20	90.35	8.27
2	DRECHSLERA 2	97.08	93.92	95.50	2.74
3	DRECHSLERA 3	97.95	53.11	75.53	31.71
4	DRECHSLERA 4	92.25	76.92	84.59	10.84
5	D. BICOLOR	97.86	73.37	85.62	17.31
6	ALTERNARIA 1	78.62	26.24	52.43	37.04
7	ALTERNARIA 2	90.60	76.10	83.35	10.25
8	ALTERNARIA 3+GONATOBOTRYS SIMPLEX	23.66	11.11	17.39	8.87
9	E. LINEATUM	97.84	74.40	86.12	16.57
10	E. LINEATUM (inject culm)	61.64	61.27	62.46	0.26
11	E. LINEATUM (inject infl. st.)	41.18	44.83	43.01	2.58
12	PEYRONELLAEA SP.	97.50	67.53	82.52	21.19
13	FUSARIUM 1	83.16	57.84	73.00	21.44
14	FUSARIUM 2	85.21	50.00	67.61	24.90
15	FUSARIUM 3	88.31	96.76	92.54	5.98
16	unidentified fungus #1	76.20	60.36	68.28	11.20
17	unidentified fungus #2	97.08	39.36	68.22	40.81
18	unidentified fungus #2 (inject culm)	83.90	94.33	89.12	7.37
19	CONTROL 1	96.25	82.93	89.59	9.42
20	CONTROL 2	30.83	85.88	58.36	38.93
21	CONTROL 3	57.95	17.93	37.94	28.30
22	CONTROL 4	83.24	29.02	56.13	38.34

APPENDIX 2.5

Expt. II — THE F RATIOS OF THE SEED YIELDS — (Cf: Fig. 2.4)

	ISOLATE (spray inoc. unless otherwise stated)	F RATIO*	SIGNIF.AT 5% **SIG-NIFI-CANCE	SIGNIF.AT 5% LOWEST SIG. F RATIO	SIGNIF.AT 1.0% SIG-NIFI-CANCE	SIGNIF.AT 1.0% LOWEST SIG. F RATIO	SIGNIF.AT 0.1% SIG-NIFI-CANCE	SIGNIF.AT 0.1% LOWEST SIG. F RATIO
1	DRECHSLERA 1	5.2045	-		-		-	
2	DRECHSLERA 2	11.9306	+		+		-	
3	DRECHSLERA 3	6.8681	+		-		-	
4	DRECHSLERA 4	6.7716	+		-		-	
5	D. BICOLOR	34.1902	+		+		+	
6	ALTERNARIA 1	3.7625	-		-		-	
7	ALTERNARIA 2	13.1134	+		+		-	
8	ALTERNARIA 3+GONATOBOTRYS SIMPLEX	3.8559	-		-		-	
9	E. LINEATUM	2.9666	-	5.32	-	11.26	-	25.42
10	E. LINEATUM (inject culm)	0.4364	-		-		-	
11	E. LINEATUM (inject infl. st.)	19.6899	+		+		-	
12	PEYRONELLAEA SP.	0.0224	-		-		-	
13	FUSARIUM 1	3.8559	-		-		-	
14	FUSARIUM 2	19.0236	+		+		-	
15	FUSARIUM 3	7.1272	+		-		-	
16	unidentified fungus #1	-144.6688	-		-		-	
17	unidentified fungus #2	2.4994	-		-		-	
18	unidentified fungus #2 (inject culm)	0.6087	-		-		-	
19	ALL TREATMENTS (1-18)	5.0265	+	4.08	-	7.31	-	12.61
20	D. SPP. 1-4 (inclusive)	14.7316	+	4.60	+	8.86	-	17.14
21	D. SPP. 1-4 + D. BICOLOR	20.0000	+	4.49	+	8.53	+	16.12

* Degrees of Freedom $n_1=1$; $n_2=8$ ** + = Significance; - = Nonsignificance

APPENDIX 2.6

Expt. II — THE F RATIOS OF THE LESION INDICES — (Cf: Fig.2.4)

	ISOLATE (spray inoc. unless otherwise stated)	F RATIO*	SIGNIF.AT 5% **SIG-NIFI-CANCE	SIGNIF.AT 5% LOWEST SIG. F RATIO	SIGNIF.AT 1.0% SIG-NIFI-CANCE	SIGNIF.AT 1.0% LOWEST SIG. F RATIO	SIGNIF.AT 0.1% SIG-NIFI-CANCE	SIGNIF.AT 0.1% LOWEST SIG. F RATIO
1	DRECHSLERA 1	96.2666	+		+		+	
2	DRECHSLERA 2	121.9140	+		+		+	
3	DRECHSLERA 3	141.1200	+		+		+	
4	DRECHSLERA 4	141.1200	+		+		+	
5	D. BICOLOR	141.1200	+		+		+	
6	ALTERNARIA 1	4.8765	-		-		-	
7	ALTERNARIA 2	0.2666	-		-		-	
8	ALTERNARIA 3+GONATOBOTRYS SIMPLEX	0.2666	-		-		-	
9	E. LINEATUM	2.4000	-	5.32	-	11.26	-	25.42
10	E. LINEATUM (inject culm)	1.2191	-		-		-	
11	E. LINEATUM (inject infl. st.)	1.2191	-		-		-	
12	PEYRONELLAEA SP.	1.2191	-		-		-	
13	FUSARIUM 1	4.8765	-		-		-	
14	FUSARIUM 2	0.3200	-		-		-	
15	FUSARIUM 3	2.8800	-		-		-	
16	unidentified fungus #1	1.2191	-		-		-	
17	unidentified fungus #2	2.8800	-		-		-	
18	unidentified fungus #2 (inject culm)	2.8800	-		-		-	
19	ALL TREATMENTS (1-18)	3.7928	-	4.08	-	7.31	-	12.61
20	D. SPP. 1-4 (inclusive)	413.8700	+	4.60	+	8.86	+	17.14

* Degrees of Freedom $n_1=1$; $n_2=8$ ** + = Significance; - = Nonsignificance

APPENDIX 2.7

Expt. II — THE F RATIOS OF THE SEED GERMINABILITIES — (Cf: Fig. 2.2)

	ISOLATE (spray inoc. onto parental plants unless otherwise stated)	F RATIO*	SIGNIF.AT 20%		SIGNIF.AT 10%		SIGNIF.AT 5%	
			**SIG-NIFI-CANCE	LOWEST SIG. F RATIO	SIG-NIFI-CANCE	LOWEST SIG. F RATIO	SIG-NIFI-CANCE	LOWEST SIG. F RATIO
1	DRECHSLERA 1	1.7016	-		-		-	
2	DRECHSLERA 2	2.3618	+		-		-	
3	DRECHSLERA 3	0.3783	-		-		-	
4	DRECHSLERA 4	1.0996	-		-		-	
5	D. BICOLOR	1.1643	-		-		-	
6	ALTERNARIA 1	0.1042	-		-		-	
7	ALTERNARIA 2	0.9916	-		-		-	
8	ALTERNARIA 3+GONATOBOTRYS SIMPLEX	3.4560	+		+		-	
9	E. LINEATUM (inject culm)	1.2160	-	1.95	-	3.46	-	5.32
10	E. LINEATUM (inject infl. st.)	0.0017	-		-		-	
11	E. LINEATUM	0.5903	-		-		-	
12	PEYRONELLAEA SP.	0.8757	-		-		-	
13	FUSARIUM 1	0.2818	-		-		-	
14	FUSARIUM 2	0.0890	-		-		-	
15	FUSARIUM 3	1.9694	+		-		-	
16	unidentified fungus #1	0.1145	-		-		-	
17	unidentified fungus #2	0.0918	-		-		-	
18	unidentified fungus #2 (inject culm)	1.5654	-		-		-	

* Degrees of Freedom $n_1=1$; $n_2=8$ ** + = Significance; - = Nonsignificance

APPENDIX 2.8

CORRELATION COEFFICIENTS

Expt. II SEED YIELD AND LESION INDEX (Cf: Fig.2.5)

DEGREES OF FREEDOM	ZERO ORDER CORR. COEF.	SIGNIF. AT 10% *SIG-NIFI-CANCE	SIGNIF. AT 10% LOWEST SIG. CORR. COEF.	SIGNIF. AT 5% *SIG-NIFI-CANCE	SIGNIF. AT 5% LOWEST SIG. CORR. COEF.	SIGNIF. AT 1.0% *SIG-NIFI-CANCE	SIGNIF. AT 1.0% LOWEST SIG. CORR. COEF.	SIGNIF. AT 0.1% *SIG-NIFI-CANCE	SIGNIF. AT 0.1% LOWEST SIG. CORR. COEF.
20	0.48171	+	0.3598	+	0.4227	-	0.5368	-	0.6524

Expt. II SEED YIELD AND SEED GERMINABILITY

20	0.00000	-	0.3598	-	0.4227	-	0.5368	-	0.6524

Expt. II LESION INDEX AND SEED GERMINABILITY (Cf: Fig. 2.6)

20	0.42029	+	0.3598	-	0.4227	-	0.5368	-	0.6524

* + = Significance; - = Nonsignificance

APPENDIX 3.1

SEED YIELD DATA

Expt. III — GREENHOUSE SPRAY AND POINT INOCULATIONS — (Cf: Fig.3.9)

	ISOLATE	METHOD OF INOCULATION	SEED YIELD/PLANT (g.d.wt.) REP. I	REP. II	REP. III	REP. IV	MEAN	ST. DEVI-ATION
1	D. BICOLOR	spray	0.40	0.44	0.29	0.29	0.36	0.07
2	D. BICOLOR	toothpick	0.27	0.31	0.30	0.42	0.33	0.07
3	CONTROL	toothpick	0.59	0.42	0.32	0.97	0.58	0.29
4	DRECHSLERA 1	spray	1.03	0.91	0.57	0.29	0.70	0.29
5	DRECHSLERA 1	toothpick	0.52	0.30	0.26	0.38	0.37	0.11
6	CONTROL	toothpick	1.36	1.63	0.74	0.89	1.16	0.41
7	DRECHSLERA 5L	spray	0.67	0.73	0.40	0.40	0.55	0.17
8	DRECHSLERA 5L	toothpick	0.38	1.33	0.18	0.43	0.58	0.51
9	CONTROL	toothpick	1.00	1.56	0.44	0.82	0.96	0.47
10	DRECHSLERA 6C	spray	1.00	0.57	0.48	0.53	0.65	0.24
11	DRECHSLERA 6C	toothpick	0.78	0.22	0.41	0.20	0.40	0.27
12	CONTROL	toothpick	1.48	0.71	1.00	0.71	0.98	0.36
13	FUSARIUM 1	spray (dry)	1.18	1.13	0.48	0.59	0.85	0.36
14	FUSARIUM 4	spray (dry)	0.89	0.42	0.40	0.33	0.51	0.26
15	FUSARIUM 4	spray	1.06	0.92	0.83	0.82	0.91	0.11
16	E. LINEATUM	spray	1.13	1.15	0.42	0.75	0.86	0.35
17	E. LINEATUM	spray + inject	0.97	0.37	0.09	0.28	0.43	0.38
18	CONTROL	spray + inject	0.90	0.73	0.23	0.20	0.52	0.35
19	CONTROL	spray	1.33	1.27	1.69	0.60	1.22	0.45
20	CONTROL	spray	1.13	1.80	0.83	0.67	1.11	0.50

APPENDIX 3.2

SEED YIELD DATA

Expt. IV GREENHOUSE POINT INOCULATIONS (Cf: Fig.3.12)

(0.5 ml inoculum injected into the base of the culm)

	ISOLATE	SEED YIELD/PLANT (g.d.wt.) REP I	REP II	REP III	REP IV	MEAN	ST. DEVI-ATION
1	D. BICOLOR	0.25	0.25	0.53	0.23	0.32	0.14
2	DRECHSLERA 1	0.10	0.14	0.06	0.07	0.09	0.04
3	DRECHSLERA 5L	0.12	0.16	0.16	0.11	0.14	0.03
4	DRECHSLERA 6C	0.09	0.19	0.13	0.19	0.15	0.05
5	FUSARIUM 1	0.14	0.04	0.05	0.09	0.06	0.08
6	FUSARIUM 4	0.03	0.02	0.01	0.03	0.02	0.01
7	E. LINEATUM	0.48	0.69	0.56	0.36	0.52	
8	CONTROL (water)	0.53	0.55	0.94	0.50	0.63	0.21
9	CONTROL (untreated)	0.40	0.74	0.61	0.57	0.58	0.14

APPENDIX 3.3

THE PERCENTAGE LOSSES IN SEED YIELD

Expt. III GREENHOUSE SPRAY AND POINT INOCULATIONS (Cf: Fig.3.14)

The per cent losses in seed yield are based on the means of 4 replicates for each treatment relative to the mean of the controls.

	ISOLATE	METHOD OF INOCULATION	PER CENT LOSS IN YIELD
1	D. BICOLOR	spray	70.50
2	D. BICOLOR	toothpick	43.10
3	DRECHSLERA 1	spray	42.60
4	DRECHSLERA 1	toothpick	68.10
5	DRECHSLERA 5L	spray	54.92
6	DRECHSLERA 5L	toothpick	39.58
7	DRECHSLERA 6C	spray	46.72
8	DRECHSLERA 6C	toothpick	59.18
9	FUSARIUM 1	spray DRY	30.33
10	FUSARIUM 4	spray DRY	58.20
11	FUSARIUM 4	spray	25.41
12	E. LINEATUM	spray	29.50
13	E. LINEATUM	spray + inject	17.25

APPENDIX 3.4

THE PERCENTAGE LOSSES IN SEED YIELD

Expt. IV GREENHOUSE POINT INOCULATIONS (Cf: Fig.3.14)

(0.5 ml inoculum injected into the base of the culm)

The per cent losses in seed yield are based on the mean of 4 replicates for each treatment relative to the mean of the controls injected with sterile distilled water.

	ISOLATE	PER CENT LOSS IN YIELD
1	D. BICOLOR	49.21
2	DRECHSLERA 1	85.71
3	DRECHSLERA 5L	77.78
4	DRECHSLERA 6C	76.19
5	FUSARIUM 1	90.48
6	FUSARIUM 4	96.83
7	E. LINEATUM	17.50
8	CONTROL (untreated)	7.94

APPENDIX 3.51

LESION INDEX CURVE

Expt. III DRECHSLERA BICOLOR FIRST INOCULATION (Cf: Fig. 3.1)

HOURS AFTER INOCULATION	LESION INDEX				MEAN	ST. DEVI-ATION
	REP I	REP II	REP III	REP IV		
12	0	0	0	0	0	0
16	0	0	0	0	0	0
20	6.00	4.75	6.50	4.75	5.20	0.34
24	6.50	4.75	6.50	4.75	5.63	0.39
40	8.00	6.00	7.00	7.00	7.00	0.82
48	8.25	7.00	7.75	7.50	7.88	0.39
60	8.75	7.75	8.00	7.75	8.06	0.24
64	9.25	8.25	8.50	8.75	8.69	0.43
69	9.50	8.25	8.75	8.75	8.81	0.52
87	9.50	8.25	8.37	9.00	8.88	0.52
108	9.60	8.75	9.25	9.25	9.21	0.35
120	9.70	8.80	9.30	9.30	9.28	0.37
144	9.80	8.85	9.40	9.40	9.36	0.39

DRECHSLERA BICOLOR SECOND INOCULATION

10	0	0	0	0	0	0
16	2.25	3.00	2.10	2.50	2.46	0.39
24	7.50	7.75	7.75	7.00	7.50	0.35
37	8.25	8.50	8.60	8.00	8.34	0.27
64	9.20	9.00	9.10	9.35	9.16	0.15
89	9.25	9.15	9.35	9.36	9.28	0.10
120	9.35	9.16	9.50	9.40	9.35	0.14
138	9.36	9.20	9.75	9.45	9.44	0.23

APPENDIX 3.52

LESION INDEX CURVE

Expt. III DRECHSLERA 1 FIRST INOCULATION (Cf: Fig.3.2)

HOURS AFTER INOCULATION	LESION INDEX				MEAN	ST. DEVIATION
	REP I	REP II	REP III	REP IV		
12	0	0	0	0	0	0
16	0	0	0.50	0	0.13	0.25
18½	4.50	3.50	1.50	6.00	3.88	1.89
21	6.00	6.50	2.75	6.75	5.50	1.86
24	7.50	7.20	6.00	7.20	7.00	0.63
38½	7.75	8.00	7.50	7.75	7.75	0.21
48	8.00	8.50	7.75	8.25	8.13	0.33
60	8.25	8.75	7.75	8.25	8.25	0.41
72	8.30	8.80	7.80	8.30	8.30	0.41
96	8.40	8.90	7.80	8.75	8.46	0.46
136	8.45	9.00	7.90	8.80	8.54	0.48

DRECHSLERA 1 SECOND INOCULATION

10	0	0	0	0	0	0
13	3.00	1.00	1.00	0	1.25	1.26
18	5.00	5.50	3.50	0.50	3.44	2.25
24	8.25	8.50	5.95	6.90	7.40	1.20
41	8.35	8.85	6.95	7.25	7.85	0.90
65	8.55	9.10	7.00	7.30	7.99	1.00
96	8.60	9.30	7.10	7.45	8.11	1.02
114	8.75	9.45	7.15	7.50	8.21	1.07
135	8.80	9.50	7.20	7.60	8.28	1.06

APPENDIX 3.53

LESION INDEX CURVE

Expt. III DRECHSLERA 5L FIRST INOCULATION (Cf: Fig.3.3)

HOURS AFTER INOCULATION	LESION INDEX				MEAN	ST. DEVIATION
	REP I	REP II	REP III	REP IV		
12	0	0	0	0	0	0
16	3.50	3.00	0	3.50	2.50	1.68
$17\frac{1}{4}$	5.75	5.00	4.00	5.60	5.09	0.79
20	8.00	7.50	4.00	7.50	6.75	1.85
24	8.25	7.75	4.50	8.25	7.19	1.80
40	8.30	8.25	5.00	8.75	7.63	1.76
48	8.60	8.50	6.00	8.80	7.98	1.32
88	8.80	8.60	6.00	8.85	8.06	1.38
120	8.85	8.70	6.00	8.90	8.11	1.41

DRECHSLERA 5L SECOND INOCULATION

10	0	0	0	0	0	0
16	4.25	3.95	2.95	3.25	3.60	0.60
24	6.25	8.25	3.75	7.00	6.31	1.90
41	6.75	8.65	4.00	7.85	6.81	2.03
72	8.85	8.75	4.50	8.15	7.56	2.06
90	8.90	8.80	4.55	8.20	7.61	2.06
111	8.95	8.83	4.75	8.25	7.70	1.99
138	9.00	8.90	4.75	8.25	7.73	2.07

APPENDIX 3.54

LESION INDEX CURVE

Expt. III DRECHSLERA 6C FIRST INOCULATION (Cf: Fig.3.4)

HOURS AFTER INOCULATION	LESION INDEX				MEAN	ST. DEVI-ATION
	REP I	REP II	REP III	REP IV		
12	0	0	0	0	0	0
16	6.50	6.00	4.00	6.00	5.63	1.12
21	8.00	7.00	7.00	7.25	7.31	0.47
24	8.75	7.25	8.50	7.30	7.95	0.79
40	8.90	7.30	8.60	7.50	8.08	0.79
72	8.95	7.30	8.75	7.60	8.15	0.82
111	9.25	7.60	8.95	7.70	8.35	0.83

DRECHSLERA 6C SECOND INOCULATION

10	0	0	0	0	0	0
17	4.50	3.75	3.00	3.50	3.69	0.62
24	7.25	7.10	6.95	6.75	7.01	0.21
48	8.00	7.80	7.90	8.55	8.26	0.49
66	8.90	7.90	8.00	8.65	8.36	0.49
87	8.95	8.00	8.10	8.70	8.44	0.46
114	9.00	8.10	8.10	8.75	8.49	0.46
136	9.10	8.15	8.15	8.80	8.55	0.48

APPENDIX 3.55

LESION INDEX CURVE

Expt. III — FUSARIUM 1 FIRST INOCULATION — (Cf: Fig. 3.5)

(WITHOUT HUMIDIFICATION)

HOURS AFTER INOCULATION	LESION INDEX				MEAN	ST. DEVI-ATION
	REP I	REP II	REP III	REP IV		
10	0	0	0	0	0	0
16	2.50	2.00	2.00	3.50	2.50	0.71
24	4.00	3.75	4.35	3.75	3.99	0.26
41	7.50	7.25	7.50	6.50	7.19	0.47
64	8.50	8.25	8.45	7.85	8.26	0.30
89	8.60	8.35	8.60	7.95	8.38	0.31
112	8.70	8.50	8.75	8.10	8.51	0.30
137	8.85	8.60	8.85	8.20	8.63	0.31

FUSARIUM 1 SECOND INOCULATION

(WITHOUT HUMIDIFICATION)

8	0	0	0	0	0	0
16	3.75	3.50	3.50	3.65	3.60	0.12
24	6.20	6.20	6.35	5.00	5.91	0.62
42	7.30	7.95	7.30	7.20	7.44	0.34
64	7.65	8.10	7.55	7.40	7.68	0.30
96	7.85	8.25	7.80	8.20	8.03	0.23
118	7.90	8.30	7.83	8.25	8.07	0.24
136	7.95	8.40	8.84	8.25	8.11	0.26

APPENDIX 3.56

LESION INDEX CURVE

Expt. III — FUSARIUM 4 FIRST INOCULATION — (Cf: Fig.3.6)

(WITHOUT HUMIDIFICATION)

HOURS AFTER INOCULATION	LESION INDEX				MEAN	ST. DEVI-ATION
	REP I	REP II	REP III	REP IV		
8	0	0	0	0	0	0
11	0	0	0	3.75	0.94	1.87
37	8.25	7.75	6.75	8.00	7.69	0.66
48	8.35	7.80	6.78	8.70	7.91	0.84
64	8.95	7.85	7.85	8.75	8.35	0.58
89	9.00	7.90	7.90	8.75	8.39	0.57
112	9.10	7.95	8.00	8.80	8.46	0.58
137	9.25	8.15	8.15	8.95	8.63	0.56

FUSARIUM 4 SECOND INOCULATION

(WITHOUT HUMIDIFICATION)

10	0	0	0	0	0	0
15	3.00	3.20	2.25	2.75	2.80	0.41
24	6.00	6.10	4.80	5.75	5.46	0.99
42	6.95	6.75	6.75	6.25	6.68	0.30
64	7.85	7.50	7.75	7.75	7.71	0.15
90	8.30	8.00	7.95	8.20	8.11	0.17
112	8.40	8.07	8.00	8.30	8.18	0.20
144	8.45	8.01	8.10	8.35	8.23	0.21

APPENDIX 3.57

LESION INDEX CURVE

Expt. III — FUSARIUM 4 FIRST INOCULATION — (Cf: Fig. 3.7)

HOURS AFTER INOCULATION	LESION INDEX				MEAN	ST. DEVIATION
	REP I	REP II	REP III	REP IV		
10	0	0	0	0	0	0
16	0	0.5	1.00	0	0.38	0.48
41	4.01	4.25	6.50	4.00	4.69	1.21
64	6.00	7.00	8.00	5.50	6.63	1.12
90	6.85	7.20	8.25	6.50	7.20	0.76
112	6.95	7.35	8.35	6.70	7.34	0.73
137	7.00	7.45	8.40	6.75	7.40	0.73

FUSARIUM 4 SECOND INOCULATION

16	0	0	0	0	0	0
24	2.00	0.50	0.75	1.50	1.19	0.69
48	4.00	2.50	3.00	3.90	3.35	0.75
64	4.15	2.60	3.10	4.10	3.49	0.76
96	4.20	2.75	3.15	4.15	3.56	0.73
118		UNCHANGED			3.56	0.73
136		UNCHANGED			3.56	0.73

APPENDIX 3.58

LESION INDEX CURVE

Expt. III ENTYLOMA LINEATUM FIRST INOCULATION (Cf: Fig.3.8)

HOURS AFTER INOCULATION	LESION INDEX				MEAN	ST. DEVI-ATION
	REP I	REP II	REP III	REP IV		
16	0	0	0	0	0	0
24	0	0	0	0	0	0
36	3.50	2.88	1.50	3.25	2.81	0.90
40	4.25	3.00	3.25	4.00	3.63	0.60
61	4.50	3.00	3.30	4.25	3.76	0.72
88	4.55	3.00	3.40	4.30	3.81	0.63
113	UNCHANGED				3.81	0.63

ENTYLOMA LINEATUM SECOND INOCULATION

10	0	0	0	0	0	0
18	0.75	0.50	0.75	1.00	0.75	0.20
24	3.20	2.50	2.25	2.75	2.68	0.41
39	4.25	3.50	2.75	3.10	3.40	0.64
66	4.35	3.65	2.85	3.25	3.53	0.64
90	4.40	3.66	2.85	3.30	3.55	0.65
114	UNCHANGED				3.55	0.65
136	UNCHANGED				3.55	0.65

APPENDIX 3.59

THE LESION INDEX

Expt. III GREENHOUSE SPRAY INOCULATIONS (Cf: Fig. 3.9)

(Final Lesion Indices of the Lesion Index Curves)

	ISOLATE	LESION INDEX				MEAN	ST. DEVI-ATION
		REP I	REP II	REP III	REP IV		
1	D. BICOLOR	9.36	9.20	9.75	9.45	9.44	0.22
2	DRECHSLERA 1	8.80	9.50	7.20	7.60	8.28	1.06
3	DRECHSLERA 5L	9.00	8.90	4.75	8.25	7.73	2.01
4	DRECHSLERA 6C	9.10	8.15	8.15	8.80	8.55	0.48
5	FUSARIUM 1 (spray DRY)	7.95	8.40	7.84	8.25	8.11	0.26
6	FUSARIUM 4 (spray DRY)	8.45	8.01	8.10	8.35	8.23	0.21
7	FUSARIUM 4	4.20	2.75	3.15	4.15	3.56	0.73
8	E. LINEATUM	4.40	3.66	2.85	3.30	3.55	0.65

The controls were virtually free of lesions so that they were each given an arbitrary lesion index of 2.0 for purposes of conducting an Analysis of Variance (Cf: Appendix 3.8).

APPENDIX 3.6

CORRELATION COEFFICIENT

Expt. III SEED YIELD AND LESION INDEX (Cf: Fig.3.11)

		SIGNIF. AT 10%		SIGNIF. AT 5%		SIGNIF. AT 1.0%		SIGNIF. AT 0.1%	
DEGREES OF FREEDOM	ZERO ORDER CORR. COEF.	*SIG-NIFI-CANCE	LOWEST SIG. CORR. COEF.	*SIG-NIFI-CANCE	LOWEST SIG. CORR. COEF.	*SIG-NIFI-CANCE	LOWEST SIG. CORR. COEF.	*SIG-NIFI-CANCE	LOWEST SIG. CORR. COEF.
8	0.88732	+	0.5494	+	0.6319	+	0.7646	+	0.8721

* + = Significance; - = Nonsignificance

APPENDIX 3.7

Expt. III

THE F RATIOS OF THE SEED YIELDS

(Cf: Fig.3.10)

	ISOLATE (and method of inoculation)	F RATIO*	SIGNIF.AT 5%		SIGNIF.AT 1.0%		SIGNIF.AT 0.1%	
			**SIG-NIFI-CANCE	LOWEST SIG. F RATIO	SIG-NIFI-CANCE	LOWEST SIG. F RATIO	SIG-NIFI-CANCE	LOWEST SIG. F RATIO
1	D. BICOLOR (spray)	14.1723	+		+		-	
2	D. BICOLOR (toothpick)	2.9069	-		-		-	
3	DRECHSLERA 1 (spray)	3.4210	-		-		-	
4	DRECHSLERA 1 (toothpick)	13.6266	+		-		-	
5	DRECHSLERA 5L (spray)	7.6264	+		-		-	
6	DRECHSLERA 5L (toothpick)	1.1750	-	5.99	-	13.74	-	35.51
7	DRECHSLERA 6C (spray)	5.0530	-		-		-	
8	DRECHSLERA 6C (toothpick)	6.4201	+		-		-	
9	FUSARIUM 1 (spray DRY)	1.6903	-		-		-	
10	FUSARIUM 4 (spray DRY)	7.4599	+		-		-	
11	FUSARIUM 4 (spray)	1.8118	-		-		-	
12	E. LINEATUM (spray)	1.5833	-		-		-	
13	E. LINEATUM (spray+inject)	0.1135	-		-		-	

* Degrees of Freedom $n_1=1$; $n_2=6$ ** + = Significance; - = Nonsignificance

APPENDIX 3.8

Expt. III — THE F RATIOS OF THE LESION INDICES — (Cf: Fig.3.10)

	ISOLATE (and method of inoculation)	F RATIO*	SIGNIF.AT 5%		SIGNIF.AT 1.0%		SIGNIF.AT 0.1%	
			**SIG-NIFI-CANCE	LOWEST SIG. F RATIO	SIG. NIFI-CANCE	LOWEST SIG. F RATIO	SIG. NIFI-CANCE	LOWEST SIG. F RATIO
1	D. BICOLOR (spray)	4146.3370	+		+		+	
2	DRECHSLERA 1 (spray)	139.5062	+		+		+	
3	DRECHSLERA 5L (spray)	32.4190	+		+		+	
4	DRECHSLERA 6C (spray)	752.0157	+	5.99	+	13.74	+	33.51
5	FUSARIUM 1 (spray DRY)	2215.5548	+		+		+	
6	FUSARIUM 4 (spray DRY)	3641.4788	+		+		+	
7	FUSARIUM 4 (spray)	18.5235	+		+		-	
8	E. LINEATUM (spray)	22.4731	+		+		-	

The high F Ratios here compared to Expt. II, Fig. 2.4 are owing to the fact that lesions were more or less completely absent from control plants. These were each given an arbitrary lesion index of 2.0 for the purpose of conducting the Analyses of Variance.

* Degrees of Freedom $n_1=1$; $n_2=6$ ** + = Significance; - = Nonsignificance

APPENDIX 3.9

Expt. IV — THE F RATIOS OF THE SEED YIELDS — (Cf: Fig.3.13)

	ISOLATE	F RATIO*	SIGNIF.AT 5%		SIGNIF.AT 1.0%		SIGNIF.AT 0.1%	
			**SIG-NIFI-CANCE	LOWEST SIG. F RATIO	SIG-NIFI-CANCE	LOWEST SIG. F RATIO	SIG-NIFI-CANCE	LOWEST SIG. F RATIO
1	D. BICOLOR	6.2421	+		-		-	
2	DRECHSLERA 1	18.7931	+		+		-	
3	DRECHSLERA 5L	22.1506	+		+		-	
4	DRECHSLERA 6C	20.2995	+	5.99	+	13.74	-	35.51
5	FUSARIUM 1	26.7699	+		+		-	
6	FUSARIUM 4	34.1712	+		+		-	
7	E. LINEATUM	0.7977	-		-		-	
8	CONTROL (inject water)	0.1592	-		-		-	

* Degrees of Freedom $n_1=1$; $n_2=6$ ** + = Significance; - = Nonsignificance

APPENDIX 4.1

Expt. VI THE BACTERIAL FREQUENCY (Cf: Fig.4.1)

	ISOLATE** (inoc. onto parental plts.)	NO. OF HALF SEEDS PER SAMPLE OF 8 SHOWING THE PRESENCE OF BACTERIA		MEAN NO.	ST. DEVIATION
		REP I	REP II		
1	DRECHSLERA 1	4 6 4 7	4 6 5 7	5.37	1.30
2	DRECHSLERA 2	7 4 4 6	7 3 8 4	5.37	1.84
3	DRECHSLERA 3	8 6 8 3	8 3 8 3	5.87	2.47
4	DRECHSLERA 4	4 3 5 3	6 3 7 2	4.12	1.72
5	D. BICOLOR	7 8 7 8	7 8 7 8	7.50	0.52
6	ALTERNARIA 1	7 8 7 8	7 8 7 7	7.37	0.50
7	ALTERNARIA 2	5 6 4 6	3 7 3 8	5.25	1.83
8	ALTERNARIA 3+GONATOBOTRYS SIMPLEX	7 8 7 8	7 7 4 8	6.87	1.24
9	E. LINEATUM	4 4 8 4	8 5 8 8	6.12	2.02
10	E. LINEATUM (inject culm)	8 0 8 0	8 0 8 0	4.50	3.96
11	E. LINEATUM (inject infl. st.)	8 8 8 8	8 8 8 8	8.00	0
12	PEYRONELLAEA SP.	3 8 4 8	4 5 0 8	5.00	2.87
13	FUSARIUM 1	6 8 7 8	3 8 4 8	6.50	2.00
14	FUSARIUM 2	0 7 0 4	0 6 4 8	3.25	3.01
15	FUSARIUM 3	3 6 3 5	7 5 6 6	5.12	1.45
16	unidentified fungus #1	7 8 7 8	7 8 7 7	7.37	0.50
17	unidentified fungus #2	2 2 3 6	2 5 2 6	3.50	1.84
18	unidentified fungus #2 (inject culm)	3 6 4 7	5 7 5 8	4.62	2.32
19	CONTROL 1	1 8 0 8	8 6 1 8	5.00	3.66
20	CONTROL 2	6 7 2 8	4 8 7 8	6.25	2.18
21	CONTROL 3	6 6 7 6	7 7 6 7	6.50	0.52
22	CONTROL 4	4 6 1 5	7 5 6 6	4.76	1.97

** spray inoc. unless otherwise stated

APPENDIX 4.2

Expt. VI THE MUCORALEAN FREQUENCY (Cf: Fig.4.2)

	ISOLATE** (inoc. onto parental plts.)	NO. OF HALF SEEDS PER SAMPLE OF 8 SHOWING THE PRESENCE OF MUC. REP I	REP II	MEAN NO.	ST. DEVI-ATION
1	DRECHSLERA 1	3 0 3 1	5 1 5 1	2.37	1.92
2	DRECHSLERA 2	0 0 0 0	0 0 0 1	0.12	0.34
3	DRECHSLERA 3	2 1 1 2	0 0 1 0	0.87	0.83
4	DRECHSLERA 4	4 0 2 0	2 1 2 0	1.37	1.40
5	D. BICOLOR	2 0 3 0	4 0 4 0	1.62	1.84
6	ALTERNARIA 1	3 0 3 0	3 0 4 0	1.62	1.76
7	ALTERNARIA 2	1 0 4 0	2 0 2 0	1.12	1.45
8	ALTERNARIA 3+GONATOBOTRYS SIMPLEX	0 0 0 0	0 0 0 0	0	0
9	E. LINEATUM	0 0 0 0	0 1 0 0	1.14	0.37
10	E. LINEATUM (inject culm)	2 0 2 2	3 0 0 0	1.12	1.24
11	E. LINEATUM (inject infl. st.)	5 0 3 0	5 0 5 0	2.25	2.49
12	PEYRONELLAEA SP.	0 0 0 0	0 0 0 0	0	0
13	FUSARIUM 1	0 0 0 0	0 0 0 0	0	0
14	FUSARIUM 2	2 0 1 0	4 0 2 0	1.06	1.42
15	FUSARIUM 3	0 0 0 0	0 0 0 0	0	0
16	unidentified fungus #1	3 0 4 0	5 0 2 0	1.75	2.04
17	unidentified fungus #2	3 0 5 0	1 0 3 1	1.62	1.84
18	unidentified fungus #2 (inject culm)	0 0 0 0	0 0 0 0	0	0
19	CONTROL 1	0 0 0 0	0 0 0 0	0	0
20	CONTROL 2	0 3 0 3	0 4 0 3	1.62	1.76
21	CONTROL 3	0 0 0 0	0 0 0 0	0	0
22	CONTROL 4	0 0 0 0	0 0 0 0	0	0

** spray inoc. unless otherwise stated

APPENDIX 4.3

Expt. VI — THE FUNGAL FREQUENCY (EXCLUDING MUC.) — (Cf: Fig. 4.3)

	ISOLATE** (inoc. onto parental plts.)	NO. OF HALF SEEDS PER SAMPLE OF 8 SHOWING THE PRESENCE OF FUNGI		MEAN NO.	ST. DEVI-ATION
		REP I	REP II		
1	DRECHSLERA 1	0 4 0 3	0 5 1 5	2.25	2.25
2	DRECHSLERA 2	4 5 6 7	8 6 7 6	6.12	1.24
3	DRECHSLERA 3	2 3 0 5	0 6 5 7	3.50	2.67
4	DRECHSLERA 4	0 0 4 2	0 0 0 2	1.00	1.50
5	D. BICOLOR	3 0 4 0	1 0 2 0	1.25	1.58
6	ALTERNARIA 1	5 3 3 4	4 4 1 2	3.25	1.28
7	ALTERNARIA 2	7 1 1 0	2 0 3 0	1.75	2.37
8	ALTERNARIA 3+GONATOBOTRYS SIMPLEX	0 1 0 1	1 2 0 0	0.62	0.74
9	E. LINEATUM	0 1 0 6	0 4 0 5	2.00	2.56
10	E. LINEATUM (inject culm)	2 6 2 3	2 7 8 5	4.37	2.44
11	E. LINEATUM (inject inf. st.)	4 5 6 8	3 8 3 5	5.25	1.97
12	PEYRONELLAEA SP.	0 0 0 0	0 0 0 0	0	0
13	FUSARIUM 1	6 6 5 8	6 8 3 7	6.12	1.64
14	FUSARIUM 2	5 1 4 0	2 0 6 0	2.00	2.06
15	FUSARIUM 3	4 0 0 1	3 1 4 0	3.00	1.19
16	unidentified fungus #1	1 1 1 2	1 1 1 1	1.12	0.34
17	unidentified fungus #2	1 0 0 0	4 0 1 0	0.75	1.38
18	unidentified fungus #2 (inject culm)	2 0 5 0	5 0 3 0	1.87	2.23
19	CONTROL 1	7 5 1 4	0 8 1 3	5.00	3.66
20	CONTROL 2	3 5 2 7	0 5 5 5	4.00	2.20
21	CONTROL 3	0 0 1 0	1 0 0 0	0.25	0.45
22	CONTROL 4	8 0 8 0	8 0 8 0	4.00	4.27

** spray inoculated unless otherwise stated

APPENDIX 4.4

CORRELATION COEFFICIENTS

Expt. VI and II — MEAN BACTERIAL FREQUENCY AND SEED GERMINABILITY — (Cf: Fig.4.1)

DEGREES OF FREEDOM	ZERO ORDER CORR. COEF.	*SIG-NIFI-CANCE	LOWEST SIG. CORR. COEF. (SIGNIF. AT 10%)	*SIG-NIFI-CANCE	LOWEST SIG. CORR. COEF. (SIGNIF. AT 5%)	*SIG-NIFI-CANCE	LOWEST SIG. CORR. COEF. (SIGNIF. AT 1.0%)	*SIG-NIFI-CANCE	LOWEST SIG. CORR. COEF. (SIGNIF. AT 0.1%)
20	0.42941	+	0.3598	+	0.4227	-	0.5368	-	0.6524

Expt. VI and II — MEAN MUCORALEAN FREQUENCY AND SEED GERMINABILITY — (Cf: Fig.4.2)

20	0.06484	-	0.3598	-	0.4227	-	0.5368	-	0.6524

Expt. VI and II — MEAN FUNGAL FREQUENCY (Excluding Mucorales) AND SEED GERMINABILITY — (Cf: Fig.4.3)

20	0.08663	-	0.3598	-	0.4227	-	0.5368	-	0.6524

* + = Significance; - = Nonsignificance

APPENDIX 4.5

CORRELATION COEFFICIENTS

Expt. VI and II — MEAN BACTERIAL FREQUENCY AND SEED YIELD

DEGREES OF FREEDOM	ZERO ORDER CORR. COEF.	SIGNIF. AT 10% *SIG-NIFI-CANCE	SIGNIF. AT 10% LOWEST SIG. CORR. COEF.	SIGNIF. AT 5% *SIG-NIFI-CANCE	SIGNIF. AT 5% LOWEST SIG. CORR. COEF.	SIGNIF. AT 1.0% *SIG-NIFI-CANCE	SIGNIF. AT 1.0% LOWEST SIG. CORR. COEF.	SIGNIF. AT 0.1% *SIG-NIFI-CANCE	SIGNIF. AT 0.1% LOWEST SIG. CORR. COEF.
20	0.01423	-	0.3598	-	0.4227	-	0.5368	-	0.6524

Expt. VI and II — MEAN MUCORALEAN FREQUENCY AND SEED YIELD (Cf: Fig. 4.4)

20	0.42039	+	0.3598	-	0.4227	-	0.5368	-	0.6524

Expt. VI and II — MEAN FUNGAL FREQUENCY (Excluding Mucorales) AND SEED YIELD

20	0.07569	-	0.3598	-	0.4227	-	0.5368	-	0.6524

* + = Significance; - = Nonsignificance

APPENDIX 4.6

CORRELATION COEFFICIENTS

Expt. VI and II — MEAN BACTERIAL FREQUENCY AND LESION INDEX

DEGREES OF FREEDOM	ZERO ORDER CORR. COEF.	SIGNIF. AT 10% *SIG-NIFI-CANCE	SIGNIF. AT 10% LOWEST SIG. CORR. COEF.	SIGNIF. AT 5% *SIG-NIFI-CANCE	SIGNIF. AT 5% LOWEST SIG. CORR. COEF.	SIGNIF. AT 1.0% *SIG-NIFI-CANCE	SIGNIF. AT 1.0% LOWEST SIG. CORR. COEF.	SIGNIF. AT 0.1% *SIG-NIFI-CANCE	SIGNIF. AT 0.1% LOWEST SIG. CORR. COEF.
20	0.01483	-	0.3598	-	0.4227	-	0.5368	-	0.6524

Expt. VI and II — MEAN MUCORALEAN FREQUENCY AND LESION INDEX

20	0.23138	-	0.3598	-	0.4227	-	0.5368	-	0.6524

Expt. VI and II — MEAN FUNGAL FREQUENCY (Excluding Mucorales) AND LESION INDEX

20	0.04857	-	0.3598	-	0.4227	-	0.5368	-	0.6524

* + = Significance; - = Nonsignificance

APPENDIX 4.7

DORMANCY STUDIES

The After Ripening of Wild Rice Seed

The dormancy of wild rice seed prevents the immediate use of new seed for experimentation in the fall. It has to be stored in conditions of water saturation for about 4½ to 5 months before appreciable quantities can be germinated. This was demonstrated in the following experiment.

Wild rice seed (Algot Johnson strain) was obtained from the tillers of greenhouse grown plants. It was divided into 2 samples and stored under different conditions.

Sample 1

Ten 100 g subsamples in water in sealed polyethylene bags were frozen by storage in a freezer at $-10^{o}C$.

Sample 2

2000 g were stored in tap water at $2^{o}C$ in a polystyrene tank.

Approximately 150 seeds were withdrawn from each sample for germination tests at intervals. Each seed sample was divided in 4 glass petri plates containing nonsterile tap water, 35 to 40 seeds per plate. The plates were incubated

in a growth chamber with a 14 hour day of incandescent and florescent lighting at 22°C and a night temperature at 17°C. After 10 days the samples were removed and their germinabilities determined by counting the number of germinated and ungerminated seeds.

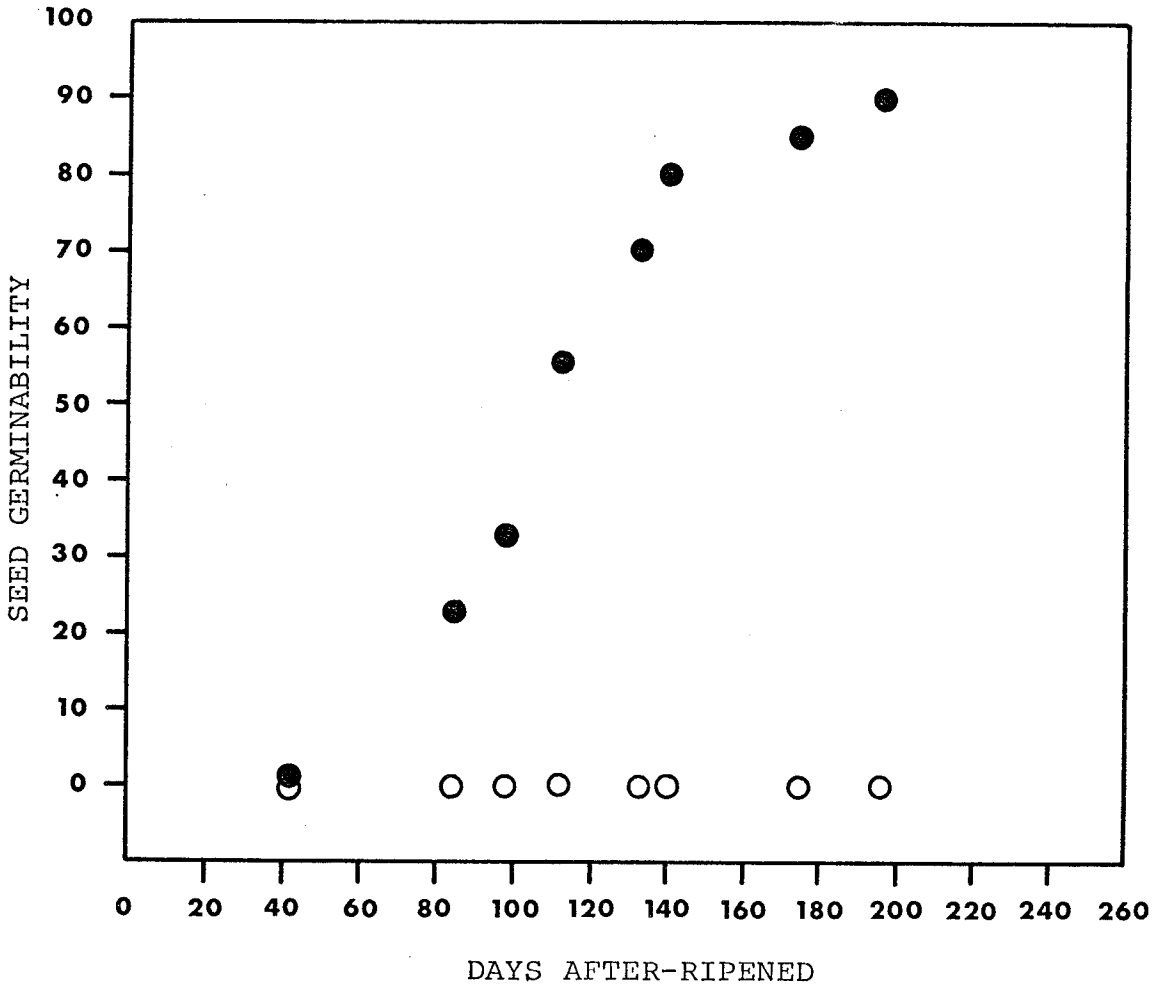
None of the seed stored in ice germinated at any time during the experiment. However, sample 2 showed progressively better germinability with each germination test. The results are presented graphically in Fig. 5.

It is probable that storage in ice resulted in dehydration of the seed and consequently a loss in viability. The very high germination rate reached after 7 months storage was not expected, however. Less than 10 per cent of the seed remained ungerminated. In natural stands it has been assumed that a moderately substantial portion of the seed remains dormant in spring for a further 12 months. The fact that wild rice stands are known to recover in the following season after plants in the floating leaf stage have been killed by flooding in early spring (p. 83) provides circumstantial evidence for the presence of reserve dormant seed for growth the following year. It is conjectured that the Algot Johnson strain of wild rice used in this experiment may differ from the natural varieties in this respect, unless in natural stands less than 10 per cent is enough to ensure growth the next season. Alternatively, the strong

FIG. 5.1

The germinability of wild rice seed samples (Algot Johnson type) after-ripened in water and ice for various storage periods.

FIG. 5.1


SEED GERMINABILITY
100
90
80
70
60
50
40
30
20
10
0
0
20
40
60
80
100
120
140
160
180
200
220
240
260
DAYS AFTER-RIPENED
● : STORAGE IN WATER AT 2°C
○ : STORAGE IN ICE AT -10°C

lighting conditions in combination with the ideal temperatures may have in some way caused germination of seed which under natural conditions might have remained dormant. In nature the seed is commonly buried partially or completely in lake floor mud in conditions of relatively poor light and in temperatures which fluctuate relatively slowly. This buffering effect afforded by the ambient mud and lake water contrasts with the experimental conditions where the transition from dormancy to conditions of seed growth amounted to a temperature and light shock treatment. In view of the findings by the author concerning the ultrasonication of wild rice seed, it is likely that the temperature shock treatment is an important factor; it is possible that a light factor related to the activation of seed phytochrome may also be operative.

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