CHARACTERISTICS OF TWO POPULATIONS OF *FUSARIUM ROSEUM* 'GRAMINEARUM' IN EASTERN AUSTRALIA

by

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PREFACE

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The work reported in this thesis is original and my own, except where specifically noted in the text.

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ABSTRACT

1. *Fusarium roseum* 'Graminearum' was the predominant fungus associated with stalk rot of maize in eastern Australia in the 1972, 1973 and 1974 growing seasons. All isolates of this pathogen were of the Group 2 type. Thus Group 2 contrasts with Group 1 which is normally isolated from crown rot of wheat and grasses.

Other fungi isolated in order of frequency were *Diplodia maydis*, *F. moniliforme* 'Subglutinans', *Bipolaris sorokiniana*, *Nigrospora oryzae*, *F. roseum* 'Semitectum', *F. moniliforme*, *F. roseum* 'Equiseti', *F. roseum* 'Concolor', *Macrophomina phaseolina*, *Rhizoctonia* sp., *F. roseum* 'Acuminatum', *F. oxysporum*, *F. solani*, *F. tricinctum* and *F. roseum* 'Heterosporum'. The relative isolation frequencies of the fungi varied according to the seasonal conditions. Stalk rots were not of major importance in 1973, a relatively dry growing season. However, in 1974, a wet growing season, stalk rot diseases were common in all areas investigated.

2. Isolates of *F. roseum* 'Graminearum', derived mainly from wheat and maize but also from other sources and from various regions of eastern Australia, were examined for perithecia formation, colony characteristics, fertility, colony growth, conidia production and conidia size. The distribution of the fungus in field colonized maize and wheat plants was also studied.

The Group 1 isolates did not produce perithecia, were heterothallic and very infertile, had a mean colony growth of 4.4 cm per 3 days (range, 3.9-5.1) and produced relatively large numbers of conidia. In contrast,

Group 2 isolates were homothallic and produced perithecia readily, had a mean colony growth of 5.4 cm per 3 days (range, 4.7-6.1) and produced relatively low numbers of conidia.

Group 1 isolates were found to be commonly associated with crowns and roots of plants and Group 2 isolates were commonly associated with aerial plant parts.

3. The ability of a number of Group 1 and Group 2 isolates to produce the fungal hormone, zearalenone was assessed. Group 1 isolates produced three to four times more zearalenone than Group 2 isolates. In addition, a culture which had previously produced perithecia but had lost that ability following numerous transfers, produced no detectable zearalenone. The results provided good evidence that the observed difference in perithecia formation was directly related to the ability to produce zearalenone.

4. The pathogenicity to wheat, maize and carnations of Group 1 isolates from crown rot affected wheat plants and Group 2 isolates from stalk rot affected maize plants was tested. Pathogenicity of 11 other isolates from teosinte, carnations, pearl millet, wheat and barley scab, banana, ginger and common wheat grass was also assessed. The results indicated that pathogenic specialization exists within *F. roseum* 'Graminearum'. Wheat isolates were the most pathogenic to wheat, carnation isolates were the most pathogenic to carnations and all maize isolates were pathogenic to maize while those from wheat and common wheat grass were not as pathogenic to maize. Moreover, Group 2 isolates were more pathogenic when inoculated in aerial

plant parts , and the Group 1 isolates were more pathogenic when inoculated in plant parts in soil . Inoculations on wheat seedlings in sterile field soil demonstrated that the inherent pathogenicity to wheat seedlings of isolates from wheat and maize were similar.

5. Some factors which could contribute to the observed pathogenic differences between isolates from wheat and maize to wheat seedlings in field soil were examined. . Conidia volume, germination rate and inherent germinability in the soil were studied. The Group 1 isolates had the largest volume, the most rapid germination and the highest inherent germinability. Pathogenicity was positively correlated with conidium volume and inherent germinability. In addition, the inherent germinability and conidium volume were positively correlated. Thus, it was established that pathogenic behaviour of conidia of Group 1 and Group 2 reflected differences in conidia morphology.

TABLE OF CONTENTS

INTRODUCTION

The fungus *Fusarium roseum* sensu Snyder and Hansen 'Graminearum' is a member of the complex species *F. roseum* (Snyder and Hansen, 1945). Alternatively it has been classified as *Fusarium graminearum* Schw. (Wollenweber and Reinking, 1935; Booth, 1971). This fungus causes seedling blight of many gramineous species (Dickson, 1947; Cook, 1968), crown rot of wheat (*Triticum aestivum* L.) and other grasses (McKnight and Hart, 1966; Cook, 1968), stalk rot of many crops, particularly maize (*Zea mays* L.)(Edwards, 1936; Dickson, 1947; Kingsland and Wernham, 1962; Rintelin, 1967), head scab of most cereals (Tu, 1929; Anderson, 1948; Schroeder and Christensen, 1963) and stub die-back of carnations (*Dianthus caryophyllus* L.)(Tammen, 1958; Dorworth and Tammen, 1969; Nelson, Pennypacker, Toussoun and Horst, 1975). In addition, it has been isolated from various plant parts of other genera such as *Coffea, Lycopersicon, Pisum, Trifolium and Solanum* (Sprague, 1950; Simmonds, 1966; Booth, 1971). Although the fungus has a wide host range, it incites only two disease syndromes, air-borne diseases of above ground stalks and heads and a soil-borne disease of crowns and roots. These diseases caused by *F. roseum* 'Graminearum' result in significant reductions in yield and grain quality and have been reported in Africa, Australia, China, Europe, India, Japan, Korea, New Zealand, North and South America and the U.S.S.R. (Booth, 1971). Consequently this pathogen has been the subject of considerable research in many countries (Tu, 1929; Bennett, 1931; Eide, 1935; Oswald, 1949; Garrett, 1956, 1963; Butler, 1961; Colhoun and Park, 1964; Burgess, 1967; Purss, 1969, 1971; Mesterhazy, 1974).

Studies on the pathogenicity of *F. roseum* 'Graminearum' to various hosts have consistently demonstrated a significant degree of variability between isolates (Tu, 1929; Eide, 1935; Ullstrup, 1935; Nisikado, Matsumoyo and Yaminti, 1935; Oswald, 1949) and the environmental conditions for maximum disease expression (Dickson, 1923; Bennett, 1931). However, these authors did not obtain evidence for physiological specialization in *F. roseum* 'Graminearum' and considered that the variability was an expression of the normal variability within a population. No attempt was made by these authors to correlate pathogenicity of cultures to host of origin or the disease syndrome from which the cultures were isolated.

Some workers have attempted to relate pathogenicity to host of origin. However, the results of cross-inoculation experiments with isolates from wheat and maize (Koehler, Dickson and Holbert, 1924), wheat and rice (*Oryza sativa* L.)(Ikeya, 1933) and wheat and carnations (Tammen, 1958), indicated that there was no physiological specialization in *F. roseum* 'Graminearum'. In contrast, Purss (1969, 1971) found evidence that severe crown rot of wheat was caused by isolates from crown rot affected wheat plants but not by isolates from stalk rot of maize. Further, isolates from both wheat and maize were equally pathogenic to maize plants. Purss concluded that physiological specialization existed within *F. roseum* 'Graminearum'.

Variability amongst isolates of *F. roseum* 'Graminearum' is not confined to pathogenicity but has been observed in respect to perithecia formation, another ecologically important characteristic. The ability of some isolates to form the perfect state (*Gibberella zeae* (Schw.) Petch) while others cannot, was observed by Adams (1921). He reported that although isolates from maize readily formed perithecia, a culture from wheat did not. Similarly, Eide (1935) reported that some cultures of *F. roseum* 'Graminearum' were able to form perithecia while others could not. Hynes (1924) described in detail two cultures, one from oat crown rot in Australia and one from the U.S.A. The Australian isolate was unable to produce perithecia in culture, however the one from the U.S.A. was able to do so within 10 to 14 days. Purss (1969) working in Queensland, noted a relationship of perithecia formation to host plant. He found isolates from stalk rot of maize readily formed perithecia in the field and in culture, however those from crown rot of wheat did not.

The degree of variability in cultural morphology, particularly colour and colony type, has also been examined by several workers. Hynes (1924) found that the two cultures which he studied differed in colony colour and cultural form. Eide (1935) and Ullstrup (1935) both reported variation in colony colour and form, particularly between cultures which had been continually sub-cultured.

An extensive research programme concerning the ecology, physiology and taxonomy of the complex species *F. roseum* is being undertaken at The University of Sydney. Included in this programme is a detailed study of

F. roseum 'Graminearum'. The results of preliminary investigations by Burgess (pers. comm.) supported Purss' contention that pathogenic specialization existed within this organism. It was also found that isolates from wheat stem bases affected by crown rot, did not form perithecia in culture, whereas those from maize stems affected by stalk rot, formed perithecia on suitable media. I therefore initiated a study on the nature of variability within *F. roseum* 'Graminearum' to determine whether these differences in pathogenic ability and the ability to form perithecia could be related to the existence of discrete populations within the fungus.

The concept that an organism, such as *F. roseum* 'Graminearum' may consist of discrete populations was recognized by Turessen (1930). He concluded that:

a) widely distributed species exhibit variation which varies from place to place within their habitat,

b) this variation can be largely correlated with observable habitat differences, and

c) the correlated variation was the result of natural selection of particular genotypes from the pool of genetic variability within a species.

Thus, Turessen (1930) presented evidence that variability within an organism was not necessarily random but related to the existence of discrete populations. This does not imply that members within a population are uniform but rather that the variability between populations is of a greater order of magnitude than variability within populations. Hamilton (1967) defined such populations, selected on an ecogeographical basis, as clines*. Thus if populations can be defined within an organism, they will probably reflect differences in the econiches occupied.

The concept that taxonomic categories consist of discrete populations is a principle that has often eluded workers in mycology (Hudson, 1970). Most studies on fungal variability have focused attention on variability between individual isolates rather than between populations. However, populations (micro-evolutionary units) have been shown to exist within several fungal species. Duncan and McDonald (1967) were able to distinguish these populations in *Auricularia auricula* (Hook) Underw. based on host of origin, spore morphology and interfertility of geographically isolated populations. Loveless (1971) distinguished populations of *Claviceps purpurea* (Fr. ex Fr.)Tul. based on conidia size which he found was related to host of origin. Shepherd and Pratt (1973) found isolates of *Phytophthora drechsleri* Tucker from several geographic regions which differed in respect to growth rate. The role of geographic isolation as a selective mechanism which contributes to the development of discrete populations has been well documented (Boughey, 1971).

The adoption of the above concepts as a basis for assessing variability in *F. roseum* 'Graminearum' necessitated the collection of isolates from as many sources as possible and from different environments. Consequently, these studies were preceded by and were concurrent with systematic surveys of the Fusaria associated with maize stalk rot in eastern Australia (Francis and Burgess, 1975) and similar surveys of wheat crown rot in eastern Australia

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* Cline: when a population is exposed to an environmental gradient there will be a corresponding gradient in selection pressure. The subsequent gradients of biotypes or races within a population are known as clines.

(Burgess, Wearing and Toussoun, 1975). This enabled the collection of isolates from diverse geographic zones. From these isolates (approximately 4000), a random selection of cultures was examined in respect to perithecia formation, cultural morphology, colony growth, conidia morphology and pathogenicity to wheat, maize and carnations. Zearalenone production by selected isolates was also measured. These data were then used in a detailed analysis of variability within *F. roseum* 'Graminearum'.