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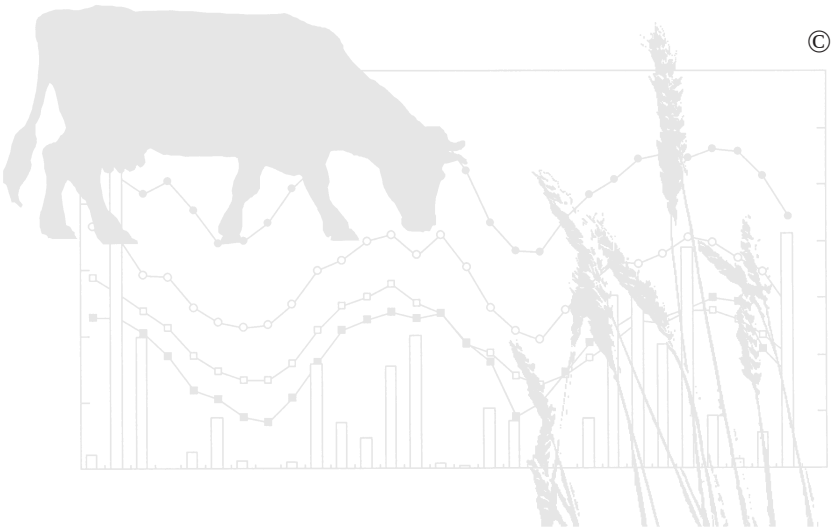

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Effects of plant residue, soil characteristics, cotton cultivars and other crops on fusarium wilt of cotton in Australia

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Summary. The incidence and severity of fusarium wilt of cotton in glasshouse trials increased when levels of plant residue in the soil were increased by the incorporation of whole cotton plants (6-week-old seedlings dried out for a further 6 weeks) into the soil. In non-residue-supplemented potting mix, disease incidence was <50%, but ranged from 74 to >90% in residue-supplemented potting mix. The disease was significantly affected by soil microflora and soil type, but not affected by soil pH in the range 4.0−8.5. Although the same amount of inoculum was added to both autoclaved and untreated soils, the disease was less severe in autoclaved soils than in untreated soils regardless of soil type. Among the 4 clay soils investigated, the disease was less severe in a grey sandy clay and a brown heavy clay than in a dark grey heavy clay. Compared with the soils collected at the end of the 1995–96 growing season, the disease became more severe in the soil of plots planted to the most susceptible cotton cultivar Siokra 1-4, but less severe in the soil of plots planted to the less susceptible cotton cultivar DP90 at the end of the 1996–97 growing season. However, no significant differences were observed in the soil of plots planted to cotton cultivar Siokra L22, sorghum, maize or soybean.

Introduction

Fusarium wilt of cotton (*Gossypium hirsutum* L.), caused by *Fusarium oxysporum* Schlecht. f. sp. *vasinfectum* (Atk.) Snyd. and Hans. (*Fov*), is a relatively new disease in Australia. It was first detected in the Brookstead area of the Darling Downs of Queensland in 1993 and now occurs in Queensland and New South Wales (Kochman *et al*. 1994). There are at least 2 pathotypes, one represented by the isolates obtained from the Darling Downs and the other by those from the Boggabilla area of New South Wales (Kochman 1995). Little is known about their origins. DNA fingerprint analyses showed that these 2 pathotypes were different from each other and distinct from all the overseas races of *Fov* studied so far (S. Bentley pers. comm.). This indicates that they perhaps originated independently from endemic populations of local *Fusarium oxysporum* which adapted and became prominent in response to wide-scale planting of highly susceptible cotton cultivars. Some common characteristics were observed among the isolates from the Darling Downs, including pathogenicity similar to race 6 of *Fov*, production of detectable volatile compounds of a specific odour when grown on a starch substrate, distinctive pigmentation on an aesculin-containing medium and inclusion in a single and unique vegetative compatibility group (Davis *et al*. 1996).

The most effective method of control of the disease has been to grow resistant cultivars (Hillocks 1992). However, most of the current Australian commercial cotton cultivars are very susceptible or only moderately tolerant (Kochman *et al*. 1994). Therefore, additional strategies must be sought to limit the development of the disease in infested fields.

Cotton stalks are usually retained in fields and ploughed into the soil after harvest. In fields infested with fusarium wilt the residue derived from infected plants contains a large number of pathogenic propagules (Trujillo and Snyder 1963), which may serve as a reservoir of the pathogen and contribute to its local population when incorporated into the soil. *Formae speciales* of *Fusarium oxysporum* are able to colonise crop residue (Smith and

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Snyder 1975) and the residue derived from healthy plants can also increase the soil population of *Fov* (Koshkelova and Muratmukhamedov 1971). Removal of plant residue has been suggested as a potential measure to prevent the accumulation of soilborne fungal pathogens in soil (Takeuchi 1987). However, there appear to be no direct investigations concerning the contribution of plant residue to disease development.

It has long been known that fusarium wilt of cotton is influenced by the soil environment. Some races of *Fov* are more prevalent in sandy or loamy soils than in clay soils and the disease is more severe in acidic soils than in neutral or alkaline soils (Hillocks 1992). Most soils known to be suppressive to fusarium wilt are associated with high pH (Scher and Baker 1980) and fusarium wilt of tomato has been effectively controlled by elevating soil pH with lime (Woltz and Jones 1973). In contrast, little work has been conducted on the effect of soil microflora in general although fusarium wilt of cotton is enhanced by the presence of nematodes in some types of soil (Minton and Minton 1966; DeVay *et al.* 1997).

Crop rotation is a well-established strategy for the management of plant diseases caused by soilborne pathogens, aiming to eliminate pathogens by removing potential substrates and encouraging lysis of pathogens by other soil microorganisms (Campbell 1994). It is difficult to make any clear conclusions concerning the effect of crop rotation on fusarium wilt of cotton because it may vary with the crop, the race of *Fov* and the soil environment involved in different situations (Nash and Snyder 1967; Goshaev 1972). Although other crops should be the most appropriate candidates in rotational practice, it is important to investigate the effect of the growth of different cotton cultivars on the disease as cotton is often grown in the same field for several consecutive years.

The current study was undertaken under glasshouse conditions to determine the effects of plant residue, soil microflora, soil type and pH on fusarium wilt of cotton. The influence of the growth of different cotton cultivars or other crops on the process of disease development under field conditions was also examined, by comparing the disease severities of plants grown under glasshouse conditions in field soils.

Materials and methods

Soils with different levels of plant residue incorporated

Soil collected from an infested field in the Cecil Plains area of the Darling Downs of Queensland, where serious disease and high levels of *Fov* had been observed, was used as the inoculum of *Fov* in this experiment. It was air-dried at 20–25°C for 1 week, ground and sieved through a 2 cm screen. Potting mix (Baker 1957) was inoculated by adding the soil at a weight : weight ratio of 1 soil : 19 potting mix and mixing thoroughly in a rotating cement mixer and then distributed into 15 cm plastic pots.

The potting mix in each pot was sown with 3 seeds of cotton cultivar Siokra 1-4. The plants were grown in a glasshouse at 18–23°C for 6 weeks. After the symptoms of fusarium wilt had been assessed, all plants were strictly confined to the pots in which they were grown. Plants which had been removed for the purpose of disease assessment were returned to their original pots. Then the pots and plants were maintained in a sheltered place for 6 weeks without being watered.

Twelve groups of 5 pots chosen at random were established. The plants and soil from the 5 pots within a group were treated as a single experimental unit with the soil, combined and re-distributed into the pots. Each group of 5 pots was used to form 1 of 3 replicates for 1 of 4 treatments. The 4 treatments were designated as follows: T1, whole-plant-supplemented and mixed, in which the leaves, shoots and roots (mainly taproots and a few attached lateral roots) of uprooted plants were cut into sections about 0.5 cm long and mixed thoroughly into the combined soil from the 5 pots; T2, whole-plant-supplemented and unmixed, which was the same as treatment T1 except that the sections were distributed evenly on top of the combined soil after it had been replaced in the pots; T3, lateral-root-supplemented, in which all the uprooted plants were removed, leaving only detached lateral roots in the soil; and T4, non-residue, in which the plant residue remaining in the soil was reduced to a minimum level by sieving the soil through a 0.5 cm screen.

In summary, the whole plant residue was retained in the soil of treatments T1 and T2, only detached lateral roots in the soil of treatment T3 and no residue in the soil of treatment T4.

Soils with different characteristics

Four types of soil used in this experiment were collected from commercially cropped fields in the Brookstead and Cecil Plains areas of the Darling Downs: soil S_B, pH 6.96, grey sandy clay from Brookstead (27°46'S, 151°28'E); soil S_F, pH 5.32, brown heavy clay from Formartin (27°26'S, 151°25'E); soil S_N, pH 6.54, dark grey heavy clay from Norwin (27°33'S, 151°24'E); and soil S_T, pH 5.07, deep red clay from Toowoomba (27°34'S, 151°57'E). They were air-dried at 20–25°C for 1 week, ground and sieved through a 2 cm screen. These soils were tested to ensure that no *Fov* was present by growing cotton cultivar Siokra 1-4 in them and assessing for the symptoms of fusarium wilt. Autoclaved soils were prepared by sterilising these soils at 121°C for 2 h on each of 2 consecutive days. The pH values of the corresponding autoclaved soils were 6.99, 5.32, 6.74 and 5.44 respectively. The water content was determined by drying 200 g of soil in a 500 mL beaker in an oven at 65°C for 24 h and comparing the weights of the soil before and after drying.

Four samples of each soil were adjusted to different pH values (4.0, 5.5, 7.0 and 8.5 respectively) by the addition of either H_2SO_4 or $Ca(OH)_2$ according to the method of Anderegg and Murray (1988). The pH was determined by suspending the soil in 0.01 mol $CaCl_2$/L solution (1 : 2, soil : $CaCl_2$) and measuring with a potentiometer and thin-glass electrode (McLean 1982).

A strain of Australian *Fov* (TF-1), isolated from a soil sample collected from an infested field in the Cecil Plains area of the Darling Downs in 1995, was used in this experiment. It was maintained on sterile filter paper at 4°C (Correll *et al.* 1986) and recovered in modified fresh potato dextrose broth (PDB, 5% potato infusion and 1% glucose) at 25°C for 3 days before being used to

inoculate a cotton seed medium. Ginned and delinted cotton seeds, enclosed in cheesecloth bags, were soaked in a water bath at 80°C for 6 h, washed twice with tap water, distributed into 2 L flasks plugged with cheesecloth and cotton and autoclaved at 121°C for 1 h on each of 2 consecutive days. Each flask of cotton seed medium was inoculated with 5 mL of the recovered culture of TF-1 and incubated at 25°C until the fungal hyphae colonised the cotton seeds (about 4 weeks). The seeds were then removed from the flasks, air-dried completely at 20–25°C, triturated using a grinder, sieved through a 750 µm screen (Endecotts Ltd) and mixed with dry sterile fine sand at a weight : weight ratio of 1 seed powder : 9 sand. The mixture was viewed under a microscope to ensure that microconidia, macroconidia, chlamydospores and hyphae were present. The density of propagules of *Fov* was $5.0 \pm 0.5 \times 10^7$ colony-forming units per gram of the mixture, determined by counting the colonies formed by serial dilutions onto potato dextrose agar (PDA, Difco) plates.

Both autoclaved and untreated soils were inoculated by adding the mixture at a weight : weight ratio of 1 mixture : 9 soil and evenly mixing in a rotating cement mixer. The soils were then distributed into 15 cm plastic pots. There were 3 replicates in the treatments concerning soils of unadjusted pH, with 3 pots used in each replicate, but there was no replication in the treatments concerning soils of adjusted pH, with 3 pots used in each treatment. Therefore, the data obtained from the soils of adjusted pH were analysed by using either the soil types or the pH values respectively as the replicates. The interaction between soil type and pH was not considered in this experiment.

Soils sown with different cotton cultivars or other crops

Three cotton cultivars (Siokra 1-4, most susceptible; Siokra L22, moderately susceptible; and DP90, less susceptible) and 3 other crops (sorghum, maize and soybean) were used in this experiment, conducted during the 1995–96 and 1996–97 growing seasons consecutively in an infested field in the Cecil Plains area of the Darling Downs. The experiment was designed using the completely randomised plot method. There were 24 plots (4 rows, each 10 m long), which consisted of 4 replicate plots for each of the cotton cultivars or other crops grown. Soil was sampled by collecting 5 kg of soil at each of the 4 points, 2 m from either end of the 2 middle rows, in a plot and combining them to provide a bulk sample for the plot. Cotton, sorghum, maize and soybean were sown on 12 October 1995 during the 1995–96 growing season and 18 October 1996 during the 1996–97 growing season. Soil samples were collected on 27 June 1996 and 21 March 1997 respectively. They were air-dried if necessary, ground, sieved through a 2 cm screen and distributed into 20 cm plastic pots. There were 3 pots for the soil collected from each of the plots.

Pathogenicity assay, assessment of disease severity and data analysis

Seeds of cotton cultivar Siokra 1-4 were sown in those soils prepared above, with 8 seeds per 15 cm pot or 12 seeds per 20 cm pot. The plants were grown in a glasshouse at 18–23°C and disease assessment was conducted 6 weeks after sowing. Diseased plants were identified by the appearance of typical symptoms of fusarium wilt and dark brown discolouration of the leaf bases and vascular tissues. Disease severity was assessed by rating the plants on a scale of 0–4 according to their foliar wilt symptoms, in which 0 is healthy, 1 is cotyledons only wilted, 2 is ≤50% true leaves wilted, 3 is >50% but ≤90% true leaves wilted, and 4 is all leaves wilted and the plant was dead. The disease index (the mean of the ratings of individual plants) was calculated using the formula below:

$$\text{Disease index} = \frac{\sum_{\text{Rating}=1}^{4} (\text{Rating} \times \text{no. of plants with rating})}{\text{Total no. of plants investigated}}$$

Data were analysed using analysis of variance (ANOVA) in the general linear models of the MINITAB Release 11 for Windows (Minitab Inc.). Significance levels of $P = 0.05$ or $P = 0.01$ were used. The means were compared using Duncan's multiple range test when the difference was significant.

Results

Effect of plant residue on the disease development

Of the 144 plants investigated in the first generation, 49 (34.0%) were diseased. The disease index was 1.0.

There were significant increases in the disease incidences ranging from 74.4 to 91.8% observed in the residue-supplemented potting mixes (treatments T1, T2 and T3), while it was still low (43.2%) in the non-residue potting mix (treatment T4). Similar variations were also seen in the disease indices. The presence of plant residue significantly enhanced disease development as the disease indices in the residue-supplemented potting mixes, regardless of whether the residue was the whole plant (treatments T1 and T2) or only the lateral roots (treatment T3), were significantly higher than that in the non-residue potting mix (treatment T4). The disease index in the lateral-root-supplemented potting mix (treatment T3) was significantly lower than that in both of the whole-plant-supplemented potting mixes (treatments T1 and T2). Where the whole plant was used as residue, it made no difference whether the residue was placed on top of the potting mix or mixed in (Fig. 1).

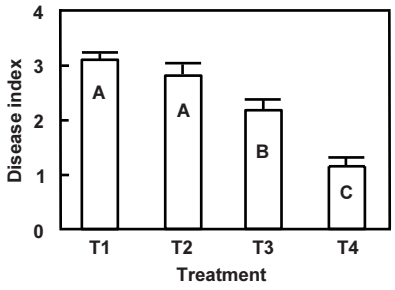

Figure 1. Disease indices of plants grown in soils subjected to different levels of plant residue (T1, whole-plant-supplemented and mixed; T2, whole-plant-supplemented and unmixed; T3, lateral-root-supplemented; and T4, non-residue). Values are the means of 3 replicates. The vertical bars indicate the standard errors. Different letters show significant differences at $P = 0.01$.

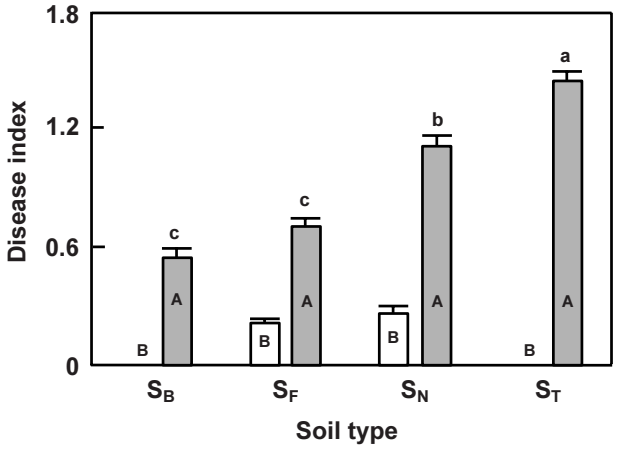

Figure 2. Disease indices of plants grown in autoclaved (open bars) and untreated (shaded bars) soils (S_B, grey sandy clay; S_F, brown heavy clay; S_N, dark grey heavy clay; and S_T, deep red clay) of unadjusted pH, in both of which the same amount of inoculum was added. Values are the mean of 3 replicates. The vertical bars indicate the standard errors. Different uppercase letters show significant differences between autoclaved and untreated soils of the same type at $P = 0.01$. Different lowercase letters on top of the bars show significant differences among the 4 types of untreated soil at $P = 0.01$.

Effects of soil characteristics on the disease

The means of disease indices of plants grown in autoclaved soils were significantly lower than those of untreated soils regardless of soil type. However, the amounts by which the disease indices were reduced varied with soil types. The reductions occurring in soils S_B and S_T were significantly greater than those in soils S_F and S_N. When the disease indices obtained from untreated soils were compared, significant differences were observed. The disease index of plants grown in soil S_T was highest, while those in soils S_B and S_F were lowest (Fig. 2).

No significant differences were observed among the disease indices of plants grown in either untreated soils (Fig. 3*a*) or autoclaved soils (Fig. 3*b*) when the values of soil pH varied in the range 4.0–8.5.

Significant differences were observed among the disease indices of plants grown in the 4 types of autoclaved soils with adjusted pH, from which the influence of both microflora and pH in soils had been reduced to a minimum level. The highest disease index occurred in soil S_N, while the lowest occurred in soils S_B and S_F (Fig. 4).

Effects of different cotton cultivars and other crops on the disease development

In comparison with the plants grown in the soils collected at the end of the 1995–96 growing season, the disease indices increased significantly in plants grown in the soils collected from the plots of cotton cultivar Siokra 1-4, but decreased significantly in plants grown in the soils collected from the plots of cotton cultivar DP90 at the end of the 1996–97 growing season. However, no significant differences were observed between the disease indices of plants grown in the soils of the plots of either cotton cultivar Siokra L22 or other crops collected at these 2 times (Fig. 5).

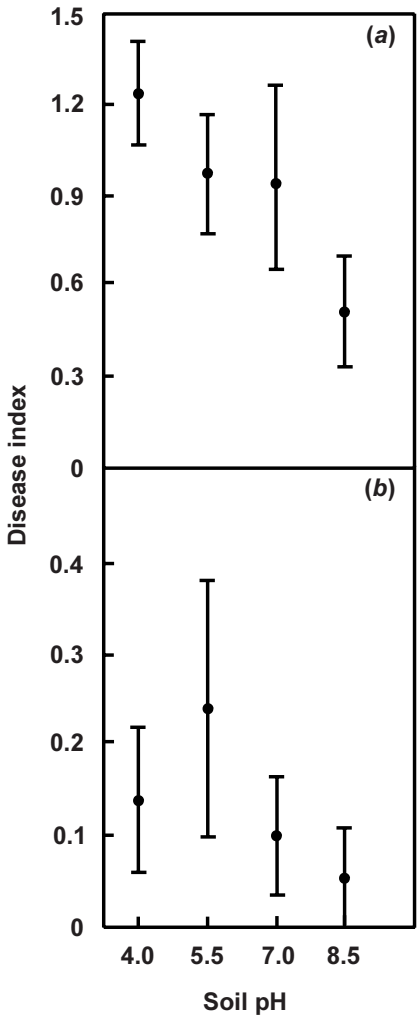

Figure 3. Disease indices of plants grown in (*a*) untreated and (*b*) autoclaved soils of adjusted pH (4.0, 5.5, 7.0 and 8.5), in both of which the same amount of inoculum was added. Values are the means of the disease indices of plants grown in the 4 types of soil of the same pH value. The vertical bars indicate the standard errors.

Discussion

The mathematical model of positive correlation between the level of pathogen and the severity of disease has long been established theoretically (Baker *et al.*

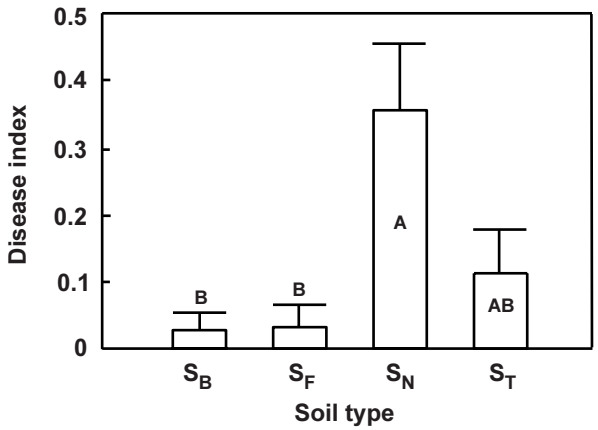

Figure 4. Disease indices of plants grown in autoclaved soils (S_B, grey sandy clay; S_F, brown heavy clay; S_N, dark grey heavy clay; and S_T, deep red clay) of adjusted pH (4.0, 5.5, 7.0 and 8.5 respectively), in both of which the same amount of inoculum was added. Values are the means of the disease indices of plants grown in the same type of soil of the 4 pH values. The bars indicate the standard errors. Different letters show significant differences at $P = 0.01$.

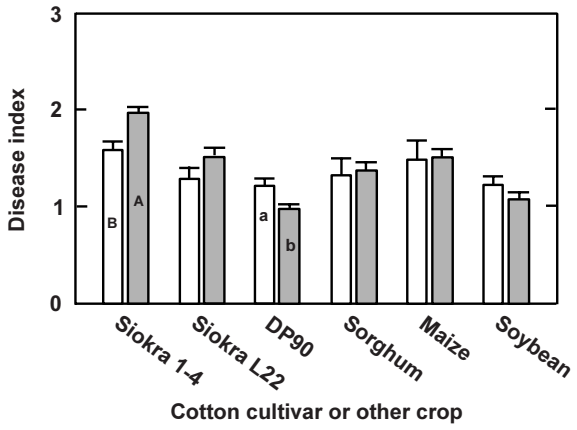

Figure 5. Disease indices of plants grown in the soils of plots planted to different cotton cultivars (Siokra 1-4, most susceptible; Siokra L22, moderately susceptible; and DP90, less susceptible) or other crops (sorghum, maize and soybean). Data are for the end of the 1995–96 season (open bars) and the end of the 1996–97 season (shaded bars). Values are the mean of 4 replicates. The bars indicate the standard errors. Different letters show significant differences at $P = 0.01$ in Siokra 1-4 bars or at $P = 0.05$ in DP90 bars.

1967). It has been verified on many interactions concerning different *formae speciales* of *Fusarium oxysporum* (Morgan and Timmer 1984; Caperton *et al*. 1986; Elmer and Lacy 1987; DeVay *et al*. 1997). There are reports that *formae speciales* of *Fusarium oxysporum* grow fast and become widely distributed in the vascular system of infected plants by means of hyphal growth and conidial production once the infection is established (Conway and MacHardy 1978; Davis *et al*. 1996). The increased incidence and severity of fusarium wilt of cotton in the residue-supplemented potting mixes in the present study was primarily due to the increase in the level of *Fov* caused directly by the incorporation of plant residue derived from the infected plants. Our results showed that the disease indices were positively correlated with the levels of plant residue incorporated (Fig. 1). This is the direct evidence that the development of fusarium wilt of cotton is enhanced by the incorporation of plant residue. It also indicates that the development of fusarium wilt of cotton can be slowed down effectively if most of the plant residue can be removed from the soil, even though this is not always practical in the field.

The relationship between soil microflora and fusarium wilt of cotton was investigated in this study. The disease indices were significantly lower in autoclaved soils than in untreated soils although the same amount of inoculum was added to both (Fig. 2), indicating that soil microflora may play some role in the process of infection and/or disease development. Additional studies are needed to examine the mechanism involved. One explanation for the phenomenon may be the absence of nematodes in the autoclaved soils, because little or no disease symptoms were observed on cotton in soils that were nematode-free but infested with *Fov* soils in previous studies (Jorgenson *et al*. 1978; Garber *et al*. 1979).

Soils suppressive to fusarium wilt have been studied for their biological control potential for many years. It has been widely accepted that certain soil microorganisms are mainly responsible for this response and some non-pathogenic *Fusarium* spp. and fluorescent *Pseudomonas* spp. have the ability to control fusarium wilt effectively under glasshouse conditions (Alabouvette 1990). However, our results showed that fusarium wilt of cotton was generally enhanced by the activities of soil microflora (Fig. 2). This is especially relevant for new areas of the disease, such as Australia, because it may take quite a long time for any antagonistic microorganisms in the soil to establish.

The availability of soil micronutrients to *Fusarium oxysporum* can be limited by high pH (Woltz and Jones

1968), while the development of soil bacteria and actinomycetes are favoured at high pH and their metabolites (toxins) and/or direct competition for organic and inorganic nutrients can result in suppression of the development of *Fusarium oxysporum* (Jones and Woltz 1981). Since most of the previous works concerning the effect of soil pH on fusarium wilt were conducted in natural soils, the reactions of soil microflora and soil type were inevitably involved in the effect of soil pH. Although the attachment of macroconidia of *Fusarium solani* f. sp. *phaseoli* to the roots of mung bean was reported to be enhanced and the disease to be increased at low pH in a hydroponic nutrient solution (Schuerger and Mitchell 1992*a*, 1992*b*), it is not possible to conclude that pH alone can affect fusarium wilt in soil, as the proximity of the pathogen to the infection courts can be maintained by the stability of the soil matrix. Our results indicated that the effect of soil pH alone on fusarium wilt of cotton was not significant in either untreated or autoclaved soils (Fig. 3). It is consistent with Scher and Baker's observation (1980), in which pH had no appreciable influence on fusarium wilt of flax in the conducive soil but did have a significant influence in the suppressive soil. The effect of soil pH on fusarium wilt may therefore be due to the indirect influence of changes in the populations of soil bacteria and actinomycetes.

Previous studies indicated that the proliferation of pathogenic *Fusarium oxysporum* was significantly affected by soil type and it was usually greater in light soils than in heavy soils (Fravel *et al.* 1996). Additionally, the virulence of pathogenic *Fusarium oxysporum* was significantly influenced by the level of micronutrients in liquid media and the inocula prepared from cultures deficient in molybdenum or zinc or oversupplied with copper were less virulent than those from complete cultures (Woltz and Jones 1968). Since the chemical composition of soil varies, it is reasonable that different effects of soil type on fusarium wilt will be observed in different studies. In the present work fusarium wilt of cotton was found to be more severe in a heavy clay (soil S_N) than in either a light clay (soil S_B) or another heavy clay (soil S_F) when the influence of soil microflora and soil pH had been reduced to a minimum level (Fig. 4).

In our study, 2-season growth of the most susceptible cotton cultivar Siokra 1-4 resulted in a significant increase in disease development. This is in agreement with field observations that fusarium wilt usually increases with the planting of susceptible cotton cultivars (Hillocks 1992).

In China the disease severity of fusarium wilt was found to be reduced significantly when a susceptible cotton cultivar was replanted in a previously heavily infested field, in which a resistant cotton cultivar had been grown successively for more than 10 years, and the same result was observed even when a large amount of the pathogen was added to the soil in advance (C. Ma and G. Jian pers. comm.). Our results indicated that the disease indices of plants grown in the soil of plots planted to the less susceptible cotton cultivar DP90 were reduced significantly, while no difference was observed in the soil of plots planted to sorghum, maize or soybean (Fig. 5). Since *Fov* can persist in a wide range of hosts parasitically or in the soil saprophytically, tolerant or resistant cotton cultivars may be more effective than other crops in limiting disease development.

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