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Understanding the Causes of Sudden Wilt of Capsicum

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Understanding the causes of sudden wilt of capsicum

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Purpose of the report

Sudden wilt is the most important disease of capsicum in Australia. Plants appear healthy until fruit set, when they suddenly begin to wilt and lose their leaves. Healthy fruit becomes shrivelled and unmarketable and most affected plants die. In most years there are only minor losses from the disease, but occasionally, as many as 25% of plants may die in the main capsicum production areas of Queensland. The purpose of this report is to describe research aimed at determining the cause of sudden wilt. This work shows that two fungi (*Pythium myriotylum* and *P. aphanidermatum*) are involved in the disease. These fungi grow at temperatures above 40°C and are capable of quickly destroying the root system of heat-stressed capsicum plants. Evidence is presented to indicate that sudden wilt is the result of severe *Pythium* root rot during hot dry weather, when soil temperatures in the raised, plastic-covered beds in which capsicum is grown may reach the high 30's or low 40's.

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Media Summary

The objective of this project was to determine the cause of sudden wilt of capsicum. This disease occurs in all capsicum production areas of Queensland and losses are as high as 25% in some years. Studies during the 2000 and 2001 seasons showed that the first symptoms of sudden wilt are a slight yellowing of leaves and an inconspicuous wilting and shrivelling of fruit. As the disease progresses, affected plants wilt completely and then defoliate. If the root system is examined carefully before above-ground symptoms are visible, some fine roots are rotted and these primary infections later spread rapidly to large roots. The end result is extensive rotting of most of the root system. Between 70 and 90% of large roots are generally rotted in plants with incipient sudden wilt symptoms, compared with fewer than 5% in healthy plants.

Surveys carried out in the Bowen and Bundaberg regions during the 2000 growing season showed that four fungi were consistently associated with rotted roots of diseased plants. *Fusarium* was recovered from both small and large roots in most fields. *Pythium*, *Rhizoctonia* and *Macrophomina* were also recovered consistently but not as frequently. Some fields had all four fungi, but more commonly only two or three fungi were present. Other potential pathogens (e.g. nematodes, bacteria or viruses) did not appear to be associated with the disease.

Laboratory and glasshouse tests with small capsicum seedlings showed that *Pythium* was the only one of the above fungi to cause severe root rotting. Seedlings inoculated with *Rhizoctonia* were stunted and had some discoloured roots, but *Fusarium* and *Macrophomina* did not cause damage. When the fungi were inoculated onto 6-week old capsicum plants at fruit-fill stage, *Pythium* was again the only fungus to produce disease symptoms. Plants began to wilt within a few days of inoculation and within 1 week, root systems were almost completely destroyed. Two species of *Pythium* (*P. myriotylum* and *P. aphanidermatum*) were capable of causing root rot, but the former species was more damaging. Both species grow well at 36-40°C.

Capsicums, like many other intensively grown vegetable crops, are grown on plastic mulch in raised beds with trickle irrigation. Measurements of soil temperature under plastic in Bowen and Bundaberg showed that temperatures of 38-40°C are common at certain times of the year, even on days when the air temperature is less than 30°C. Thus, temperature conditions that are ideal for *Pythium* root rot sometimes occur during autumn and spring in the main capsicum growing areas of Queensland. Studies of temperature, rainfall and cloud cover records from the Bureau of Meteorology for Bundaberg during the period from 1990 to 2002 suggested that sudden wilt was most severe in years when the spring was hot and dry.

Experiments in the glasshouse showed that soil temperatures of more than 30°C are detrimental to capsicum. When plants are grown in sterile soil at 35 and 40 °C, root health is poor and growth of both roots and shoots is markedly reduced. Addition of either *P. myriotylum* or *P. aphanidermatum* to plants that are already stressed by high temperatures results in severe root rotting and in some cases, death of plants.

In summary, the work done in this project has shown that when capsicum plants are grown at high temperatures, symptoms of sudden wilt can be reproduced by inoculating them with either *P. myriotylum* or *P. aphanidermatum*. However, fungal pathogens that destroy root systems at temperatures greater than 36°C are not the only factors involved. High soil temperatures, which reduce the capacity of roots to function, and carbohydrate starvation of roots, which occurs at fruit fill stage because of competition from the developing fruit, are other important exacerbating factors. Since the name ‘sudden wilt’ simply describes non-

specific above-ground symptoms that can be caused by other pathogens (e.g. *Sclerotium rolfsii*), we suggest that in future, the disease should be referred to as 'heat-induced Pythium root rot'.

The fact that both pathogens and environmental factors are involved in the sudden wilt syndrome suggests that losses from sudden wilt will not be reduced by simply concentrating on controlling *Pythium*. For example, it may also be necessary to reduce soil temperatures and increase the tolerance of the crop to heat. This project was not designed to look at control measures for the disease, but a range of possible management options are being investigated in a follow-up research project (VG02020) that commenced in January 2003.

Technical Summary

The objective of this project was to determine the cause of sudden wilt of capsicum. This disease occurs in all capsicum production areas of Queensland and losses are as high as 25% in some years. Observations in the field during the 2000 and 2001 seasons showed that the first symptoms of sudden wilt are a slight yellowing of leaves and an inconspicuous wilting and shrivelling of fruit. As the disease progresses, affected plants wilt completely and then defoliate. If the root system is examined carefully before above-ground symptoms are visible, rotted fine roots can be found and these primary infections later spread rapidly to large roots. The end result is extensive rotting of most of the root system. Between 70 and 90% of large roots are generally rotted in plants with incipient sudden wilt symptoms, compared with fewer than 5% in healthy plants.

Measurements in the raised, plastic-covered beds used for capsicum production in Queensland showed that soil temperatures of 38-40°C were common in Bowen and Bundaberg during periods of the year when sudden wilt occurs. This suggests that heat stress may be involved in the sudden wilt syndrome. Studies of temperature, rainfall and cloud-cover records from the Bureau of Meteorology for Bundaberg during the period from 1990 to 2002 also indicated that excessive heat may be a contributing factor, as disease incidence and severity was highest during the spring of hot, dry years.

Surveys for potential pathogens associated with sudden wilt showed that four fungi were consistently associated with rotted roots of diseased plants in the field. *Fusarium* was almost always recovered from roots, whereas *Pythium*, *Rhizoctonia* and *Macrophomina* were recovered consistently but not as frequently. All fungi were isolated from some fields, but more commonly only two or three were present. Laboratory and glasshouse tests with small capsicum seedlings showed that *Pythium* was the only fungus to cause severe root rotting. Seedlings inoculated with *Rhizoctonia* were stunted and had discoloured roots while *Fusarium* and *Macrophomina* did not cause damage.

Pathogenicity tests with 25 isolates of *Pythium* showed that they could be categorised into two broad groups. One highly pathogenic group reduced shoot and root weight by more than 80% and sometimes killed seedlings, while the other group reduced shoot weight by 20-80%. Taxonomic studies indicated that most of the isolates in the more pathogenic group were *P. myriotylum* or, less frequently, *P. aphanidermatum*. Both these species have optimum temperatures of about 36°C but grow well at 32-40°C. The less pathogenic group consisted mainly of species tentatively identified as *P. diclinum* and *P. dimorphum*, which have optimum temperatures of approximately 30°C.

Experiments in the glasshouse under conditions where maximum soil temperatures ranged from 35-41°C showed that both *P. myriotylum* and *P. aphanidermatum* caused severe damage to the roots of mature capsicum plants at the fruiting stage. Plants began to wilt within a few days of inoculation and within a week, root systems were almost completely destroyed. The degree of root rotting increased as inoculum density increased, and after 5 weeks, there was an inverse polynomial relationship between shoot and root weight and inoculum density.

Experiments in the glasshouse showed that soil temperatures of more than 30°C were detrimental to capsicum. When plants were grown in pasteurised soil at 35 and 40 °C, root health was poor and growth of both roots and shoots was markedly reduced. Addition of either *P. myriotylum* or *P. aphanidermatum* to plants that were already stressed by high temperatures resulted in severe root rotting and in some cases, death of plants. Experiments in which *P. myriotylum* and either *Fusarium*, *Macrophomina* or *Rhizoctonia* were inoculated alone or together onto plants growing at various temperatures showed that fungi other than

Pythium were not pathogenic under the test conditions and did not increase the level of damage caused by *P. myriotylum*.

These results are consistent with the hypothesis that sudden wilt is a response to the severe root rotting which occurs when *P. myriotylum* and *P. aphanidermatum* attack plants that are stressed by heat when soil temperatures are greater than about 35°C. Assimilate deprivation of roots, which occurs during fruit-fill because carbohydrate reserves are diverted to developing fruit, is also an exacerbating factor. Thus, the disease is the result of an interaction between specific pathogens and the environment. Since the name 'sudden wilt' simply describes non-specific above-ground symptoms that can be caused by other pathogens (e.g. *Sclerotium rolfsii*) and provides no information about the etiology of the disease, we suggest that, in future, the disease should be referred to as 'heat-induced Pythium root rot'.

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Chapter 1: Sudden wilt of capsicum and other similar diseases: a review

Introduction

The genus *Capsicum* is a member of the *Solanaceae* and originated in Central and Southern America. The genus consists of at least 25 wild species and five species that were domesticated independently in different regions of the Americas. These five cultivated species (*C. annuum*, *C. pubescens*, *C. baccatum*, *C. chinense* and *C. frutescens*) contain many cultivars that produce fruit varying in shape, pungency and colour (Bosland and Votava, 2000). In Australia, *C. annuum* is the most commonly cultivated species (Meurant *et al.*, 1999).

When capsicum is used for fruit production, it is grown as an annual, with a cropping period of 3 - 6 months. In Queensland, the major production areas are Bowen and the Burdekin in the dry tropics, and a sub-tropical region around Bundaberg. In Bowen and the Burdekin, crops are planted from March to August, while Bundaberg has two main planting times: February to March and then July to September.

Typical production systems for capsicum involve transplanting seedlings of hybrid varieties into raised beds covered by plastic mulch, the use of trickle irrigation and close monitoring of nutrition. In Bundaberg, many growers previously fumigated the soil with methyl bromide, but with the phase-out of methyl bromide in recent years, some growers have turned to metham sodium or are not fumigating at all. A few growers in the Bowen/Burdekin area also use metham sodium, primarily for weed control.

Although some growers feel that they need to fumigate, there are relatively few important soil-borne diseases of capsicum crops in Queensland. Diseases caused by *Verticillium* spp. and *Ralstonia solanacearum* are rarely seen, while losses caused by *Sclerotium rolfsii* are generally restricted to a few scattered plants in some fields. Root-knot nematode (*Meloidogyne incognita*) is more important, but losses are largely restricted to sandy loam soils around Bundaberg. The major production constraint is a poorly understood disease known as sudden wilt, which has caused losses of up to 50% in some fields in some years (Olsen *et al.*, 1992). The following review presents the limited literature available on sudden wilt of capsicum and discusses literature on a similar disease of cucurbits.

Sudden wilt of capsicum

Published literature on this disease is limited. There are two articles in industry magazines from Australia (Olsen *et al.,* 1992; Olsen and Barnes, 1999) and an abstract on a similar disease in Israel (Krikun *et al.*, 1981). There are also three extension publications that describe the current knowledge on symptoms and management of the disease in Queensland (Persley *et al.*, 1989; Persley, 1994; Meurant *et al.*, 1999). Dr. Mike Stanghellini of the University of California, Riverside reported that he had never seen the disease in the USA. However, he did state that two pathogens, *Pythium aphanidermatum*, and *Phytophthora capsici*, are major root rot pathogens of capsicum in the USA (pers. com., 2000).

Symptoms

Losses from sudden wilt occur quickly and unexpectedly. Plants appear healthy until fruit set and then wilt suddenly. Above-ground symptoms only occur after fruit set. Initially, plants wilt during the warmest part of the day (Olsen and Barnes, 1999) and if conditions are not suitable for rapid disease development, signs of general leaf yellowing may be apparent. Under suitable conditions, all leaves drop off and only small red fruit are retained on the stem and branches. Affected plants die or produce small, shrivelled, unmarketable fruit. When the root systems of affected plants are inspected, feeder roots and many of the larger roots are rotted.

Pathogens involved

In Australia, *Pythium* spp., *Fusarium* spp., *Rhizoctonia* spp. and *Macrophomina* spp. are commonly isolated from the rotted roots of sudden wilt affected capsicum (Olsen and Barnes, 1999). *Pythium* was thought to be the primary causal organism (Persley *et al.*, 1989) but Olsen and Barnes (1999) suggested that the four main pathogens may work together to cause the disease. Olsen (1996) surveyed healthy capsicum crops in the Bowen/Burdekin region and found that *Pythium aphanidermatum* was the most commonly isolated *Pythium* species. Stanghellini (pers. com., 2000) also stated that *P. aphanidermatum* was a major pathogen of capsicum in Arizona and California and that *Macrophomina* is a serious pathogen of stressed plants. Pathogenicity studies on *Pythium* spp. isolated from capsicum in Florida (Chellemi *et al.*, 2000) implicated *P. aphanidermatum* and *P. myriotylum* in capsicum root rot. Both pathogens reduced shoot and root weight, and disease severity increased significantly as temperature increased.

In Israel, *Pythium elongatum* together with high chloride levels in plant tissue were thought to cause root rot and wilt in capsicum (Krikun *et al.*, 1981). However, elevated chloride content in plant tissue is also observed with avocado affected by Phytophthora root rot (Whiley *et al.*, 1986). Thus the changes in chloride concentration observed by Krikun *et al.* (1981) may have been a consequence of root rot rather than a cause.

Phytophthora capsici causes a root and crown rot of capsicums that results in rapid wilting and blight of leaves, fruit and stems (Ristaino and Johnston, 1999). Phytophthora blight is one of the most economically important diseases of capsicum in the Americas and Eurasia (Adorada *et al.*, 2000), but there is no record of this disease on capsicum in Australia.

Predisposing conditions

In the Australian literature, there are various theories on the conditions that pre-dispose capsicum plants to sudden wilt. Since the disease has been worse in Bundaberg during dry years such as 1991 (Olsen *et al.*, 1992) and 1997, one hypothesis relates to a failure of crop residues to break down, so that pathogen propagules subsequently survive on this organic matter (Olsen *et al.*, 1992). Since most of the pathogens associated with sudden wilt of capsicum are competent saprophytes, the colonisation and subsequent build-up of inoculum on organic matter when the crop is irrigated prior to planting may also be involved. The latter is probably more likely than the former because crop residues may not be colonised when insufficient moisture is available. Another confounding factor with this hypothesis is that there are more clear days and increased solar radiation during dry years. Thus, soil temperatures are likely to be higher than in wet, cloudy years. Olsen *et al.* (1992) also states that there was speculation that *Pythium* spp. vectored by fungus gnats may have infected nursery seedlings and contributed to the disease in 1991. However, this theory does not explain the widespread occurrence of the disease in fields where seedlings were obtained from non-infected nurseries.

Plant stress is another popular theory for pre-disposition of plants to sudden wilt. High soil temperatures under black plastic (instead of the standard white plastic) may result in conditions that are unfavourable for root growth and favourable to pathogens such as *Pythium* spp. (Olsen and Barnes, 1999). *P. aphanidermatum* and *M. phaseolina* have optimal temperatures of 32-37°C (Gubler and Davis, 1996) and 32- 34°C (Mihail, 1992) respectively. However Bakker and van Uffelen (1988) demonstrated that a 24 hour mean temperature between 21 and 23°C was optimal for vegetative growth of one cultivar of capsicum, while Gosselin and Trudel (1986) showed that another cultivar produced more shoot dry weight and

leaf area with a constant root-zone temperature of 30°C than at 24 or 36°C. Losses to sudden wilt in Bundaberg appear to be greater in winter-spring planted crops on black plastic, possibly because of its soil heating effect. The disease is not observed in crops planted in autumn on white plastic. In Bowen, where all plantings are on white plastic, losses are greater in the early plantings (late summer-early autumn), when temperatures are higher, than in plantings in late winter and early spring. The difference in disease severity between regions in autumn-planted crops on white plastic may be due to the fact that autumn temperatures in Bowen are higher than in Bundaberg.

Soil moisture is often thought to contribute to sudden wilt, as it can affect root growth and plant stress. Over-watering at the seedling stage starves roots of oxygen and does not encourage roots to grow in search of water. This combined with under-watering when the crop is setting fruit results in moisture stress at a time of high water demand (Olsen *et al.*, 1992). Soil moisture can also affect the activity of root pathogens. High soil moisture favours infection by *Pythium* spp. (Hendrix and Campbell, 1973), while losses from *Macrophomina* spp. increase when plants suffer moisture and high temperature stress (Olaya *et al.*, 1996).

In the last 15 - 20 years, many growers have moved from open-pollinated to hybrid varieties. These hybrids produce high yields but are reported to have relatively small root systems compared to open-pollinated varieties such as Green Giant (Olsen *et al.*, 1992). However, this has not been documented conclusively. In addition, the hybrid varieties tend to have a shorter, more intense period of fruit-set from open-pollinated (R. Wright, pers. comm.). This may put additional stress on the plant during fruit-fill.

The phenomenon of root death, which has been noticed in hydroponically grown cucumber and tomato, may be relevant to sudden wilt of capsicum, as it causes plants to wilt despite the absence of common pathogens. A review by Van der Vlugt (1989) indicated that root death occurs soon after flowering in some hydroponically grown crops that have a cyclic pattern of fruit production. The size of the root system reaches a minimum at the time of maximum fruit load, possibly because of competition for assimilates between fruit and roots. Since plants with less fruit lose fewer roots, it appears that root growth is opposed to fruit growth (Van der Vlugt, 1989). Attempts to transmit this problem via inoculation have been unsuccessful and therefore the phenomenon appears to be abiotic (Daughtrey and Schippers, 1980).

Varietal susceptibility

No published research can be found on differences in varietal susceptibility to sudden wilt of capsicum. However, Olsen *et al.* (1992) reported some differences between five cultivars tested. Domino was the most resistant and Cordoba the least resistant, but it is not clear how susceptibility was assessed.

Why has sudden wilt become a problem in the last 15-20 years?

Observations by growers suggest that sudden wilt has only caused economic problems in the last 15 - 20 years. In this time, there have been major changes in the capsicum production system used in Australia. Plants are now watered using trickle irrigation and beds are covered in low density polyethylene, whereas in the past, crops were grown in beds of bare soil and watered by overhead or flood irrigation. This has greatly changed the soil environment. Soil covered with plastic generally remains hotter than bare soil and may therefore be more favourable to pathogens with high temperature optima, as well as being less favourable to root growth during the warmer times of the year. The use of trickle irrigation and computerised methods of monitoring soil moisture have allowed growers to water more precisely and only apply water and nutrients to a depth that is needed. It is possible that this practice restricts the volume of soil exploited by the root system.

As indicated previously, the varieties grown have also changed in the last 15 years. While hybrids exhibit hybrid vigour and produce high yields, they may be more susceptible to sudden wilt.

Prior to about 1985, capsicum crops were established by direct seeding or by transplanting bare rooted seedlings grown in seedling beds. Capsicums are now raised as transplants in small cells of potting media. When the roots reach the bottom of the cell they are air-pruned, resulting in the loss of the true tap root and the development of more lateral roots (Leskovar *et al.*, 1989). Transplants also exhibit a greater fruit sink demand in comparison to direct-seeded plants (Leskovar *et al.*, 1990). It is therefore possible that the increase of sudden wilt in the last 15-20 years is in some way related to the different pattern of root and shoot development in transplanted seedlings.

Sudden wilt of cucurbits

In contrast to sudden wilt of capsicum, there is much more literature on sudden wilt of melons. This disease is also known as melon collapse, vine decline or root rot, and is very similar to sudden wilt of capsicum.

Symptoms

Above ground symptoms resemble sudden wilt of capsicum. The plants appear healthy until they suddenly wilt prior to harvest. Initially, crown leaves die prematurely but this is followed by a complete and radiating collapse of the vine. These symptoms typically occur during fruit filling (Cole, 1994). The disease is apparently caused by a variety of single pathogens or disease complexes. However, regardless of the pathogens involved, above-ground symptoms are very similar and the timing of symptom development is the same (i.e. fruit set and fruit fill). Inspection of the root system generally reveals extensive rotting of large and small roots, sometimes accompanied by a crown rot.

Pathogens involved

Fungal pathogens are thought to be the primary cause of sudden wilt of melon, but the causal agents seem to depend on the region of the world in which the disease occurs.

P. aphanidermatum, together with *P. ultimum* and *P. myriotylum*, cause a severe rot of mature cucurbit plants that results in decline and rapid wilting (Gubler and Davis, 1996). *P. aphanidermatum* causes symptoms in the field but losses are greater when *Fusarium solani* is also present (Pivonia *et al.*, 1997). *M. phaseolina* also causes a root and crown rot of melon (Reuveni *et al.*, 1982; Walker, 1994) and is frequently involved in a disease complex that results in sudden wilt (Mertely *et al.* 1991; Bruton and Miller, 1998; Aegerter *et al.*, 2000).

In hot arid climates, *Monosporascus canonballus* has been shown to be the primary cause of a sudden wilt disease that is referred to as Monosporascus root rot and vine decline (Cohen *et al.*, 2000). However, this pathogen has not been recorded in Australia. In Texas, a *Stagonospora*-like fungus was isolated from melon roots in situations where the disease could not be attributed to *Monosporascus* sp. (Miller *et al.*, 1996). Subsequent pathogenicity studies showed that six isolates of this fungus were pathogenic to melon. *Rhizoctonia solani* AG-7 was commonly isolated from melons affected by “sudden wilt” in Indiana and was shown to cause lesions on roots, stems and cotyledons (Baird and Carling, 1995).

Acremonium sp. (or *Cephalosporium* sp.) causes a hypocotyl rot of melon seedlings (Gubler, 1996). It is also implicated as a cause of melon collapse in Spain (García-Jiménez *et al.*, 1994), and is associated with wilting plants in California (Bruton *et al.*, 1995; Aegerter *et al.*, 2000) and Texas (Mertely *et al.*, 1991).

Much of the literature on sudden wilt of melons suggests that more than one organism is often involved in the disease. Most of these organisms are fungi and some of them are listed in Table 1.1. However, they have not yet been shown to be the primary cause of the disease.

Table 1.1: Fungi associated with sudden wilt of melon from published articles in which the cause was either a disease complex or undetermined.

Fungus	References
Acremonium sp.	Aegerter *et al.*, 2000.
Fusarium equiseti	Eyal and Cohen, 1986.
Fusarium proliferatum	Pivonia *et al.*, 1997.
Fusarium semitectum	Bruton and Miller, 1997; Bruton and Miller, 1997a; Bruton and Miller, 1998.
Fusarium solani	Aegerter *et al.*, 2000; Bruton and Miller, 1997a; Eyal and Cohen, 1986; Mertely *et al.,* 1991; Pivonia *et al.*, 1997.
Olpidium sp.	Pivonia *et al.*, 1997.
Plectosporium tabacinum	Bruton and Miller, 1997.
Pythium spp.	Aegerter *et al.*, 2000; Bruton and Miller, 1997a; Bruton and Miller, 1998.
Rhizoctonia solani	Bruton and Miller, 1997; Bruton and Miller, 1997a; Bruton and Miller, 1998; Pivonia *et al.*, 1997; Troutman and Matejka, 1970.
Rhizopycnis vagum	Aegerter *et al.*, 2000; Bruton and Miller, 1998.
Stagonospora sp.	Bruton and Miller, 1997; Bruton and Miller, 1997a; Mertely *et al.*, 1991.

Predisposing conditions

The date of planting is known to affect the development and severity of sudden wilt diseases of melon. Sudden wilt incited by *Monosporascus* sp. is severe in Israel during the relatively warm autumn cropping season, whereas losses in crops grown in the same soil in the winter-spring season can be greatly reduced (Cohen *et al.*, 1996). This effect is thought to be related to elevated soil temperatures (Kim *et al.*, 1995). A similar scenario has been observed with a disease caused by *M. phaseolina* (Reuveni *et al*., 1982). The increase in disease severity with increasing temperatures is probably related to the temperature requirements of the pathogens, because the temperature optima for *Monosporascus canonballus*, *P. aphanidermatum*, and *M. phaseolina* are respectively 30 - 35°C (Martyn and Miller, 1996), 32-37°C (Gubler and Davis,

1996) and 32- 34°C (Mihail, 1992). However, temperature may also affect factors that induce plant stress, such as rate of fruit-fill, the number of fruit set and the rate of plant growth (Pivonia *et al.*, 1999).

Stress resulting from sub- or supra optimal irrigation and temperature can make plants more susceptible to sudden wilt, especially when *M. phaseolina* is involved (see previous capsicum section). Fruit load also affects the susceptibility of melons to sudden wilt caused by *M. cannonballus* (Pivonia *et al.*, 1997).

Conclusion

Sudden wilt of capsicum may be related to competition for assimilates between fruit and roots, because symptoms are first observed at fruit-fill. Hybrid varieties that have been selected for high yields and a concentrated harvest are exacerbating factors, together with the use of transplants rather than direct seeded plants. This disease also appears to be associated with periods of warm weather that favour pathogens with high temperature optima and are unfavourable for root growth. The use of plastic soil coverings probably exacerbates the effects of high temperature by keeping the soil warmer than bare ground.

Sudden wilt of capsicum has a similar etiology to sudden wilt of cucurbits and may eventually be found to involve several pathogens that differ in relative importance from field to field or region to region. Since the sudden wilt of capsicum and cucurbits occur at the same crop stage, are exacerbated by similar conditions and involve similar pathogens, many of the experimental techniques used with cucurbits will be applicable to studies with capsicum.

Chapter 2: Relationship between meteorological conditions and occurrence of sudden wilt

Introduction

One of the basic principles of plant pathology is that environmental conditions play a major role in determining when and where a disease occurs. Environment also greatly affects disease development. In fact, a disease will only cause significant losses if all three components of what is commonly referred to as the 'disease triangle' are present: a susceptible plant, an infective pathogen and a favourable environment. For most diseases, the environmental factors of most importance are temperature and moisture and they affect disease development by influencing the growth and susceptibility of the host, the multiplication and activity of the pathogen, and the interaction between host and pathogen as it relates to symptom development. A change in any environmental factor may favour the host or the pathogen, or it may be more favourable to one than it is to the other. As a result, disease expression will be affected.

Although there are no accurate records on the occurrence of sudden wilt of capsicum in Australia, it occurs spasmodically and its occurrence is thought to be related to weather conditions. In Bundaberg, the disease is known to have caused heavy losses in only two of the last 13 years: the spring crops of 1991 and 1997. Sudden wilt tends to occur more commonly in autumn-planted crops in Bowen and winter-planted crops in Bundaberg, and this difference is thought to be related to environmental conditions. Anecdotal evidence from growers suggests that sudden wilt is more severe in hot dry conditions. Thus, the relationship between environmental factors and the disease is explored in this chapter. The environmental conditions that occur in the main capsicum growing areas of Queensland are described, data on the temperatures that occur in soil under capsicum crops are collected and observations are made on the possible impact of meteorological conditions on the incidence and severity of sudden wilt.

Weather data for the main capsicum-growing areas

Materials and methods

Records from weather stations at Bundaberg and Bowen airports for the period from 1990 to 2002 were obtained from the Bureau of Meteorology. The data available consisted of daily maximum and minimum temperature, daily rainfall and cloud cover (taken five times a day).

Since the critical periods for the development of sudden wilt are autumn and spring, average maximum temperature, average minimum temperature, monthly rainfall, average percent daily cloud cover and number of days with more than 50% cloud cover were calculated for February to May and August to October of each year. The number of days when the temperature was over 28 and 30°C was also determined. During 1997, the Bundaberg weather station became an automatic, unmanned station and data on cloud-cover were only collected on an irregular basis. Cloud-cover data for later years are therefore incomplete.

Results

A summary of the meteorological data (Tables 2.1 & 2.2) shows that average maximum and average minimum temperatures are higher in Bowen than in Bundaberg in both autumn and spring. At those times of the year, the maximum temperature also exceeds 28°C more times in Bowen than in Bundaberg. A comparison of weather conditions at the time capsicum crops are first planted in the two regions (February in Bundaberg and March in Bowen) shows that average maximum and minimum temperatures in those two months are higher and number of days over 28 and 30°C are greater in Bowen than in Bundaberg. Similar differences occur in late winter and spring, with Bowen being hotter than Bundaberg. Rainfall is higher in Bowen during early autumn but after May, Bundaberg is wetter than Bowen. Average daily cloud cover and number of days with less than 30% cloud-cover are similar in both regions.

The variability in weather conditions from year to year and at different times of the year is apparent from the data shown in Appendices 1 A-D. Data for Bowen indicate that maximum temperatures of more than 30°C are common in March, when the first capsicum crops are usually planted. However, exceptions do occur, particularly at times when there is heavy cloud cover and very high rainfall (e.g. March of 1990, 1994 and 1997). Temperatures in spring are much lower than in autumn, and there is relatively little rainfall.

In Bundaberg, the sudden wilt year of 1991 stands out as an unusually hot year (Appendix 1D). The sky was relatively clear, and it had higher average maximum temperatures in August and September and more days above 28°C than any other year from 1990 to 2002. It was also a relatively dry year, with less than 50 mm of rain in August, September and October. The late-winter, early spring period of 1997 was also relatively hot, particularly in September, and rainfall was only 51 mm.

Table 2.1: Summary of weather conditions in late summer and autumn for Bowen and Bundaberg from 1990 to 2002.

Parameter	February		March		April		May	
	Bowen [a]	Bundaberg	Bowen	Bundaberg	Bowen	Bundaberg	Bowen	Bundaberg
Average max temp (°C)	31.5	30.1	30.9	29.6	29.3	27.5	27.1	25.0
Average min temp (°C)	24.0	21.6	22.6	20.0	20.9	17.6	18.1	15.0
# of days >28 C	26	25	29	25	23	11	9	1
# of days >30C	22	14	23	11	10	3	0	0
Total rainfall (mm)	233	141	81	71	50	46	55	88
Average daily cloud cover (%)	62	58	51	46	49	43	47	48
# of days <30% cloud cover	4	3	9	10	9	11	10	9

[a] Data for Bowen in February are shaded to indicate that this month is before the capsicum planting season (which usually does not start until March).

Table 2.2: Summary of weather conditions in late winter and spring for Bowen and Bundaberg from 1990 to 2002.

Parameter	August		September		October	
	Bowen	Bundaberg	Bowen	Bundaberg	Bowen	Bundaberg
Average max temp (°C)	25.5	23.5	27.4	25.6	29.1	26.9
Average min temp (°C)	14.6	11.2	16.7	14.0	19.8	16.8
# of days >28 C	1	1	8	4	25	7
# of days >30C	0	0	1	1	4	2
Total rainfall (mm)	27	36	9	45	18	79
Average daily cloud cover (%)	32	29	28	30	35	41
# of days <30% cloud cover	18	19	18	17	16	12

Soil temperatures under capsicum crops in Bundaberg and Bowen

Materials and methods

Temperature probes (Tiny Talk®) were placed under crops in Bundaberg and Bowen during periods of the year when sudden wilt was most likely to occur and soil temperatures were recorded every hour at depths of 5 and 15 cm. In Bundaberg, probes were inserted on 16 August 2001 into the centre of a bed covered with black plastic. The bed contained a single row of capsicums planted on 8 August 2001 and had two irrigation lines, one on each side of plants. The probes at Bowen were set up on 6 March 2002 in a bed covered with white plastic. There was a single irrigation line down the centre of the bed and probes were placed next to it. Two rows of capsicum seedlings were planted about a week later.

Daily maximum soil temperatures recorded for Bundaberg and Bowen at 5 and 15 cm were tabulated, together with daily maximum air temperatures from the Bureau of Meteorology for the same period. Regression analysis was then used to determine the strength of the relationship between air and soil temperatures.

Results

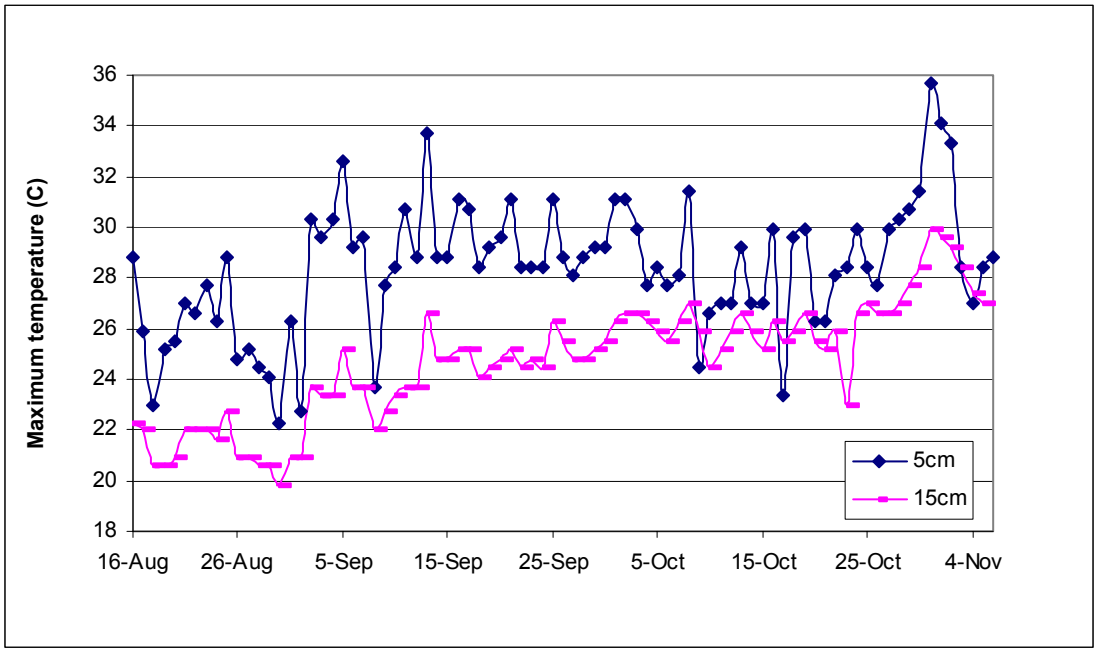
At both Bowen and Bundaberg, the average temperatures at 5 and 15 cm were similar throughout the monitoring period (Tables 2.3 & 2.4). However, daily temperature fluctuations (and therefore maximum temperatures) were much greater at 5 cm than at 15 cm.

Temperatures recorded at Bundaberg are summarised in Table 2.3 for five half-month periods. Minimum, average and average maximum temperatures all increased at both depths as the season progressed. Daily maximum temperatures at both depths also tended to increase over this time (Figure 2.1). At 15 cm, the maximum temperature in August was always less than 25°C and it remained below 28°C until the end of October. However, at 5 cm, the maximum temperature in August occasionally reached 28°C and was generally between 28°C and 31°C during September and October.

Table 2.3: Soil temperatures at Bundaberg from August to October 2001 in beds covered with black plastic and planted to capsicum.

Time	Depth	Minimum temperature	Average temperature	Maximum temperature	Average max temperature
mid – late Aug	5 cm	12.0	19.3	28.8	25.8
	15 cm	14.9	18.7	22.7	21.3
early – mid Sept	5 cm	14.5	22.5	33.7	29.0
	15 cm	17.0	21.3	26.6	23.7
mid – late Sept	5 cm	18.1	23.6	31.1	29.4
	15 cm	19.8	22.8	26.3	25.0
early – mid Oct	5 cm	19.1	24.1	31.4	27.7
	15 cm	21.6	24.5	27.0	26.0
mid – late Oct	5 cm	19.8	24.7	35.7	28.6
	15 cm	22.3	25.1	29.9	26.5

Figure 2.1: Daily maximum soil temperatures at Bundaberg from August to November 2001 in beds covered with black plastic and planted to capsicum.

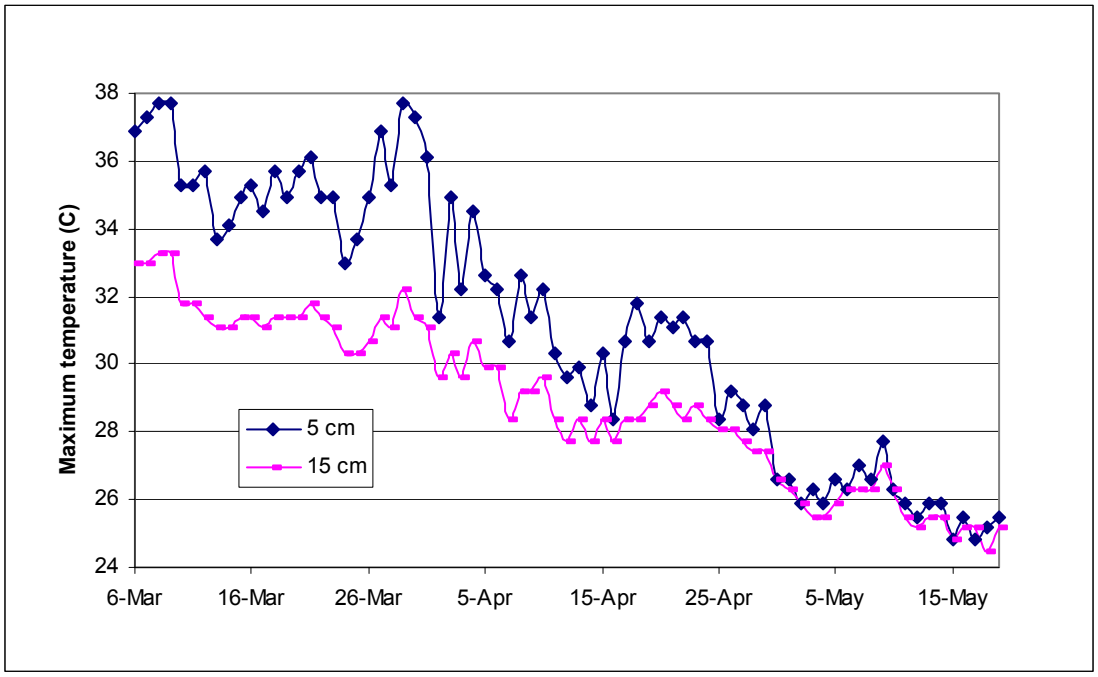


Temperatures recorded at Bowen are summarised in Table 2.4 for five half-month periods. Minimum, average and average maximum temperatures decreased at both depths as the season progressed. Daily maximum temperatures at both depths also tended to decrease over this time (Figure 2.2). During March and early April, the maximum temperature at 5 cm was greater than 34°C on most days and greater than 36°C on 9 days. At 15 cm, it was usually between 30 and 32°C. During April, maximum temperatures at 5 and 15 cm declined to below 32 and 30°C, respectively. By May, maximum temperatures at both depths were below 28°C.

Table 2.4: Soil temperatures at Bowen from March to May 2002 in beds covered with white plastic and planted to capsicum.

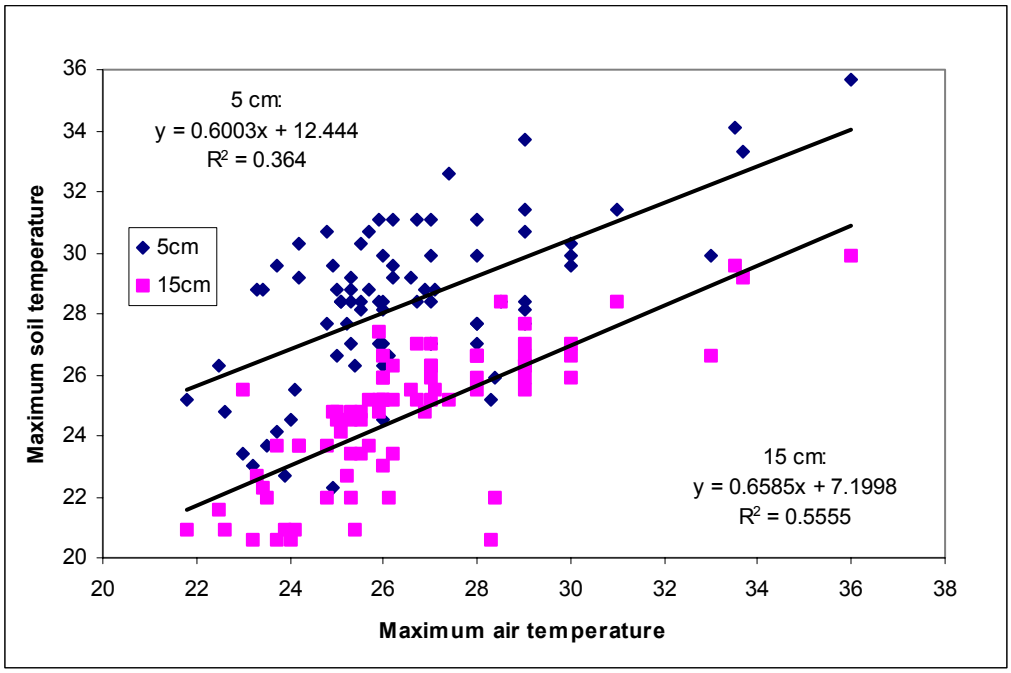
Time	Depth	Minimum temperature	Average temperature	Maximum temperature	Average max temperature
early – mid Mar	5 cm	25.2	30.2	37.7	35.9
	15 cm	28.1	30.4	33.3	32.1
mid – late Mar	5 cm	22.3	29.0	37.7	35.4
	15 cm	26.6	29.4	32.2	31.2
early – mid Apr	5 cm	23.0	27.2	34.9	31.6
	15 cm	25.9	27.8	30.7	29.1
mid – late Apr	5 cm	22.0	26.3	31.8	29.8
	15 cm	24.5	27.1	29.2	28.1
early – mid May	5 cm	19.5	24.3	27.7	26.2
	15 cm	22.7	25.1	27.0	25.9

Figure 2.2: Daily maximum soil temperatures at Bowen from March to May 2002 in beds covered with white plastic and planted to capsicum.



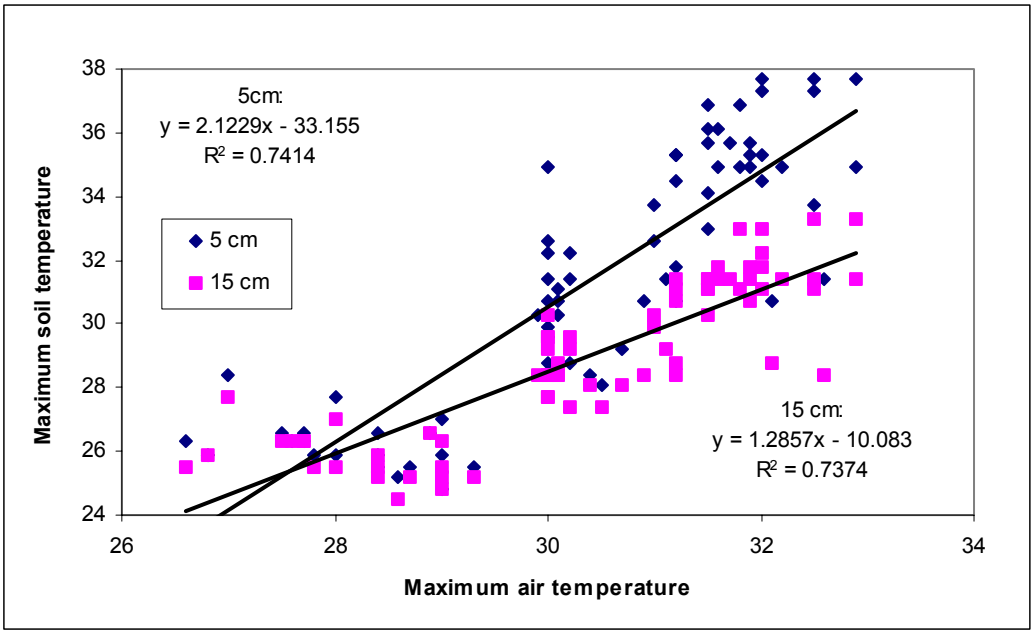
Regression analysis of maximum air and soil temperatures at Bundaberg (Figure 2.3) showed that these two parameters were related but the relationship was very weak (R^2 = 0.364 and 0.556 for depths of 5 and 15 cm respectively).

Figure 2.3: Relationship between maximum air temperatures and soil temperatures at 5 cm and 15 cm under a capsicum crop grown in beds covered with black plastic at Bundaberg.



The relationship between maximum soil temperatures and air temperatures (Figure 2.4) was stronger at Bowen than at Bundaberg (R^2 = 0.741 and 0.737 respectively). On days when the maximum air temperature was 32°C, the temperature at a depth of 5 cm under a capsicum crop typically reached 35°C. However, at 15 cm, the maximum soil temperature was usually lower than the maximum air temperature.

Figure 2.4: Relationship between maximum air temperatures and soil temperatures at 5 cm and 15 cm under a capsicum crop grown in beds covered with white plastic at Bowen.



Effect of plastic colour on soil temperature

Materials and methods

The effect of plastic colour on soil temperature, crop growth and incidence of sudden wilt was studied at Bundaberg in September and October 2002. The beds used were covered with black plastic, ran from east to west and contained a single row of capsicum plants with a single irrigation line slightly offset from the centre of the bed. The plants had been planted in late July 2002. Plots 15 m long containing 75 plants were marked out and white, low sheen paint was painted on the plastic in five replicate plots to produce the following treatments:

1. Black;
2. White on both sides of the bed from 3 September 2002;
3. White on the north side of the bed from 3 September 2002;
4. White on both sides of the bed from 2 October 2002;
5. White on the north side of the bed from 2 October 2002.

Temperature probes were placed approximately 10 cm from the edge of the bed and data loggers were used to record temperatures in selected plots at a depth of 5 cm. Daily maximum soil temperatures were compared to daily maximum air temperatures supplied by the Bureau of Meteorology and regression analysis was used to examine the relationship

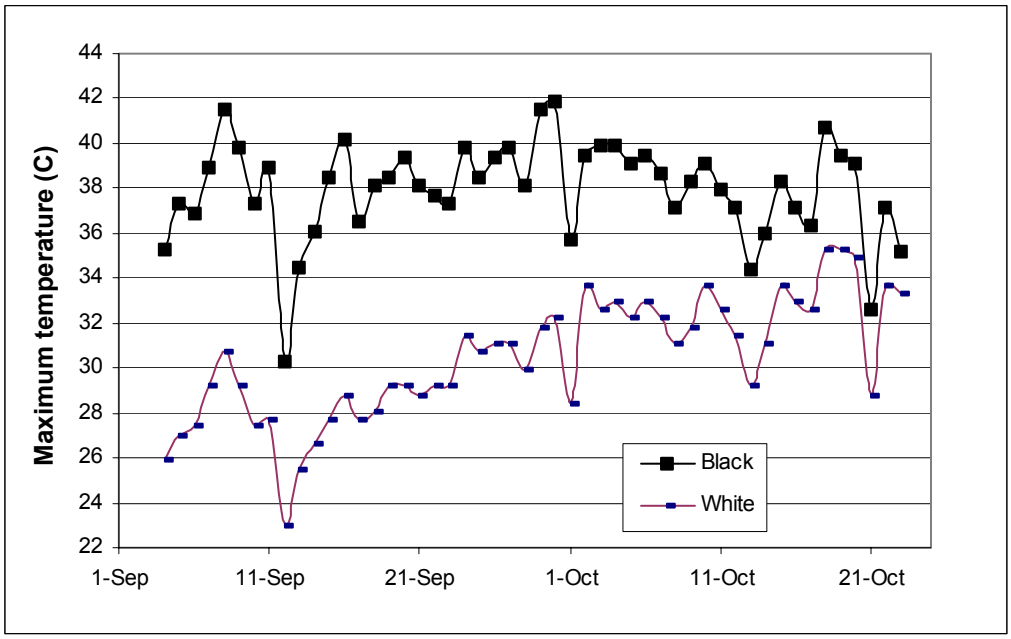
between air and soil temperatures for the different coloured plastics. On 23 October, when the crop was at fruit fill stage, differences in plant growth between treatments were noted and the number of plants with sudden wilt was counted.

Additional temperature readings were taken on 2 October, on a fine, partly cloudy day with a maximum temperature of 25°C. Such conditions are typical of those that occur in Bundaberg in early October. Soil temperatures were measured manually 10 cm from the edge of beds on both north and south sides, and in the centre of beds at depths of 5, 10 and 15 cm. Readings were taken regularly throughout the day.

Results

At a depth of 5 cm, soil temperature reached a maximum between 2 and 3 pm each day. Under the north side of black plastic, maximum temperatures usually ranged from 37-40°C in September and were approximately 10°C higher than those recorded under white plastic (Figure 2.5). In contrast, minimum soil temperatures (which occurred between 4 and 7 am) were within 1°C of each other under both coloured plastics (data not shown).

Figure 2.5: Maximum soil temperatures under the north side of black and white plastic at a depth of 5 cm in Bundaberg.



Painting the northern side of the bed white in September had almost the same impact on soil temperature at 5 cm as painting both sides white (Figure 2.6). When the northern side was

painted white and the southern side was left black, temperatures on the black southern side were initially higher than on the white northern side (Figure 2.7). However, as the crop grew and shaded the southern side, the situation was reversed and temperatures were 3 – 4 °C lower on the black southern side.

Figure 2.6: Maximum soil temperatures under the northern side of fully white and half white plastic at Bundaberg.

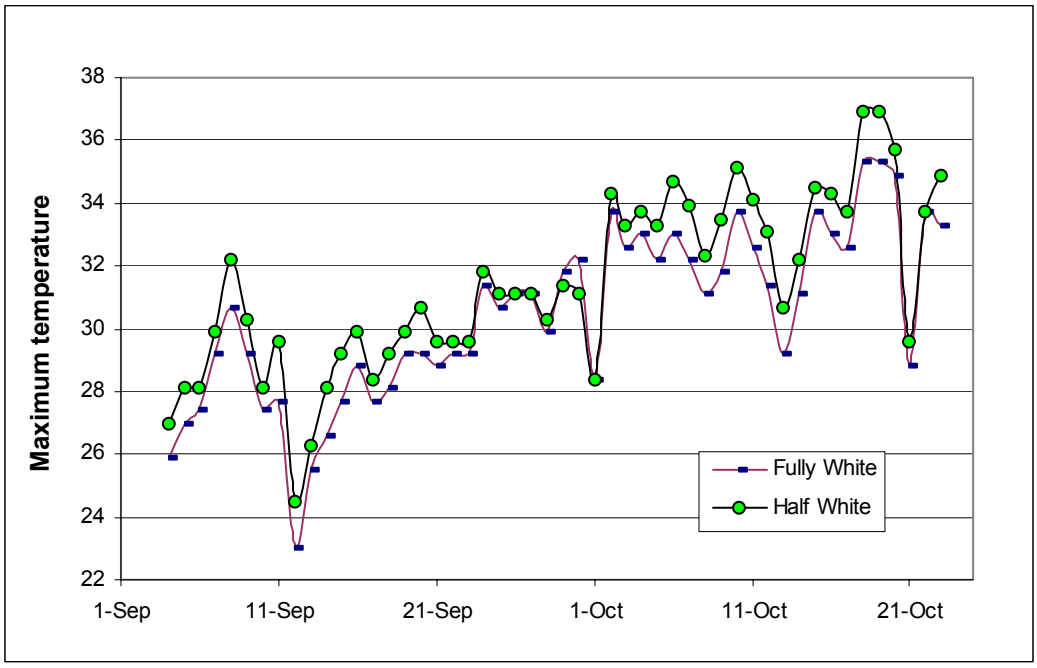
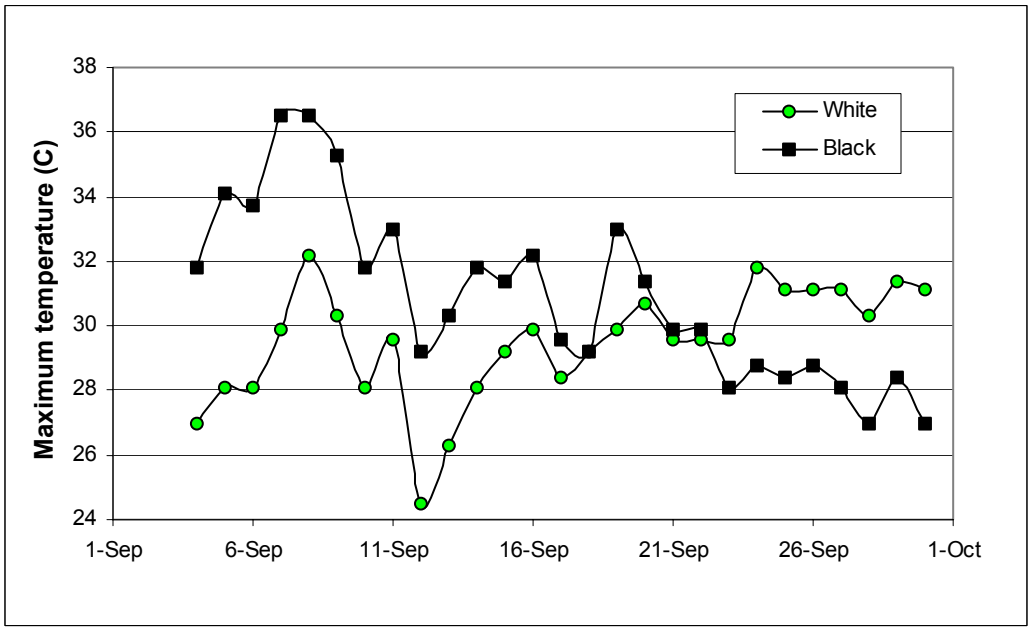
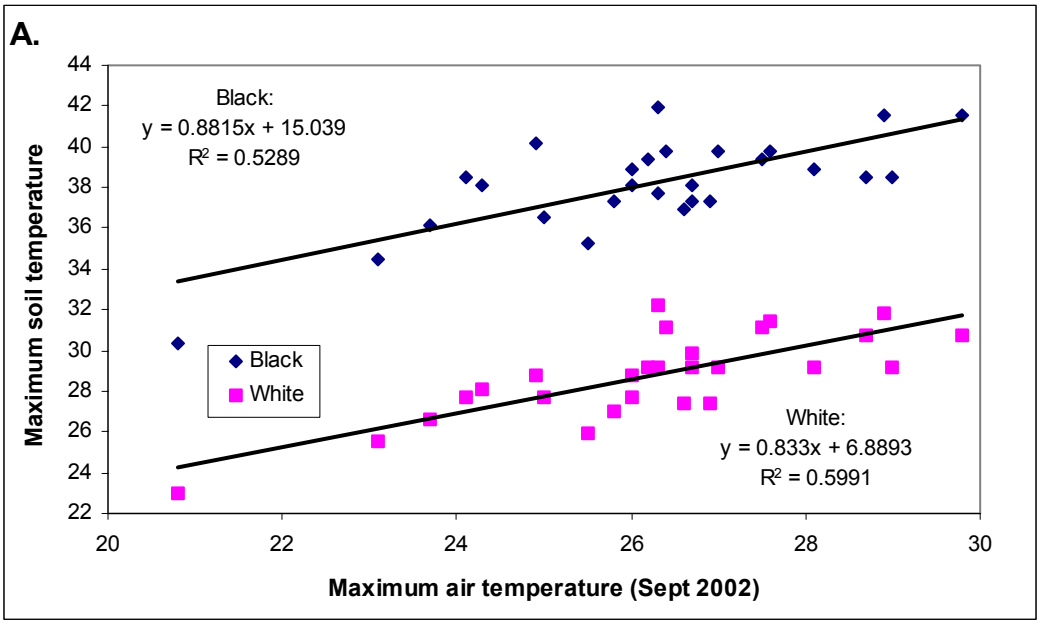


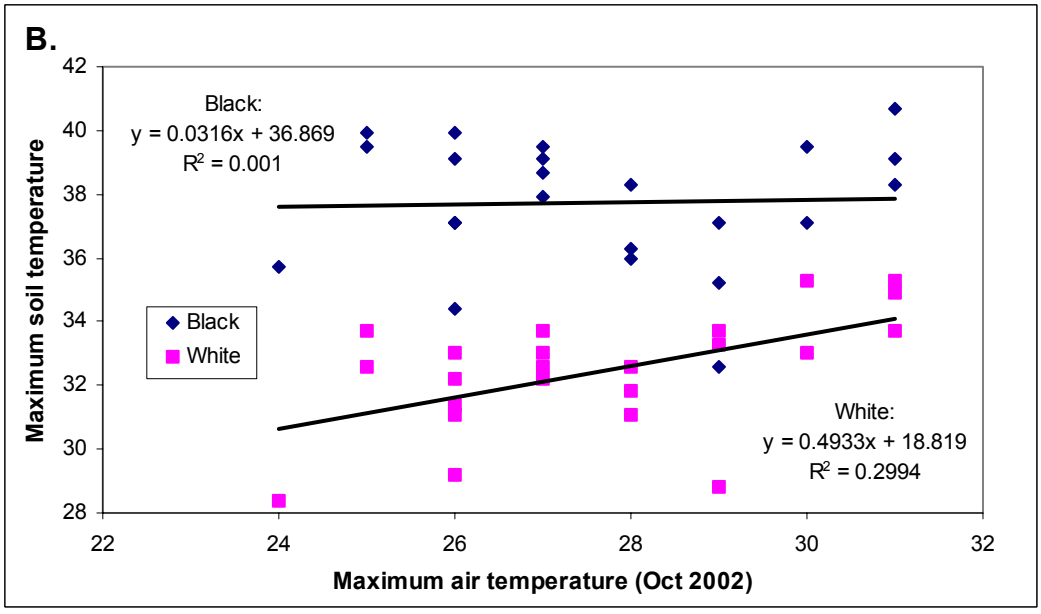
Figure 2.7: Maximum soil temperatures at a depth of 5 cm under plastic coloured white on the northern side and black on the southern side.



Plastic painted white in October affected soil temperatures in much the same way as plastic painted in September. Differences in temperatures were recorded between plastics painted in September and October, but they were never greater than 1.5°C (data not shown). As observed previously for black plastic in Bundaberg, there was a relatively weak relationship (R^2 = 0.0.529 and 0.599 respectively) between maximum air temperature and maximum soil temperatures at 5 cm under black and white plastic in September (Figure 2.8). In October, this relationship virtually disappeared (Figure 2.8).

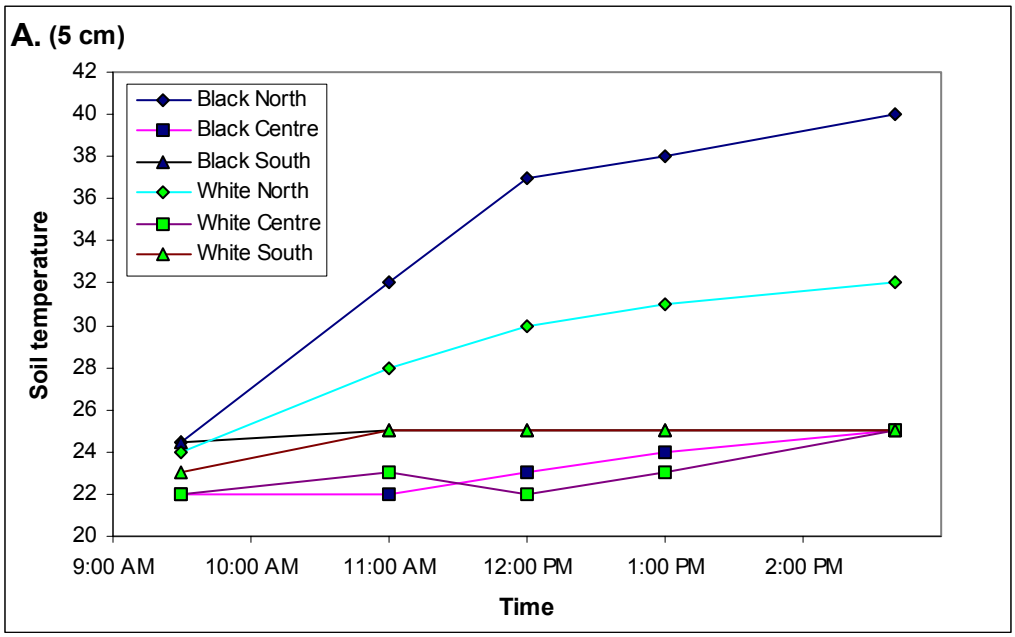
Figure 2.8: Relationship between maximum air temperatures and maximum soil temperatures at 5 cm on the northern side of a capsicum bed at Bundaberg in September (A) and October (B).

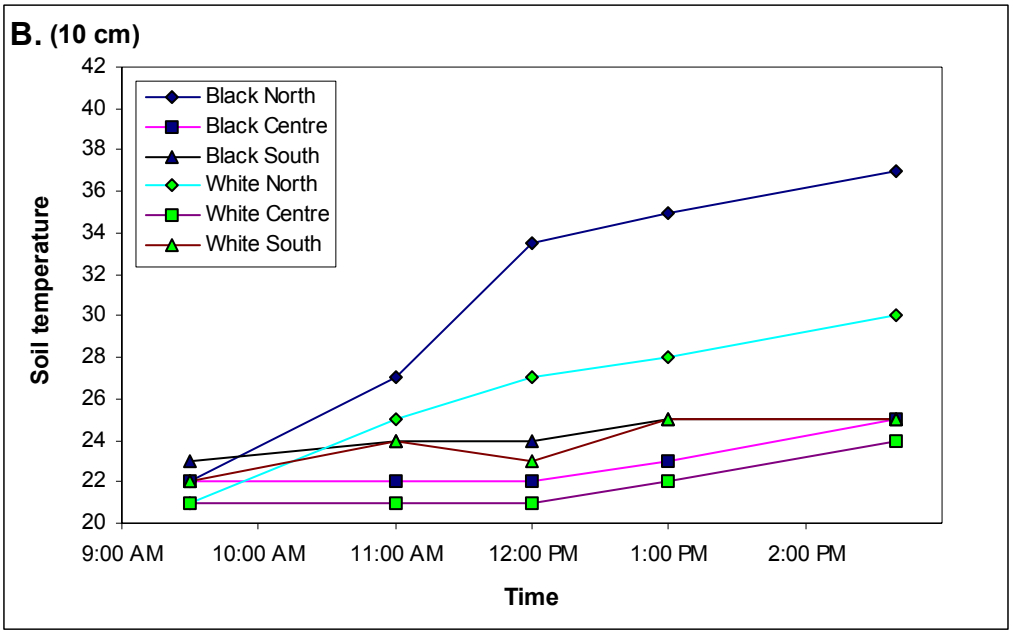


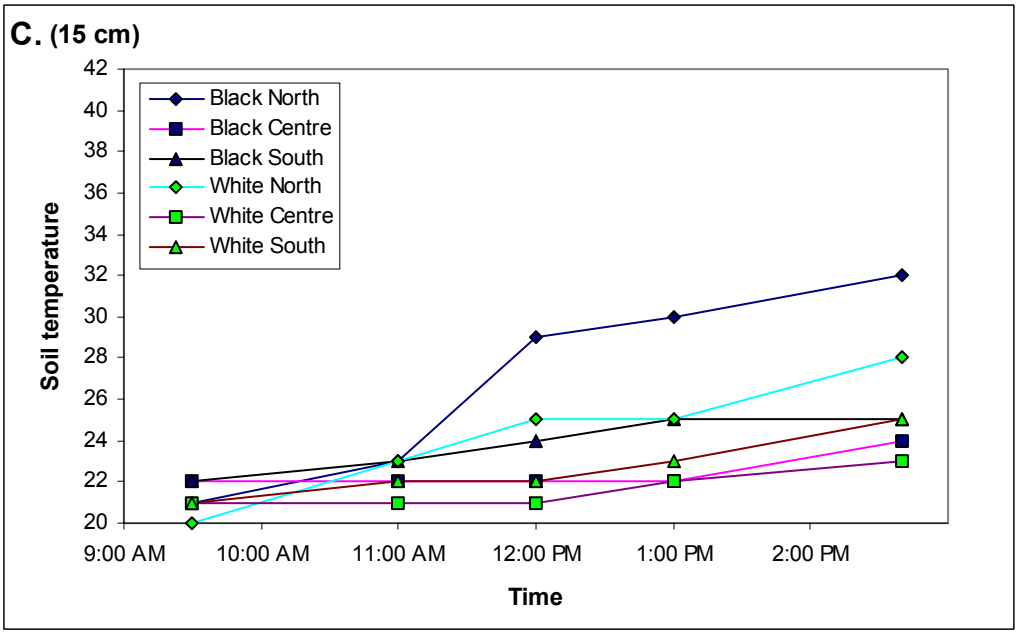


Manual soil temperature measurements taken on 2 October 2002 are shown in Figure 2.9. At 9:30am, soil temperatures at the north, centre and south sampling positions at each depth did not differ by more than 2°C, with temperature decreasing with depth. Soil temperature increased during the day but increases were only a few degrees at the centre and south sampling positions, and temperatures did not rise above 23 - 25°C at any depth. Temperatures were lowest in the centre of the bed and these temperatures, and those on the southern side, were not affected by plastic colour. Temperature was always highest at all depths on the northern sides of both black and white plastic, with black always hotter than white.

Figure 2.9: Effect of sampling position and plastic colour on soil temperatures at 5 cm (A), 10 cm (B) and 15 cm (C) on 2 October 2002 in a bed planted to capsicum at Bundaberg.







No diseased plants were observed during the course of the experiment and there were no obvious differences in growth of capsicum plants resulting from differences in plastic colour.

Discussion

The data presented in this chapter indicate that high soil temperatures often occur in the main capsicum-growing areas of Queensland. Temperatures in the mid to high 30's are common at a depth of 5 cm, particularly on the northern sides of plastic-covered beds used for capsicum production. In Bowen, where white plastic is used throughout the year, the reflective properties of the plastic are not good enough to negate the high temperatures that invariably

occur in the tropics in March and early April, when the first capsicum crops are planted. In the cooler climate of Bundaberg, high soil temperatures only occur when black plastic is used on winter-planted crops. The heat-absorbing characteristics of black plastic are advantageous in winter and early spring when low soil temperatures limit plant growth, but later in the season soil temperatures are raised to levels that are sub-optimal for plant growth (see chapter 6).

Our studies indicated that soil temperatures in beds used for capsicum production are not uniform, but vary with compass direction, distance from the edge of the bed, depth from the soil surface, time of the day, and crop age. Temperatures on the northern side of beds are much higher than the centre or southern side, and soil near the surface is hotter than at depth. Areas under the plant or shaded by plants are cooler than exposed areas, which means that excessive temperatures are most likely to occur soon after planting, when the canopy is insufficient to provide adequate shade. Also, the position of the shaded areas changes during the day as the sun passes from east to west. Thus, the temperatures to which capsicum root systems are exposed are never constant. At any one time, some roots may be growing in soil at temperatures suitable for growth while others are being affected by excessive heat.

Because records of soil temperature are not readily available, we checked to see whether air temperature could be used to predict soil temperature. However, the relationship between air and soil temperature was not strong enough or consistent enough to be useful. A close linear relationship could perhaps be expected in beds without plants, and the poor relationship obtained in our studies was probably due to the increasing amount of shade provided by the capsicum crop as the season progressed. Other meteorological parameters such as cloud cover, wind speed and wind direction may also influence soil temperature.

Although temperature and rainfall varied during the year and from year to year, we found it difficult to relate the occurrence and severity of sudden wilt outbreaks to particular weather conditions. The main problem was that there are no reliable records on the incidence of sudden wilt, and we had to rely on anecdotal evidence. Growers in Bowen indicated that sudden wilt occurred in most years and that it was most common in crops planted early in the year. Although there could be many reasons for this, this pattern of occurrence suggests that high temperatures may be involved in the disease syndrome. Bowen is situated in the tropics and temperatures are always high, particularly in summer. Our data showed that soil temperatures of 34-38°C are common at depths of 5 cm in Bowen during March.

In Bundaberg, there was a general consensus amongst growers that sudden wilt rarely causes problems in crops planted in summer. Thus, the etiology of the disease appears to differ in Bundaberg and Bowen, despite the fact that crops in both districts are planted on white plastic. One possible reason for this is temperature. At the start of the planting season (early February in Bundaberg and early March in Bowen), it is hotter in Bowen than in Bundaberg.

There are reliable records to indicate that sudden wilt was severe in Bundaberg during the spring of 1991 and 1997. Although this number of outbreaks is insufficient to draw firm conclusions about factors that may have associated with these outbreaks, the meteorological data show that the spring of both these years was amongst the hottest and driest of the last 13 years. Thus, it is possible that the negative effects of high soil temperatures on root growth are involved in the disease. Although capsicum crops are irrigated, it is also possible that moisture stress during periods of low rainfall plays a role, as the standard of irrigation management may be more critical under such conditions than when some of the crop's moisture needs are supplied by rainfall.

When black plastic was painted white in early September, we demonstrated that soil temperatures were reduced markedly without any negative impact on crop growth. Not surprisingly, most of this reduction could be achieved by painting only the northern side of beds, as the southern side is shaded to some extent at that time of the year. This observation has important practical implications, because it provides growers in Bundaberg with a relatively inexpensive way of reducing the impact of the excessive soil temperatures that can occur under black plastic in spring.

Chapter 3: Soil-borne pathogens associated with healthy and sudden wilt-affected capsicum crops

Introduction

There have been no systematic surveys for pathogens associated with sudden wilt of capsicum. *Pythium* spp., *Fusarium* spp., *Macrophomina phaseolina* and *Rhizoctonia solani* have been isolated from diseased plants (Olsen and Barnes, 1999) but it is not known whether they are consistently associated with the disease. Capsicum crops in the Bowen and Bundaberg regions were therefore surveyed to identify potential pathogens that associated with sudden wilt and determine whether soil fumigation, crop age and planting date affected the range of organisms present. Since sequential samples were taken from some crops, the temporal development of the disease was also studied.

Materials and Methods

Ten crops (eight from Bundaberg and two from Bowen) selected on the basis of planting date and soil fumigation practices (Appendix 2A) were sampled two or three times during the growing season. A further six crops from the Bundaberg / Murgon region and nine from Bowen and Gumlu (Appendix 2B) were sampled when growers or crop management consultants indicated that symptoms of sudden wilt were present.

Five healthy and five diseased plants were randomly selected in each field. Sudden wilt-affected plants were typically in the early stages of the disease (i.e. they were just beginning to wilt and had lost some leaves or showed incipient leaf yellowing). The number of large roots (i.e. roots more than 3 mm in diameter arising from the crown) and the number of plants with visible collar rot was counted and crowns and stems were examined for crown lesions and vascular staining, respectively. Small roots were rated for root-rotting using a scale where 1 = 0-5%, 2 = 6-25%, 3 = 26-50%, 4 = 51-75% and 5 = 76-100% of the roots rotted.

Where possible, four pieces of infected tissue were taken from small and large roots of each plant. Root tissue was surface sterilised in 1% NaOCl (small roots for 1.5 minutes and large roots for up to 3 minutes), then rinsed twice in sterile distilled water before being blotted dry on sterile tissue. Ten pieces were then plated onto both PDA + S (potato dextrose agar amended with streptomycin 0.12 g L^{-1}) and 3P (cornmeal agar amended with 0.05 g L^{-1} penicillin; 0.05 g L^{-1} polymixin and 0.025 g L^{-1} pimaricin, Eckert and Tsao, 1962). At the last

two sampling times, tissue plated on 3P was not sterilised with NaOCl, but was washed twice in sterile distilled water. For crown and stem tissue, the lower 7cm of stem was flamed in 98% ethanol and then split longitudinally with a sterile knife. Two pieces of crown or vascular tissue were then plated onto the above media.

Fungi were identified and the number of pieces of tissue colonised by each genus was recorded. Pure cultures of *Fusarium* spp. were stored on filter paper and CLA (carnation leaf agar) in sterile distilled water while other fungi were stored on PDA in sterile distilled water.

Results

Observations on disease development

Symptoms were never observed in young plants prior to fruit set and the first sign of disease was a slight yellowing of the leaves. This was followed by minor wilting of the leaves, especially in the heat of the day, and some fruit shrivelling. The final stage was the collapse of the plant, with leaves dying and only small flaccid red fruit remaining on the stem and branches. By the time above-ground symptoms were apparent, the root systems were either severely rotted or rotted on one side up into the crown. Mature plants usually had between 10 and 17 large roots growing from the crown, regardless of variety. Small roots grew mainly from the large roots and rotting was first apparent in these roots, even in healthy plants. Infection in large roots appeared to emanate from rotted small roots and lesions then coalesced to girdle the large root. Girdling lesions eventually extended up the root and completely rotted it to the crown. Once large roots were rotted, the cortex was easily peeled away to leave an intact but discoloured stele.

Rotted large roots were not always observed in any of the crops surveyed. However, rotted small roots were always present, with symptom severity increasing as plants matured (Appendix 2C). The degree of rotting of small and large roots was similar in non-fumigated crops and in crops treated with methyl bromide or metham sodium. However, more large roots were rotted in crops planted in winter and spring in Bundaberg than in Bundaberg crops planted in autumn. Crown rot and vascular staining was negligible in Bundaberg and was not observed in Bowen.

Of the 10 crops selected for temporal observation, two crops from Bowen had both healthy and sudden wilt-affected plants. Wilted plants had more rotted roots than healthy plants, with a root-rot rating greater than 4 for small roots and more than 75% of large roots rotted. The

severity of root rotting was initially lower in the field treated with metham sodium, but later in the season, there was no difference between fields. Crown rot was observed in plants showing symptoms of sudden wilt but vascular staining was never observed.

Fusarium was the organism most commonly isolated from large and small roots in both Bundaberg and Bowen, regardless of whether plants were healthy or affected by sudden wilt (Appendix 2D). *Pythium* was also common, as it was isolated from all but two fields. *Fusarium* and *Pythium* were both recovered from fumigated and non-fumigated fields. *Macrophomina* was found in both healthy and sudden wilt-affected plants from Bowen, while *Rhizoctonia* was isolated only from sudden wilt-affected plants. No fungal pathogens were isolated from crown tissue and *Fusarium* was isolated from the vascular tissue of only one healthy plant.

Observations of sudden wilt-affected crops

Except for one crop at Bundaberg, more than 75% of the large roots of plants affected by sudden wilt were rotted and the root rot rating for small roots was greater than 3.4 (Appendix 2E). Both large and small roots of plants affected with sudden wilt were more severely rotted than their healthy counterparts in the same field (Appendix 2E). The mean root-rot rating for small roots was 1.7 for healthy plants compared with 4.0 for sudden wilt-affected plants. Only 7% of the large roots of healthy plants were rotted, whereas 84% of roots on sudden wilt-affected plants were rotted. The severity of root rotting was similar in Bundaberg and Bowen and did not appear to be affected by planting time or whether fields were fumigated.

Fusarium and *Pythium* were commonly isolated from roots of both healthy and sudden wilt-affected crops, including crops fumigated with methyl bromide (Appendix 2F). *Macrophomina* and *Rhizoctonia* were also present, but were recovered less frequently. *Fusarium* was obtained from vascular tissue at five sites and other unidentified fungi were isolated occasionally. In most cases, however, fungi were not recovered from stained vascular tissue. Crown rot occurred in eight fields and isolations from crown tissue yielded *Fusarium* and *Pythium* in five and one field respectively. Crown lesions were generally associated with extensive root-rot, suggesting that infection had moved from diseased roots into the crown tissue.

Isolates retained for further study

A total of 389 fungal isolates were obtained from capsicum tissue during the surveys and from informal plant collections. *Fusarium* and *Pythium* were the most frequently isolated

pathogens, as they were present in 92% and 72% of the fields surveyed. *Macrophomina* and *Rhizoctonia* were recovered from about one-third of sites (Table 3.1).

Table 3.1: Percentage of capsicum fields surveyed from which various fungal pathogens were isolated.

Fungus	Roots of healthy plants (16 sites) *	Roots of sudden wilt-affected plants (17 sites) *
Pythium	69	71
Fusarium	94	94
Macrophomina	19	53
Rhizoctonia	6	47

* Although 25 sites were sampled, some sites had both healthy and sudden wilt-affected plants.

Discussion

Sudden wilt was not a major problem during the survey period, as it was difficult to find wilted plants in most crops. Nevertheless, close inspection showed that there were usually some diseased plants in crops at the fruit fill stage. Observations made during the surveys clearly showed that severe root rotting always occurred before plants collapsed with sudden wilt. In most cases, 70-90% of the large roots were rotted in plants with incipient sudden wilt symptoms compared with fewer than 25% in healthy plants. Rotting appeared to start in small roots and move progressively into the large roots. Since crown rot could usually be traced back to a root that had rotted back to the crown, crown rot is probably an advanced stage of root-rot. When vascular staining occurred, it was generally light in colour and of limited distribution. It did not appear to be associated with the disease, as vascular tissue quickly darkened when stems were split due to oxidisation or some other similar process.

Fusarium was the fungus most commonly associated with rotted capsicum roots, but *Pythium* was also widely distributed, whether plants were affected by sudden wilt or not. Although *Pythium* was isolated from 72% of sites, this was probably an under-estimate because evidence obtained after the survey work was completed suggested that growth from infected plant tissue was inhibited when NaOCl was used to surface-sterilise tissue. The lack of a relationship between the presence of these fungi and sudden wilt was largely due to the fact that healthy plants always had some rotted roots. *Pythium* and *Fusarium* were generally not recovered from healthy roots on healthy plants. *Macrophomina* and *Rhizoctonia* were found much less frequently and were often not recovered from sudden wilt-affected plants.

Pythium and *Fusarium* were found at similar frequencies in all production regions whereas both *Macrophomina* and *Rhizoctonia* were isolated more frequently from Bowen and Gumlu

than from Bundaberg and Murgon. However, this conclusion is based on a relatively small number of samples and may have been confounded by fumigation. There were five fumigated sites at Bundaberg and only one at Bowen and neither *Macrophomina* nor *Rhizoctonia* were isolated from any of the fumigated sites. The range of pathogens present did not appear to be influenced by planting season or cultivar.

Chapter 4: Pathogenicity of various fungi on capsicum

Introduction

During the survey phase of this project (see chapter 3), we confirmed that severe root rotting occurs when capsicum plants are affected by sudden wilt. We also found that *Pythium*, *Fusarium*, *Rhizoctonia* and *Macrophomina* spp. were associated with the disease. In this chapter, isolates of these fungi from various locations were screened to identify those able to cause root rotting. In the first stage of a three-step process, newly germinated capsicum seedlings were exposed to fungi on water agar and pathogenic and non-pathogenic isolates were differentiated. Pathogenic isolates were then inoculated onto seedlings in small containers to determine whether they were capable of rotting roots of plants growing in soil. Finally, capsicum plants at fruit-fill stage were challenged with some of the isolates that were pathogenic in the two previous tests.

Agar test

Materials and Methods

Capsicum (cv. Target) seeds were sterilised in 50% ethanol for 2 minutes and plated onto water agar. At the same time, 90 mm Petri plates containing water agar were inoculated with four cubes of agar from fungal cultures grown on PDA. When seeds had germinated, one seed was placed next to each cube and plates were incubated at room temperature (about 25°C). After 3, 5, 7, 10 and 12 days, the degree of root rotting was evaluated using a scale where 1 = entire root white and healthy, 2 = half the root brown and 3 = root entirely brown. Isolates were considered pathogenic if three or four seedlings had root rot ratings of 2 or 3 after 12 days.

Results

Results (Table 4.1) showed that 56 of 58 isolates of *Pythium* were pathogenic, with most isolates totally rotting roots in 3-5 days. Only one-third of *Fusarium* isolates were pathogenic, and those that caused damage did not rot roots as rapidly as *Pythium*. Severe root rotting was rarely observed at 7 days and pathogenicity was usually not confirmed until 12 days. *Macrophomina* and *Rhizoctonia* also took longer than *Pythium* to produce symptoms. Many isolates were not rated as pathogenic until 10 days and pathogenic isolates of *Rhizoctonia* often caused only mild browning of roots.

Table 4.1: Pathogenicity of four fungi to newly-germinated capsicum seedlings after 12 days on agar.

Genus	No. of isolates tested	No. of pathogenic isolates	% pathogenic isolates
Pythium	58	56	97
Fusarium	120	41	34
Macrophomina	11	8	73
Rhizoctonia	13	8	62

Seedling test

Materials and methods

Four week-old capsicum seedlings (cv. Target) with two leaves above the cotyledons were grown in Speedling® cells and transplanted into pots containing 200 mL of inoculated or non-inoculated pasteurised potting mix. Inocula of *Pythium* and *Rhizoctonia* were produced by growing the fungi at 28°C for 15 days on CMS1 (cornmeal 3 g; sand 97 g; water 15 mL). *Macrophomina* was grown at the same temperature for the same time on CMS2 (cornmeal 14.2 g; sand 75 g; water 19.7 mL), while *Fusarium* was grown on PDA at 28°C for 7 days. Potting mix was inoculated with *Pythium*, *Rhizoctonia* or *Macrophomina* by incorporating CMS into soil at 10 g L^{-1}. Conidia of *Fusarium* were suspended in sterile water and incorporated at a rate of 5 x 10^5 conidia per mL of soil. To standardise tests run at different times, *Pythium* isolates SW82 and SW325 were included as standards in each experiment. Potting mix containing non-inoculated CMS1 or CMS2 was used as a control and each isolate was tested on three replicate plants. Soil temperature was recorded with a data logger (Gemini Tiny Talk®) placed in a pot at a depth of 8 cm.

Plants were grown in a glasshouse, harvested after 3-4 weeks and the wet weight of roots and shoots was measured. Roots were then surface sterilised in 1% NaOCl for 2 minutes, rinsed in sterile distilled water and 5-10 pieces of tissue were plated onto PDA + S. Roots inoculated with *Pythium* were washed in sterile distilled water and plated onto 3P.

Results

Tests were done over a nine-week period and each test took 3 – 4 weeks. During this time, the average weekly soil temperature was generally greater than 26°C and maximum soil temperatures ranged from 28.8 to 38.5°C (Table 4.2).

Table 4.2: Soil temperatures when fungi were being tested for pathogenicity to capsicum seedlings in pots.

Period	Minimum	Average	Maximum	Average maximum
Week 1	23.4	26.4	31.4	29.1
Week 2	22.3	26.8	37.3	30.1
Week 3	25.2	27.7	36.5	33.3
Week 4	24.8	28.6	36.5	34.9
Week 5	24.1	28.0	34.1	32.2
Week 6	21.3	27.2	36.5	32.7
Week 7	21.3	28.3	38.5	34.7
Week 8	20.2	25.6	32.6	30.3
Week 9	18.1	22.4	28.8	25.6
Weeks 1 - 9	18.1	26.8	38.5	31.4

The results of individual experiments are shown in Tables 4.3, 4.4 and 4.5. The data suggest that the experiments were done under similar conditions using plants at the same stage of growth, as the shoot and root weights of the control plants were similar in all three experiments. Also, the two *Pythium* isolates used as standards (SW 82 and SW325) responded consistently in the three experiments, with total plant biomass being reduced by 83-97% and 45-68% of the controls, respectively.

Pythium isolates fell into two broad groups (Tables 4.3 and 4.4). One highly pathogenic group, consisting of *P. myriotylum* and *P. aphanidermatum* (see chapter 5), reduced shoot and root weight by more than 80% and sometimes killed plants. A less pathogenic group, consisting of *P. dimorphum* and *P. diclinum*, reduced shoot and root weight by 20-80% (Figure 1 A and B). Both groups often rotted the primary root up into the crown and affected plants sometimes produced adventitious roots above crown lesions. These secondary roots were usually white and lesion-free, probably because they had formed just prior to harvest. In experiment 3, where temperatures during the last week were much lower than previous weeks, there were signs of recovery in plants inoculated with the standard isolates of *Pythium*.

Table 4.3: Pathogenicity of various fungal isolates on capsicum seedlings after 3-4 weeks in pots (Experiment 1: 2–26 February 2001).

Isolate	Genus ♦	Shoot weight (g)		Reduction in shoot weight relative to control (%) *	Root weight (g)		Reduction in root weight relative to control (%)
CMS 1	Control 1	1.33	a	0	1.33	a	0
CMS 2	Control 2	1.43	a	0	1.40	a	0
SW 256	*Macrophomina*	1.33	a	7	1.30	a	7
SW 337	*Rhizoctonia*	0.63	bc	53	0.49	bc	63
SW 357	*Rhizoctonia*	0.62	bc	53	0.47	bc	65
SW 334	*Rhizoctonia*	0.57	c	57	0.43	bc	68
SW 350	*Rhizoctonia*	0.56	cd	58	0.52	bc	61
SW 178	*Pythium* 2	0.29	efgh	78	0.29	cdef	78
SW 111	*Pythium* 2	0.32	defg	76	0.30	cde	77
SW 135	*Pythium* 2	0.47	cdef	65	0.43	bc	68
SW 75	*Pythium* 2	0.48	cdef	64	0.41	bc	69
SW 213	*Pythium* 2	0.51	cde	62	0.32	cd	76
SW 325	*Pythium* 2	0.54	cd	59	0.49	bc	63
SW 226	*Pythium* 2	0.58	c	56	0.44	bc	67
SW 89	*Pythium* 2	0.82	b	38	0.56	b	58
SW 204	*Pythium* 1	0.24	fghi	82	0.09	defg	93
SW 85	*Pythium* 1	0.04	i	97	0.03	g	98
SW 82	*Pythium* 1	0.07	hi	95	0.05	fg	96
SW 100	*Pythium* 1	0.08	ghi	94	0.05	fg	96
SW 73	*Pythium* 1	0.10	ghi	92	0.06	fg	95
SW 80	*Pythium* 1	0.11	ghi	92	0.06	efg	95
SW 201	*Pythium* 1	0.11	ghi	92	0.10	defg	92
SW 224	*Pythium* 1	0.13	ghi	90	0.08	efg	94
SW 88	*Pythium* 1	0.13	ghi	90	0.10	defg	92
SW 103	*Pythium* 1	0.15	ghi	89	0.09	defg	93
SW 90	*Pythium* 1	0.15	ghi	89	0.10	defg	92
SW 176	*Pythium* 1	0.15	ghi	89	0.15	defg	89
SW 92	*Pythium* 1	0.16	ghi	88	0.09	defg	93
SW 93	*Pythium* 1	0.19	ghi	86	0.13	defg	90
SW 74	*Pythium* 1	0.18	ghi	86	0.12	defg	81
SW 83	*Pythium* 1	0.20	ghi	85	0.10	defg	92
SW 98	*Pythium* 1	0.21	ghi	84	0.12	defg	91
l.s.d. (P = 0.05)		0.241			0.239		

* Numbers within the same column that are followed by the same letters are not significantly different (P = 0.05).

♦ Horizontal lines separate isolates into broad groups based on their identity and pathogenicity. Highly pathogenic isolates of *Pythium* (*eg*. SW82,) are designated *Pythium* 1 and some were identified as *P. myriotylum* or *P. aphanidermatum*. Less pathogenic isolates (*eg*. SW325) are designated as *Pythium* 2 and some were identified as *P. dimorphum* or *P. diclinum* (see chapter 5). However, SW 213 was *P. myriotylum*.

Table 4.4: Pathogenicity of various fungal isolates on capsicum seedlings after 3-4 weeks in pots (Experiment 2: 23 February to 16 March 2001).

Isolate	Genus ♦	Shoot weight (g)	Reduction in shoot weight relative to control (%)	Root weight (g)	Reduction in root weight relative to control (%)
CMS 1	Control 1	1.33 a	0	1.33 abc	0
CMS 2	Control 2	1.40 a	0	1.43 a	0
SW 245	*Macrophomina*	1.43 a	0	1.27 bcd	11
SW 349	*Macrophomina*	1.40 a	0	1.40 ab	2
SW 343	*Macrophomina*	1.37 a	2	1.30 abcd	8
SW 128	*Macrophomina*	1.37 a	2	1.30 abcd	9
SW 265	*Macrophomina*	1.33 a	5	1.27 bcd	11
SW 10	*Macrophomina*	1.33 a	5	1.33 abc	7
SW 339	*Macrophomina*	1.30 a	7	1.30 abcd	8
SW 259	*Macrophomina*	1.30 a	7	1.23 cd	14
SW 116	*Macrophomina*	1.23 a	12	1.17 d	18
SW 276	*Macrophomina*	1.23 a	12	1.27 bcd	11
SW 354	*Rhizoctonia*	0.65 bc	51	0.53 ef	50
SW 273	*Rhizoctonia*	0.64 bc	52	0.60 e	55
SW 254	*Rhizoctonia*	0.64 bc	52	0.52 efg	61
SW 22	*Rhizoctonia*	0.61 bc	54	0.54 ef	59
SW 118	*Rhizoctonia*	0.56 bc	58	0.46 efgh	65
SW 319	*Rhizoctonia*	0.54 bc	59	0.47 efgh	65
SW 165	*Pythium* 2	0.67 b	50	0.52 efg	61
SW 187	*Pythium* 2	0.57 bc	57	0.42 fgh	68
SW 325	*Pythium* 2	0.49 bc	63	0.43 fgh	68
SW 66	*Pythium* 2	0.49 bc	63	0.34 h	74
SW 67	*Pythium* 2	0.45 c	66	0.37 gh	72
SW 97	*Pythium* 1	0.19 d	85	0.08 i	94
SW 206	*Pythium* 1	0.15 d	89	0.08 i	94
SW 235	*Pythium* 1	0.08 d	94	0.12 i	91
SW 82	*Pythium* 1	0.08 d	94	0.06 i	95
SW 249	*Pythium* 1	0.05 d	96	0.03 i	98
l.s.d (P = 0.05)					
	isolate *vs* isolate	0.213		0.160	
	isolate *vs* control	0.184		0.138	

* Numbers within the same column that are followed by the same letters are not significantly different (P = 0.05).

♦ Horizontal lines separate isolates into broad groups based on their identity and pathogenicity. Highly pathogenic isolates of *Pythium* (*eg*. SW82,) are designated *Pythium* 1 and some were identified as *P. myriotylum* or *P. aphanidermatum*. Less pathogenic isolates (*eg*. SW325) are designated as *Pythium* 2 and some were identified as *P. dimorphum* or *P. diclinum* (see chapter 5).

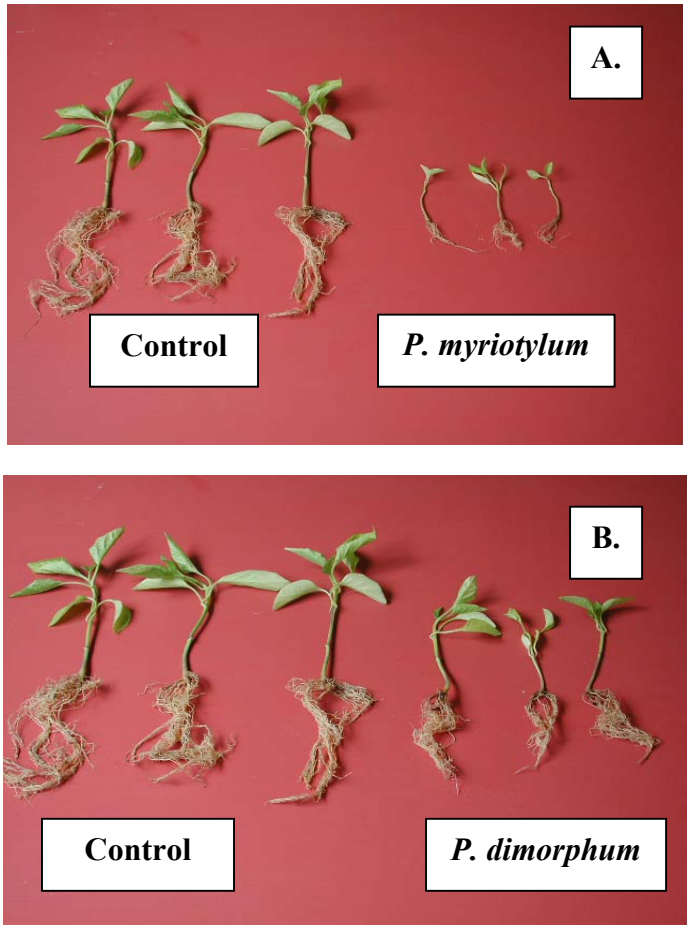
Table 4.5: Pathogenicity of various fungal isolates on capsicum seedlings after 3-4 weeks in pots (Experiment 3: 9 March – 5 April 2001).

Isolate	Genus ♦	Shoot weight (g)		Reduction in shoot weight relative to control (%)	Root weight (g)		Reduction in root weight relative to control (%)
CMS 1	Control	1.32	a-c	0	1.25	a	0
SW 275	*Fusarium*	1.40	a	0	1.33	a	0
SW 33	*Fusarium*	1.37	ab	0	1.27	a	0
SW 20	*Fusarium*	1.33	abc	0	1.23	a	2
SW 274	*Fusarium*	1.33	abc	0	1.23	a	2
SW 43B	*Fusarium*	1.30	abc	2	1.27	a	0
SW 260	*Fusarium*	1.30	abc	2	1.27	a	0
SW 43A	*Fusarium*	1.27	abc	4	1.30	a	0
SW 86A	*Fusarium*	1.27	abc	4	1.30	a	0
SW 86B	*Fusarium*	1.27	abc	4	1.33	a	0
SW 143	*Fusarium*	1.27	abc	4	1.33	a	0
SW 139	*Fusarium*	1.27	abc	4	1.23	a	2
SW 270	*Fusarium*	1.27	abc	4	1.20	a	4
SW 247	*Fusarium*	1.23	abc	7	1.27	a	0
SW 248	*Fusarium*	1.23	abc	7	1.23	a	2
SW 130	*Fusarium*	1.23	abc	7	1.20	a	4
SW 99	*Fusarium*	1.20	abc	9	1.23	a	2
SW 244	*Fusarium*	1.20	abc	9	1.13	a	10
SW 250	*Fusarium*	1.17	bc	12	1.27	a	0
SW 231	*Fusarium*	1.17	bc	12	1.23	a	2
SW 246	*Fusarium*	1.17	bc	12	1.23	a	2
SW 35	*Fusarium*	1.13	c	14	1.17	a	6
SW 325	*Pythium* 2	0.71	d	45	0.55	b	46
SW 82	*Pythium* 1	0.23	e	83	0.19	c	85
l.s.d. (P = 0.05)							
	isolate *vs* isolate	0.213			0.228		
	isolate *vs* control	0.184			0.198		

* Numbers within the same column that are followed by the same letters are not significantly different (P = 0.05).

♦ Horizontal lines separate isolates into broad groups based on their identity and pathogenicity. The highly pathogenic isolate of *Pythium* (SW82,) is designated *Pythium* 1 and was identified as *P. myriotylum*. The less pathogenic isolate of *Pythium* (SW325) is designated as *Pythium* 2 and was identified as *P. dimorphum* (see chapter 5).

Figure 4.1: Effect of *P. myriotylum* isolate SW82 (A) and *P. dimorphum* isolate SW325 (B) on capsicum seedlings



Other than a reduction in the size of the root system, the only symptom observed on plants inoculated with *Rhizoctonia* was a brown discolouration over most of the root surface. Nevertheless, shoot and root weights were reduced by 51-59% and 50-68% respectively relative to the control (Tables 4.3 and 4.4). None of the *Macrophomina* isolates produced symptoms on roots or affected shoot weight. However, four isolates reduced root weight compared with the CMS2 but not the CMS1 control, and one isolate (SW116) reduced root weight compared with both controls (Table 4.4). Plants inoculated with *Fusarium* remained healthy and showed no symptoms on roots (Table 4.5).

When average shoot and root weights were collated across all experiments for each group of fungi (Table 4.6), *Pythium* and *Rhizoctonia* were the only fungi that were consistently pathogenic. *Pythium* was usually recovered from the distal ends of rotted primary roots of inoculated plants, while *Rhizoctonia* was recovered from all inoculated plants. *Macrophomina* was re-isolated in only three of eleven samples. *Fusarium* was isolated from most root systems, whether they were inoculated with *Fusarium* or not.

Table 4.6: Shoot and root weights of capsicum seedlings inoculated with various fungi averaged over three experiments.

Genus	No. of isolates tested	Shoot weight (g)	Root weight (g)
Control (CMS)	-	1.36	1.35
Macrophomina	11	1.33	1.29
Fusarium	21	1.26	1.25
Rhizoctonia	10	0.60	0.50
Pythium 2 (moderately pathogenic)	13	0.53	0.42
Pythium 1 (highly pathogenic)	21	0.14	0.09

Mature plant test

Materials and methods

Capsicum seedlings (cv. Target) approximately 6 weeks old were potted into 1.5 L of potting mix and grown in a greenhouse for six weeks. When fruit was starting to fill and ripen, inoculum of two isolates of *Pythium*, *Rhizoctonia* or *Macrophomina* prepared on CMS as described previously was added at 10 g L^{-1} into holes made in the potting mix with a spatula. Conidia of two isolates of *Fusarium* grown on PDA were suspended in sterile water and added at 4.3 x 10^5 conidia per mL of soil. There were nine replicates of each treatment and 18 replicate pots of the control, which received CMS without fungi at 10 g L^{-1} soil. Soil temperatures were recorded as for the previous experiment.

Three replicate plants of each treatment and six controls were harvested 1, 4 and 7 weeks after inoculation. Dry weights of shoots, fruit and roots were measured and roots were rated for root-rotting using a scale where 1 = 0-5%, 2 = 6-25%, 3 = 26-50%, 4 = 51-75% and 5 = 76-100% of the roots were rotted. Root tissue (20 pieces/treatment) was taken from the margins of root lesions in all treatments and half were surface sterilised in 1% NaOCl and rinsed in sterile distilled water while the remainder were washed in sterile distilled water for 2 minutes. The ten pieces treated with NaOCl were plated onto PDA + S and the other ten pieces were plated onto 3P. Healthy roots of *Macrophomina*-treated plants were treated in the same way after the third harvest to determine whether the fungus also colonized healthy roots.

Results

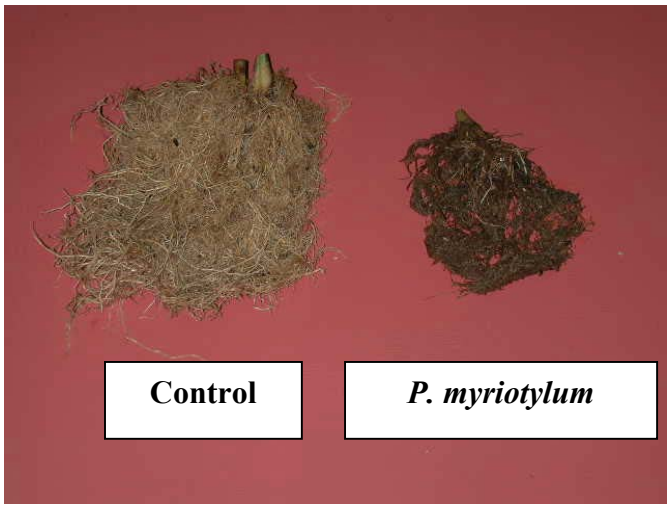
The temperature data collected during the experiment are shown in Table 4.7. Except for 3 days in the second week and one day in the third week, the maximum temperature was above 30°C every day. The minimum temperature was always less than 21°C.

Table 4.7: Summary of temperatures during an experiment on the pathogenicity of different fungal genera on mature plants.

Period	Minimum	Average	Maximum	Average maximum
Week 1	20.9	28.3	41.1	38.0
Week 2	18.4	24.6	36.5	30.7
Week 3	17.7	25.3	36.5	34.4
Week 4	16.3	24.1	38.9	34.1
Week 5	18.4	26.8	39.8	37.8
Week 6	16.3	25.8	37.3	34.3
Week 7	20.2	25.8	37.3	34.3
Weeks 1 - 7	16.3	25.7	41.1	34.6

Data for the three harvest dates are presented in Table 4.8. Analysis of variance showed that there was an interaction between isolate and harvest date for shoot and root dry weight, but for the other parameters measured, effects of isolate were significant and there was no interaction.

Figure 4.2: Roots of capsicum plants showing severe root-rotting 1 week after inoculation with *P. myriotylum* isolate SW 82.



At the first harvest, plants inoculated with *P. myriotylum* (SW 82) and *P. aphanidermatum* (SW 235) wilted during the hottest part of the day and roots were severely rotted (Figure 4.2). In contrast, rotting in control plants and plants inoculated with other genera was confined mostly to minor brown lesions located near sites of inoculation. The *Pythium*-treated plants

had higher root-rot ratings than other treatments but did not differ significantly in any of the other measured parameters.

Four weeks after inoculation, capsicum plants inoculated with both isolates of *Pythium* were still wilting in the middle of the day. Their roots were more severely rotted than at the first harvest and *P. myriotylum* significantly reduced shoot, fruit and root dry weight.

After seven weeks, plants inoculated with both isolates of *Pythium* were wilted on hot days and were not as green as the rest of the plants (Figure 4.3). Their roots were severely rotted, both shoot and root dry weights were significantly lower than control plants, and they produced significantly less fruit.

Figure 4.3: Capsicum plants showing above-ground symptoms 7 weeks after inoculation with *P. myriotylum* isolate SW82.

The inoculated fungi were successfully re-isolated from roots of all treatments at each harvest. *Pythium* was isolated from severely rotted roots on a plant inoculated with *Fusarium* isolate SW35, and so this replicate was discarded. *Fusarium* was isolated from all treatments

including the controls but no cross infection of *Macrophomina* or *Rhizoctonia* was noted. *Macrophomina* was not present on the healthy roots of inoculated plants at the third harvest.

Table 4.8: Effect of four fungi on mature capsicum plants 1, 4 and 7 weeks after inoculation.

Treatment	Shoot dry weight (g)			Root dry weight (g)			Fruit dry weight (g) (average for weeks 1, 4 & 7)	Root-rot rating (average for weeks 1, 4 & 7)
	Week 1	Week 4	Week 7	Week 1	Week 4	Week 7		
Control	8.1 h-n	11.2 ef	15.4 bc	3.2 fgh	4.3 efg	8.5 bc	15.8 a	1.5 ab
Pythium SW 82	7.3 j-o	5.5 o	6.6 l-o	2.4 gh	1.8 h	2.9 fgh	8.4 c	4.9 c
Pythium SW 235	7.0 k-o	9.7 f-k	10.4 e-h	2.0 h	3.5 e-h	3.5 e-h	9.7 bc	4.8 c
Fusarium SW 35	8.9 f-l	13.0 cde	13.9 cd	3.8 e-h	3.9 e-h	5.3 def	16.3 a	1.4 ab
Fusarium SW 275	8.3 g-o	10.2 e-i	13.8 cd	3.3 e-h	5.3 def	8.4 bc	16.5 a	1.2 a
Macrophomina SW 116	7.2 j-o	11.1 d-g	17.4 b	2.5 gh	4.3 e-h	10.3 ab	16.5 a	1.6 ab
Macrophomina SW 256	8.4 g-m	10.3 e-i	12.9 de	3.3 e-h	4.8 d-g	7.2 cd	13.0 ab	1.3 ab
Rhizoctonia SW 118	7.4 i-o	11.2 def	21.3 a	2.7 fgh	4.6 efg	11.5 a	15.9 a	1.7 b
Rhizoctonia SW 334	5.8 mno	10.0 f-j	12.9 de	2.5 gh	3.9 e-h	5.7 de	14.6 a	1.2 a
l.s.d. (P=0.05) - treatment *vs* treatment		2.87			2.58		4.41	0.40
treatment *vs* control		2.49			2.24		3.82	0.34

Effect of *Pythium* at different inoculum densities

Materials and methods

Nine-week old capsicum seedlings (cv. Target) growing in 400 mL of pasteurised potting mix were transferred to 1.5 L pots. *Pythium* (*P. myriotylum* isolate SW82 and *P. aphanidermatum* isolate SW235) was grown on CMS1 at 28°C and either 1, 5 or 10 g of inoculum of each isolate was mixed with 1 L of potting mix and this was then added to the pot so that the root ball was surrounded by, but not covered with, infested soil. There were 10 replicates of isolates SW82 and SW235, while 20 control plants received potting mix containing 5 g L^{-1} of CMS without fungus. Soil temperatures were recorded as in previous experiments.

Five plants of each treatment and ten control plants were harvested 1 and 5 weeks after inoculation. Roots were rated for root rotting using the scale previously described. Dry weights of the shoots, fruit and roots were recorded and the lengths of stems were measured at the second harvest. Fungi were recovered from 15 root samples taken from control and inoculated plants using isolation methods described previously.

Results

After plants were inoculated, soil temperatures of more than 30°C were recorded on every day except for 3 days in the last week. Temperatures for each week of the experiment are presented in Table 4.9.

Table 4.9: Weekly temperatures in pots during an inoculum density experiment with *Pythium*.

Period	Minimum	Average	Maximum	Average maximum
Week 1	16.3	25.6	37.3	33.7
Week 2	20.2	25.8	37.3	34.1
Week 3	17.4	26.2	41.1	37.6
Week 4	16.0	27.2	41.1	38.7
Week 5	15.3	22.2	34.5	31.5
Weeks 1 - 5	15.3	25.5	41.1	35.4

The results of the first and second harvests are shown in Table 4.10. Each harvest was analysed separately because, except for root rotting, treatment effects were not apparent until the second harvest.

One week after inoculation, plants inoculated with *Pythium* wilted during the hottest part of the day and roots were severely rotted. Root rotting usually extended into the crown and, in a few cases, lesions girdled the stem. Roots in the control treatment were white and healthy. Root-rot ratings were significantly higher for inoculated plants than for the controls.

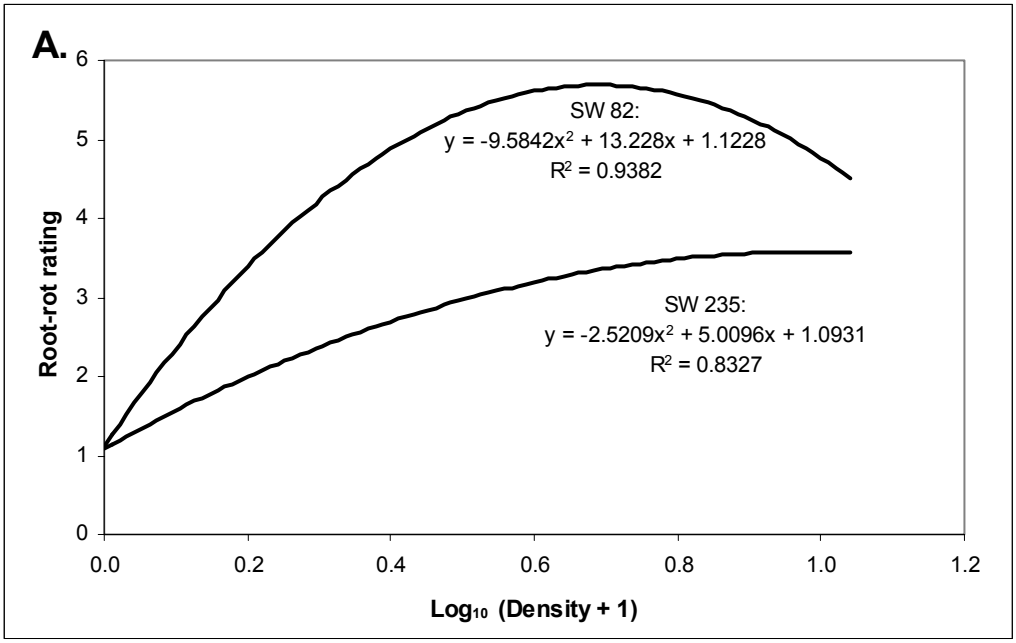
Five weeks after inoculation, plants inoculated with *Pythium* were still wilting during the day, but symptoms were less severe than at week 1. Roots were severely rotted and rotting again extended into the crown. In one case, a lesion girdled the stem. However, there were signs that infected root systems were starting to recover, as a few healthy new roots were growing from the crowns, even in the plant with a girdled stem.

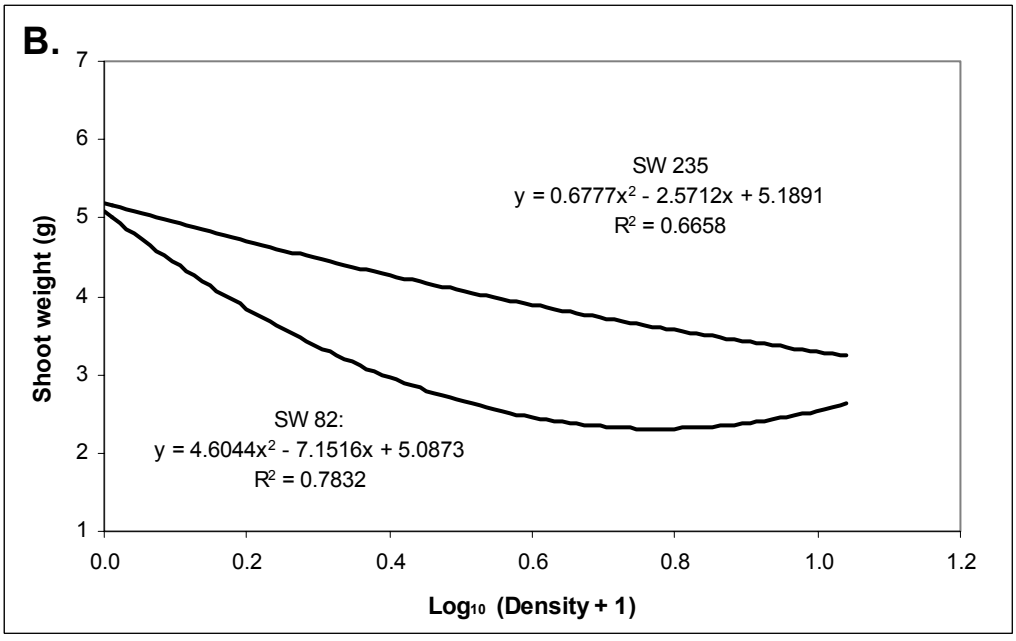
Table 4.10: Effect of two species of *Pythium* at three inoculum densities on mature capsicum plants harvested 1 and 5 weeks after inoculation.

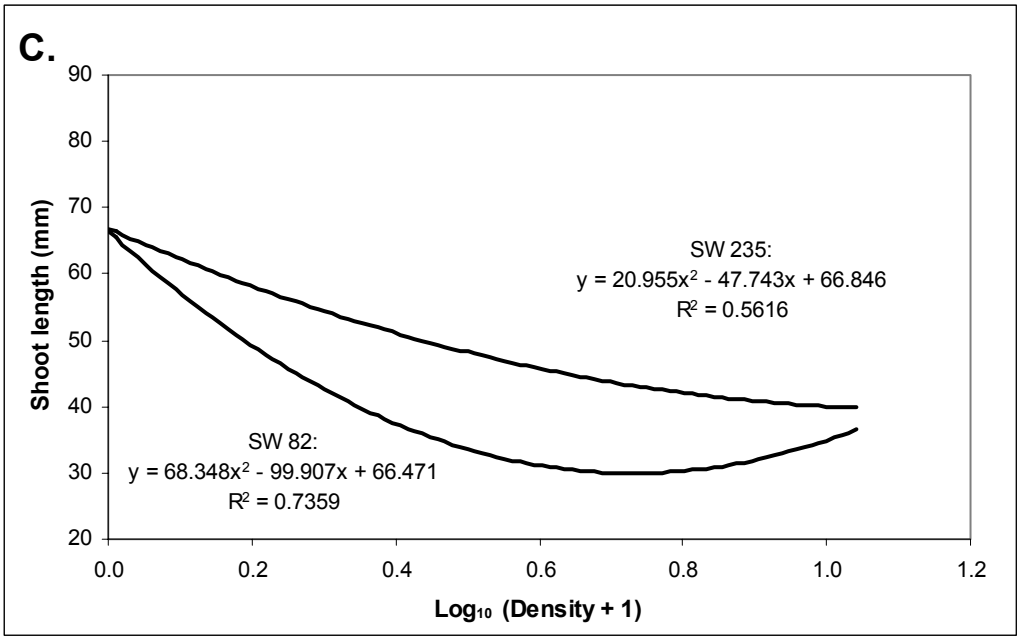
Treatment	Inoculum Density (g/L)	Shoot dry wt (g)		Fruit dry wt (g)		Stem length (cm)		Root dry wt (g)		Root-rot rating	
		Week 1	Week 5	Week 1	Week 5	Week 1	Week 5	Week 1	Week 5	Week 1	Week 5
Control		2.1	5.2 a	-	1.9	-	67.5 a	0.7 a	4.4 a	1.0 a	1.0 a
SW 82	10	1.6	2.4 e	-	0.9	-	34.2 c	0.5 ab	1.0 c	4.8 c	4.8 d
	5	1.6	2.8 de	-	1.3	-	35.0 c	0.4 b	1.2 bc	4.6 bc	5.0 d
	1	1.5	2.9 de	-	1.2	-	38.1 c	0.4 b	1.4 bc	4.8 c	4.8 d
SW 235	10	1.6	3.2 cd	-	1.6	-	38.4 c	0.5 b	1.7 bc	4.6 bc	3.8 c
	5	1.7	3.6 c	-	1.6	-	45.4 bc	0.5 b	1.9 bc	4.4 bc	3.0 b
	1	1.7	4.5 b	-	1.4	-	51.6 b	0.5 b	2.2 b	4.0 b	2.8 b
l.s.d. (P = 0.05)											
- treatment *vs* treatment		n.s.	0.704		n.s.		11.59	n.s.	1.00	0.750	0.511
- treatment *vs* control			0.610				10.04		0.87	0.649	0.442

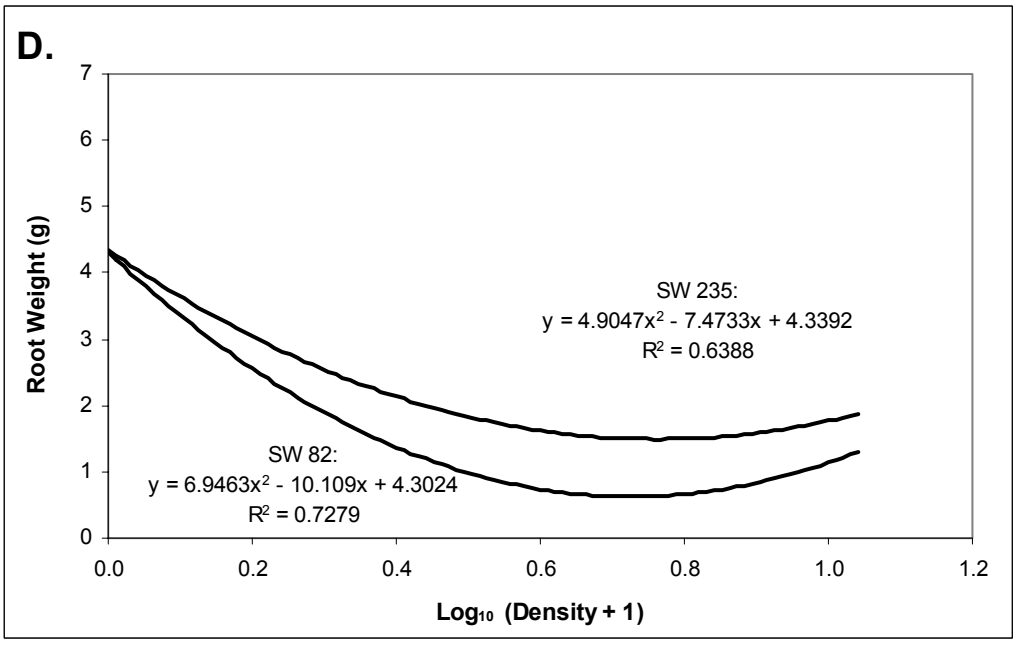
Regression analysis showed that the root rot-rating increased as inoculum density increased (Figure 4.4 A). There was also an inverse, polynomial relationship between all the plant growth parameters measured (*i.e*. shoot weight, shoot length, and root weight), and inoculum density (Figures 4.4 B-D). Isolate SW82 (*P. myriotylum*) was the most pathogenic isolate, as the amount of damage tended to reach a maximum at a relatively low inoculum density. In contrast, damage from isolate SW235 (*P. aphanidermatum*) continued to increase at inoculum densities of 5 and 10 g L^{-1}.

Figure 4.4: Regression of inoculum density and root-rot rating (A), shoot dry weight (B), stem length (C) and root dry weight (D) for isolates SW 82 (*P. myriotylum*) and SW 235 (*P. aphanidermatum*).






C.
Shoot length (mm)
90
80
70
60
50
40
30
20
0.0
0.2
0.4
0.6
0.8
1.0
1.2
Log10 (Density + 1)
SW 235:
y = 20.955x2 - 47.743x + 66.846
R2 = 0.5616
SW 82:
y = 68.348x2 - 99.907x + 66.471
R2 = 0.7359


D.
Root Weight (g)
7
6
5
4
3
2
1
0
0.0
0.2
0.4
0.6
0.8
1.0
1.2
Log10 (Density + 1)
SW 235:
y = 4.9047x2 - 7.4733x + 4.3392
R2 = 0.6388
SW 82:
y = 6.9463x2 - 10.109x + 4.3024
R2 = 0.7279

Discussion

The results of both the agar and seedling tests indicated that all the *Pythium* isolates were pathogenic to capsicum. However, levels of root damage in the seedling test varied between isolates, suggesting that some isolates were more pathogenic than others. Later studies (see chapter 5) indicated that the highly pathogenic isolates were either *P. myriotylum* or *P. aphanidermatum*. We also demonstrated that *Pythium* caused severe root rotting in mature capsicum plants, even in a situation where plants were inoculated without damaging the roots. Signs of wilting were observed within a few days of inoculation, which implicates *Pythium* in the sudden wilt syndrome. Although both isolates were highly pathogenic, our results also suggested that isolate SW82 was slightly more pathogenic than isolate SW235. Taxonomic work described later (chapter 5) indicated that the most pathogenic isolate was *P. myriotylum* while the other was *P. aphanidermatum.*

Our seedling tests and pot experiments were done at high temperatures because temperature was thought to be a major factor in the plant - pathogen interaction (see chapter 2). Also, Chellemi *et al*. (2000) had demonstrated that both *P. myriotylum* and *P. aphanidermatum* were more pathogenic to capsicum plants at 34°C than at 28°C. Our isolates of both these species were highly pathogenic at temperatures in the mid 30's, but root systems showed some signs of recovery when temperatures were cooler (*i.e.* towards the end of the inoculum density experiment). This suggests that low night temperatures are less favourable to these pathogens or more favourable to root growth than higher temperatures.

The agar test showed that many isolates of *Fusarium* were unable to rot capsicum roots in an environment that should have favoured pathogenicity. Some isolates rotted roots, but symptoms were slow to develop and no damage or stunting was seen when seedlings or larger plants growing in potting mix were inoculated with a selection of these isolates. *Fusarium* is a ubiquitous fungus, as evidenced by the cross contamination encountered with *Fusarium* in our pot tests, and this probably explains why it is consistently isolated from roots of plants affected by sudden wilt in the field.

Macrophomina produced symptoms on roots in the agar test, but did not cause damage in potting mix. This suggests that *Macrophomina* is probably not a primary pathogen of capsicum. However, it is often associated with stressed plants (M. Stanghellini, University of California, Riverside, pers. com.) and may be capable of colonising roots after other pathogens have rotted roots or the plant is water stressed.

Some of our *Rhizoctonia* isolates rotted roots on agar and caused minor damage to seedlings in potting mix. However, the two isolates tested on older plants did not damage roots. *Rhizoctonia* is often

involved in seedling diseases of vegetable crops and our results suggest that it is not a major pathogen of mature plants.

Although our results showed that *Fusarium*, *Macrophomina* and *Rhizoctonia* caused little damage to mature capsicum plants, it is possible that they are more pathogenic in a different environment (*e.g*. at temperatures lower than those used in our initial experiments). While they may not act individually to cause root-rot, it is also possible that these fungi exacerbate root rotting in the presence of other organisms. Both these possibilities are examined in more detail later in this report (see chapter 7).

Chapter 5: Identity of *Pythium* isolates pathogenic to capsicum roots

Introduction

The results of previous studies (see chapter 4) showed that most isolates of *Pythium* caused root-rot of capsicum, and some isolates severely retarded the growth of mature plants. There were also indications that some isolates were more pathogenic than others. The following studies were done in order to identify the *Pythium* species and determine whether the variability in pathogenicity had a taxonomic basis.

Material and methods

The ten isolates of *Pythium* chosen for taxonomic studies were all isolated from roots of wilted capsicum plants in the field (see chapter 3) and were pathogenic to capsicum in both the agar test and the small seedling test (see chapter 4). A representative range of isolates from various locations and with different levels of pathogenicity was included (Table 5.1).

Table 5.1: *Pythium* isolates used for taxonomic studies.

Isolate	Location	Date isolated	Level of pathogenicity in seedling test
SW82	Bundaberg	March 2000	Highly pathogenic
SW97	Bundaberg	March 2000	Highly pathogenic
SW137	Murgon	May 2000	Moderately pathogenic
SW165	Bundaberg	May 2000	Moderately pathogenic
SW187	Bundaberg	May 2000	Moderately pathogenic
SW206	Bundaberg	July 2000	Highly pathogenic
SW213	Gumlu	July 2000	Moderately pathogenic
SW235	Bowen	July 2000	Highly pathogenic
SW249	Bowen	July 2000	Highly pathogenic
SW325	Bundaberg	October 2000	Moderately pathogenic

Production of sporangia

Fresh corn leaves were cut into pieces approximately 1 cm^2 and boiled in distilled water for 10 minutes. Ten leaves were then transferred into a Petri plate containing a sterile slide and the slide was covered to a depth of 2 – 3 mm with water. Each plate was then inoculated with three PDA cubes (0.5 cm^3) from a culture of one of the *Pythium* isolates and incubated in daylight at room temperature. Two plates were prepared for each isolate.

Plates were examined under the microscope (x40) each day until sporangia were visible. Slides were then gently removed from the plates while ensuring that fungal growth and a leaf piece remained on each slide. If necessary, excess mycelium was trimmed with a sterile scalpel blade, so that material was contained in an area 40 mm x 20 mm. A drop of lactoglycerol cotton blue was added to the slide, specimens were covered with a cover slip and the morphology of sporangia was observed at a magnification of x 400.

Oospore production

Each *Pythium* isolate was grown on corn meal agar (CMA) and oospore agar (Adams, 1971) at room temperature for 2 days and then transferred to 24, 26, 28, 30 and 32°C for 3-6 days. Once oospores were produced, they were placed on slides, stained with lactoglycerol cotton blue and observed at x400. The position of oospores on the hypha, their ornamentation and details of their antheridia were noted. Twenty oospores of each isolate were then chosen at random, five from each of four slides prepared from different cultures, and their diameter, the diameter of the oogonia and the thickness of the oospore wall was measured.

Radial growth studies

Each isolate was grown on CMA in 90 mm Petri plates for 12 hours at room temperature. The growing edges of the colonies were then marked using a stereoscope microscope and at least two plates of each isolate were grown at temperatures between 3°C and 45°C for 24 hours. The growing edges of the colonies were again marked and the distance each colony had grown was measured at four different positions and averaged to determine the radial growth rate.

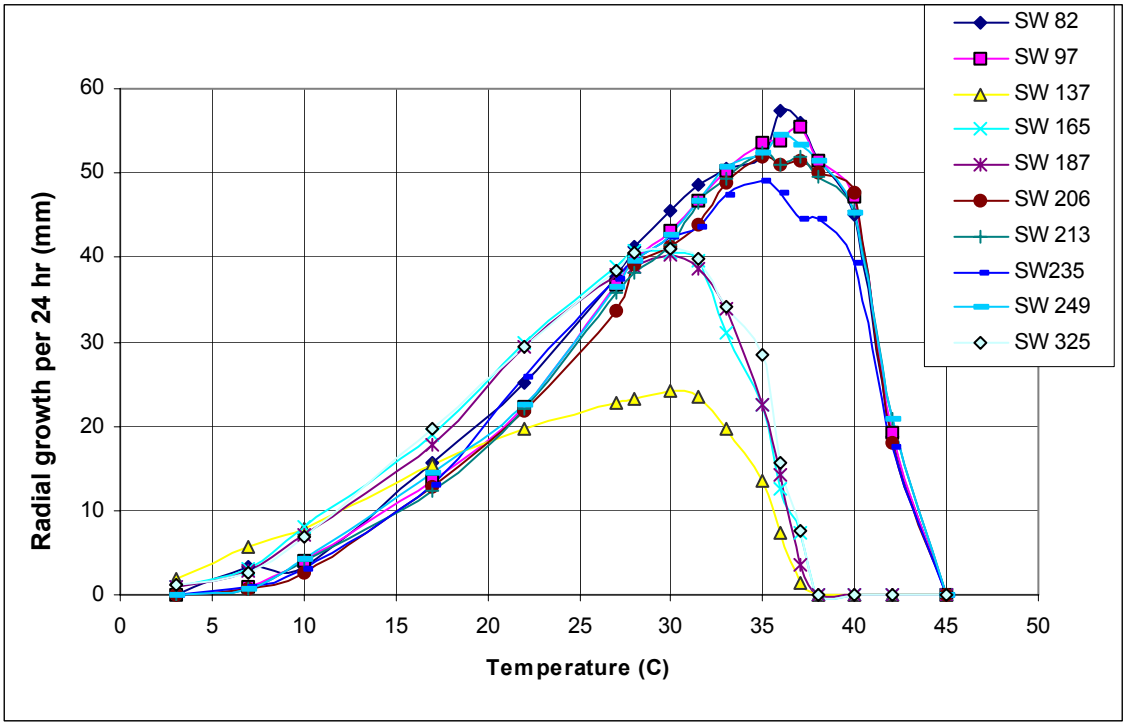
Identification process

The measurements and features of each isolate were used in conjunction with *Keys to Pythium* (Dick, 1990) to determine their identity. The first key in this publication is based on biometric measurements of oospores and oogonia and other criteria of sexual reproduction. The second key is an amendment of a key prepared by Van der Plaats-Niterink (1981), and is based on other morphological criteria. In cases where these keys gave contradictory results, two older keys were also used, one by Middleton (1943) and one by Waterhouse (1967). A database created in Microsoft Access was used to facilitate examination of the data.

Results

On the basis of radial growth studies, the isolates could be separated into three broad groups (Figure 5.1). One group grew rapidly on agar and had an optimum temperature around 36°C. The other two groups had optimum temperatures of about 30°C, but could be separated on growth rate.

Figure 5.1: Growth rates of *Pythium* spp on corn meal agar.



Cardinal temperatures and morphological parameters were used to place the ten isolates into one of four groups as shown in Table 5.2. Group 1, which contained isolate SW137, was tentatively identified as *P. diclinum* because it produced plerotic oospores (15-23μm in diameter), some of which were intercalary. However, Dick (1990) maintained that there is no such thing as true pleroticity, and based his key on the plerotic index, a relationship between the oogonium diameter and oospore diameter. When his key was used together with that of Van der Plaats-Niterink (1981), *P. diclinum* was still the most probable identity. Group 2, which contained isolates SW165, SW187 and SW 325, was identified as *P. dimorphum* or *P. undulatum* but again, this identification needs to be confirmed. These isolates did not produce oospores, despite the fact that each isolate was grown in combination with the other two. However, they did produce many large yellow-brown chlamydospores (35-45μm in diameter), and this is characteristic of both *P. dimorphum* and *P. undulatum*. Members of Groups 3 (isolate SW235) and 4 (isolates SW82, SW97, SW206, SW213 and SW249) produced obviously

aplerotic oospores and were identified using all keys as *P. aphanidermatum* and *P. myriotylum* respectively.

Table 5.2: Taxonomic characteristics of four groups of *Pythium* isolates.

	Group 1	Group 2	Group 3	Group 4
Isolate no.	SW137	SW165, SW187, SW325	SW235	SW82, SW97, SW206, SW213, SW249
Sporangia	Not observed	Not observed	Toruloid	Dendroid – toruloid
Oospores	Plerotic, smooth, 15-23 μm diameter (mean 19μm), intercalary and terminal	Not observed	Aplerotic, smooth, 18-22 μm diameter (mean 21 μm)	Aplerotic, smooth, 22-29 μm diameter (mean 25 μm).
Antheridia	1-2 per oogonium, apical attachment	None observed	1 per oogonium, intercalary	3-7 per oogonium, stalks branched
Other features		Large yellow-brown chlamydospores		
Optimum growth temperature	~ 30°C	~ 30°C	35-36°C	36-37°C
Maximum growth temperature	37°C	37°C	> 42°C	> 42°C
Identity	*P. diclinum* (tentative)	*P. dimorphum* or *P. undulatum* (tentative)	*P. aphanidermatum*	*P. myriotylum*

Discussion

The *Pythium* isolates that were highly pathogenic in previous tests (chapter 4) were identified as either *P. aphanidermatum* or *P. myriotylum.* Both species grow well at 36-40°C but are readily distinguished by differences in the number of antheridia attached to oogonia and the mode of antheridial formation. Because they have a wide host range and grow well at high temperatures, *P. aphanidermatum* and *P. myriotylum* are widely distributed in the warmer regions of the world (Van der Plaats-Niterink, 1981). In Australia, *P. aphanidermatum* causes root rot on *Brassica* and lucerne and root rot and damping-off of papaya, Rhodes grass, rockmelon, Queensland blue grass and tomato. It also causes fruit rot on cucumber, stem rot on barley, beans, tomato transplants, tobacco and maize, pod rot on beans and base rot on potato (Teakle, 1960).

P. myriotylum has been reported from several crops in Australia, mainly in warmer regions and in nursery situations. It was reported on sugarcane in Queensland, where it was mildly pathogenic

(Croft, 1988), and caused root-rot of cowpea, which is used as green manure crop in sugarcane fields (Croft, 1988a). Teakle (1956) reported *P. myriotylum* as the cause of lucerne root rot in Queensland and implicated it in taproot rot of tomato and damping-off of lupin, tobacco and pea (Teakle, 1960).

Ramsey, (1990) demonstrated that *P. myriotylum* caused significant root-rot of maize in pathogenicity tests and reduced plant dry weight. However, since it was infrequently isolated in surveys of the Atherton Tablelands, it is uncertain how much damage it causes. Pathogenicity tests on isolates from safflower done at an average maximum temperature of 33°C showed that New South Wales isolates of *P. myriotylum* prevented emergence of seedlings and were extremely pathogenic to older plants, killing a high proportion of them in both flooded and non-flooded situations (Stovold, 1973). In Darwin nurseries, *P. myriotylum* is a common root pathogen (Duff and Barnaart, 1992).

Given the widespread distribution of both *P. aphanidermatum* and *P. myriotylum* in northern Australia and the isolations we made during our surveys (see chapter 3), it is likely that these species are the most important *Pythium* species associated with capsicum crops in Queensland. However, our taxonomic studies showed that other species of *Pythium* with lower optimum temperatures are also found on capsicum roots. Although they are probably not involved in the sudden wilt syndrome, they are pathogenic to capsicum and are possibly most active in Queensland during winter. They may cause subtle forms of root rotting that are not necessarily noticeable in the field.

Chapter 6: Effect of temperature on capsicum root growth and pathogenicity of *P. myriotylum* and *P. aphanidermatum*

Introduction

Our studies of the pathogens isolated from capsicum roots implicated *Pythium aphanidermatum* and *P. myriotylum* as causes of severe root rotting in capsicum plants (see chapters 4 and 5). These pathogens have high optimal growth temperatures and can sometimes kill plants at 34°C (Chellemi *et al*. 2000). Since soil temperatures of 35-40°C occur in Queensland's capsicum growing areas at certain times of the year, we investigated the effect of temperature on the interaction between the capsicum plant and these high-temperature pathogens.

Effect of soil temperature on capsicum growth

Materials and methods

Six-week-old seedlings growing in 15 cm-high watertight pots containing 400 mL of pasteurised potting mix were placed into waterbaths at 25°C, 32°C, 34°C or 36°C. Six replicate pots were included at each temperature. Insulation was placed above the pots to minimise heat loss and the air temperature was maintained at 25°C. Plants were watered (100 mL per pot) when the soil moisture was approximately 80% of field capacity (as determined prior to the experiment). After 4 weeks, plants were harvested and the dry weight of shoots and roots was recorded.

Results

Capsicum seedlings appeared healthy at all temperatures but there was a non-significant trend for an increase in the shoot/root ratio as temperature increased. However, the dry weight of shoots and roots was significantly lower at 34 and 36°C than at 25 and 32°C (Table 6.1), and there was a noticeable reduction in the size of the root system as soil temperature increased (Figure 6.1). Roots at 34 and 36°C were shorter than roots at 25 and 32°C and some were a light brown colour rather than white.

Table 6.1: Effect of temperature on growth of capsicum.

Soil temperature	Shoot dry weight (g)	Root dry weight (g)	Shoot / root ratio
25°C	0.63	0.16	4.0
32°C	0.60	0.13	4.7
34°C	0.37	0.08	4.8
36°C	0.39	0.08	5.4
l.s.d. (P=0.05)	0.16	0.041	n.s.

Figure 6.1: Capsicum roots grown at different soil temperatures in pasteurised soil.

Pathogenicity of *P. myriotylum* and *P. aphanidermatum* at different soil temperatures

Material and methods

Three-month-old capsicum plants growing in 400 mL of pasteurised potting mix were transplanted into sealed pots that were filled with 1L of soil containing CMS (5 gL^{-1}) that was either colonised by *P. aphanidermatum* isolate SW235 or *P. myriotylum* isolate SW249, or left un-inoculated. Pots were placed into waterbaths at 25°C, 30°C, 35°C or 40°C and air temperature was maintained at 25°C. There were four replicate pots of each treatment. Plants were watered with 200 mL of water when the soil surface dried out (*i.e.* when soil moisture reached approximately 80% of field capacity, as determined prior to the experiment), and the amount of water used was recorded. After four weeks, plants were harvested and roots were rated for root rotting as previously described. Dry weights of shoots and roots were also recorded.

Results

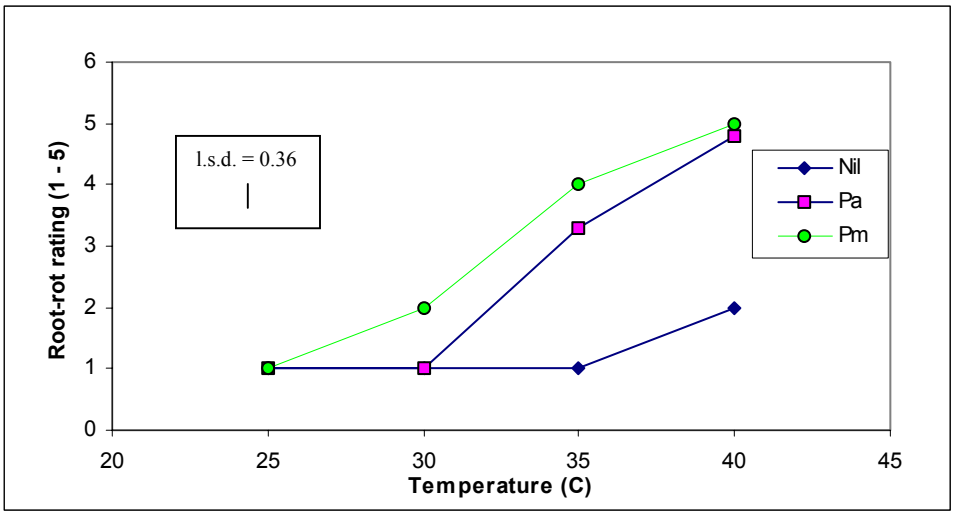
Roots of uninoculated plants grown at 35 and 40°C were smaller than those grown at 25 and 30°C, and those at 40°C were dark brown in colour (Figure 6.2). Uninoculated plants at 40°C were reduced in size but did not wilt, whereas plants inoculated with *P. aphanidermatum* or *P. myriotylum* and maintained at 40°C wilted during the day. At 35°C, only *P. myriotylum* caused wilting. When shoot weight, root weight and water use data were analysed by analysis of variance, there were significant effects of inoculation treatment and temperature but no interaction. Plants grown at 35 and 40°C had lower root and shoot weights, higher root-rot ratings and used more water than those at 25 and 30°C (Table 6.2 and Figure 6.3). Both *P. aphanidermatum* and *P. myriotylum* caused root rotting, but only the latter fungus reduced shoot weight and water usage. There was an interaction between treatment and temperature for root rotting, with root rot rating increasing markedly at high temperatures when plants were inoculated with *Pythium*.

Figure 6.2: Roots of uninoculated capsicum plants grown at different soil temperatures.

Table 6.2: Biomass production and water use of capsicum plants grown at different soil temperatures in potting mix containing corn meal sand (CMS), or CMS colonised by *P. aphanidermatum* or *P. myriotylum*.

Soil temperature or treatment	Shoot dry weight (g)	Root dry weight (g)	Water used per day (mL)
25°C	4.3	1.18	52
30°C	4.8	1.26	82
35°C	3.4	0.83	92
40°C	2.5	0.59	102
l.s.d. (P = 0.05)	0.73	0.242	11.9
Control (CMS1)	4.2	1.0	92
P. aphanidermatum	3.9	1.0	84
P. myriotylum	3.2	0.9	71
l.s.d. (P = 0.05)	0.63	n.s.	10.3

Figure 6.3: Effect of temperature on root-rotting in un-inoculated plants (Nil) and plants inoculated with *P. aphanidermatum* (Pa) or *P. myriotylum* (Pm).

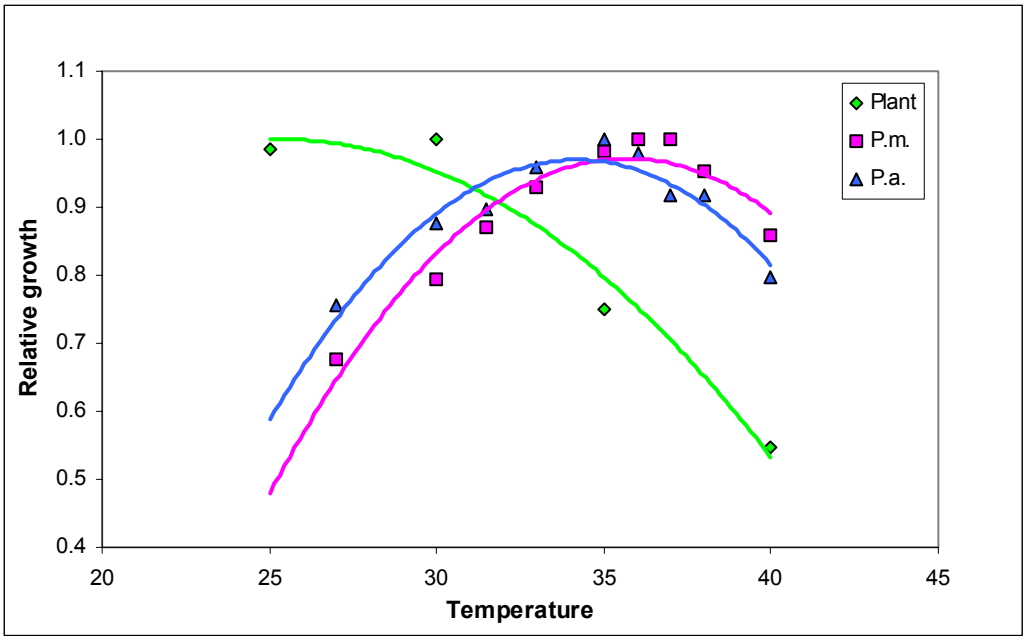


Relative effects of temperature on the growth of capsicum plants and *Pythium*

The relative growth of capsicum roots at various temperatures was calculated by dividing the root dry weight at each temperature by the root dry weight at 30°C. The data used were obtained from the second experiment in this chapter, where capsicum was grown in pasteurised soil at four temperatures. A temperature of 30°C was chosen as the optimum temperature for growth because biomass production in our experiment was similar at 25 and 30°C, and Gosselin and Trudel (1986) indicated that root-zone temperature of 30°C was more favourable to vegetative growth of capsicum than 24 or 36°C. Data for growth of *Pythium* on agar (see chapter 5, figure 5.1) were used in the same way to

determine growth rates relative to 36°C for *P. myriotylum* and relative to 35°C for *P. aphanidermatum*. The resulting curves (Figure 6.4) show that the plant is stressed at temperatures that are ideal for growth of both *P. myriotylum* and *P. aphanidermatum*.

Figure 6.4: Relative growth rates of capsicum plants, *P. myriotylum* (P.m.) and *P. aphanidermatum* (P.a.) in relation to temperature.



Effect of *Pythium* on capsicum at fluctuating temperatures

Materials and methods

Ten-week-old capsicum seedlings growing in 150 mL soil were transferred to 500 mL watertight pots that were then filled with 300 mL of soil containing 10 gL-1 CMS that had been previously inoculated with *P. myriotylum* isolate SW249 or left un-inoculated. Pots were placed in water-baths maintained at various temperatures but above-ground parts of plants were exposed to the ambient air temperature. Initially, the temperature of the water-baths was maintained at 25°C to encourage root growth but after 5 days, water-baths were adjusted to 32°C, 34°C or 36°C. Each waterbath contained ten plants of each treatment. Every afternoon, five plants of each treatment were removed from their waterbath and placed at 25°C and every morning they were returned to their original waterbaths. Thus, some plants were maintained at a constant 32, 34 or 36°C while others were exposed to those temperatures for 8 hours during the day followed by 16 hours at 25°C during the night. Each plant was irrigated with 80

mL of water when the soil surface dried out. After 3 weeks, plants were harvested and root rotting was assessed as previously described. Dry weight of shoots and roots was also measured.

Results

Inoculum of *P. myriotylum* appeared to be unevenly colonised and this caused considerable variation within treatments. Nevertheless, analysis of variance showed a significant reduction in shoot and root dry weight due to *P. myriotylum* (Table 6.3) and no effect of temperature. Root-rot ratings were higher at the constant temperatures than the fluctuating temperatures (Table 6.4), largely because the latter treatments were at a relatively low temperature at night. The three-way interaction between inoculation treatment, temperature level and temperature regime was not significant. However, the data showed that the roots of plants inoculated with *P. myriotylum* and grown at 36°C during the day and 25° at night were as badly rotted as the roots of plants kept constantly at 34 or 32°C (root-rot ratings of 3.8 compared with 3.2 and 2.5 respectively).

Table 6.3: Effect of *P. myriotylum* on shoot and root dry weight of capsicum plants grown at constant and alternating temperatures.

Inoculum	Shoot dry weight (g)	Root dry weight (g)
Nil	1.12	0.32
P. myriotylum	0.66	0.23
l.s.d. (P = 0.05)	0.219	0.082

Table 6.4: Interaction of inoculation treatment and temperature regime on root rotting of capsicum.

Inoculum	Temperature regime	
	Constant	Fluctuating
Nil	1.00	1.33
P. myriotylum	3.57	2.45
l.s.d. (P = 0.05)	0.331	

Discussion

The experimental system used for our experiments involved growing capsicum plants at high soil temperatures while maintaining their aerial parts at 25°C. This system mimics to some extent the situation to which capsicums are exposed when grown under plastic mulch in the field.

Our experiments in pasteurised soil clearly demonstrated that plants were stressed at temperatures above 30°C. The effect was most apparent at temperatures of more than 34°C, as both shoot and root

growth was reduced by more than 40% compared with growth at 25°C. However, in the absence of root pathogens, high temperatures did not have any other detrimental effects, as the foliage remained green and plants did not wilt. The susceptibility of capsicum to heat stress raises questions about whether heat tolerance could be improved. Given that the centre of origin for the genus ranges from southern USA to Mexico and Columbia, capsicum is likely to be adapted to a range of climatic conditions. It is therefore likely that some genotypes are adapted to high temperatures. A well-structured screening program may therefore identify material that could be used in a breeding program to improve the crop's tolerance to sudden wilt.

P. myriotylum caused minor root rotting at 30°C, but at 35 and 40°C, root rotting was severe. The response to *P. aphanidermatum* was similar, as it did not rot roots at 25 or 30°C, but caused severe root rotting at 35 and 40°C. These two *Pythium* species therefore appear to be most active in situations where the plant is already stressed by high temperatures. Thus our results support those of Chellemi *et al.*, (2000), who implicated high temperatures as a factor in root-rot and plant death caused by *P. myriotylum* and *P. aphanidermatum*.

Our last experiment demonstrated that soil temperatures do not have to be constantly high for *P. myriotylum* and *P. aphanidermatum* to cause root-rot in capsicum. For example, root rotting caused by *P. myriotylum* was more severe when plants were grown at 36°C for 8 hours and 25°C for 16 hours than when plants were held constantly at 32°C. This indicates that as long as the soil temperature is above 36°C during the day, these *Pythium* species have the potential to cause severe root rot. Such conditions occur under white plastic in Bowen during March and April and under black plastic in Bundaberg during September (see chapter 2).

Observations made earlier in this report (chapter 4) suggest that capsicum has the capacity to recover from root rotting by growing new adventitious roots. However, when roots are rotted by *Pythium* during periods of high daytime temperatures, night-time temperatures are usually not low enough for long enough to allow recovery. In the fluctuating temperature environment that occurs in the field, recovery is most likely to occur when a period of cool, wet or cloudy weather follows a period of intense heat. However, recovery may never occur during fruit-fill, because assimilates are being diverted from roots and shoots to the fruit. This probably explains why sudden wilt symptoms are observed only when the plant is laden with developing fruit.

In conclusion, these results demonstrate that sudden wilt of capsicum is not caused by *Pythium* alone. Assimilate deprivation of roots during fruit-fill and the detrimental effects of high soil temperatures on root function are exacerbating factors. Since the term 'sudden wilt' provides no information about the

etiology of the disease, we suggest that it would be better to refer to it as ‘heat-induced’ Pythium root rot.

Chapter 7: Interactions between *Pythium myriotylum* and other fungi

Introduction

Previously, we demonstrated that *Pythium* is often isolated from the roots of capsicum plants affected by sudden wilt in the field (see chapter 3), that *P. myriotylum* and *P. aphanidermatum* can destroy capsicum roots (see chapters 4 and 5) and that root rot symptoms are most severe when soil temperatures are greater than 32°C (see chapter 6). However, surveys for potential pathogens associated with sudden wilt (chapter 3) showed that other fungi (e.g. *Fusarium*, *Macrophomina* and *Rhizoctonia)* also occur on diseased capsicum roots in the field. Although these fungi do not produce symptoms or are only mildly pathogenic on young capsicum seedlings, and are not pathogenic to mature plants (chapter 4), it is possible that they exacerbate disease by interacting with *P. myriotylum* or *P. aphanidermatum.* Other moderately pathogenic *Pythium* species (chapter 4) may also act in the same way. Thus, this chapter reports on experiments in which capsicum plants inoculated with *P. myriotylum* were challenged with isolates of *Pythium*, *Fusarium*, *Macrophomina* or *Rhizoctonia*. Because these fungi have different optimum temperatures, experiments were carried out at temperatures of 30-36°C.

Experiment 1 (Interaction of *Pythium* spp. and *Fusarium*)

Materials and methods

This factorial experiment consisted of two treatments at transplanting (inoculated or not inoculated with *P. myriotylum*) x four types of inoculum introduced after 2 weeks (two isolates of *Pythium*, one isolate of *Fusarium* or no fungus) and two temperature regimes (30°C or 36°C for two weeks followed by 30°C until harvest) x five replicates. The experiment was set up by transplanting nine-week-old capsicum plants growing in 150 mL soil into watertight pots and adding 300 mL of pasteurised potting mix. Cornmeal sand (CMS1) colonised by *P. myriotylum* isolate SW249 or left un-inoculated had been added to the potting mix previously at 10 g L^{-1}. Pots were placed into waterbaths at 30 or 36°C in a glasshouse where the air temperature ranged from 19 to 28°C. Two weeks after transplanting, the temperature in one waterbath was lowered from 36 to 30°C. Plants were then inoculated with *Pythium* isolate SW137 or *Pythium* isolate SW165 growing on CMS, with 2.3 x 10^5 chlamydospores of *Fusarium* isolate SW35 obtained from 3- week-old cultures on CLA, or left un-inoculated. Inoculum was added by loosening soil at the edge of the pot and adding CMS or the chlamydospore suspension in sterile water. After 2 weeks, plants were harvested and root -rotting was assessed as previously described. Dry weights of shoots and roots were also recorded.

Results

Analysis of variance showed that there was a significant interaction between the treatment at transplanting, the post-plant treatment and soil temperature for both root rot rating and dry weight of shoots. Inoculation with *P. myriotylum* at transplanting increased root rotting and reduced shoot dry weight, with the effects being greater in the 36-30°C temperature regime than at a constant 30°C (Tables 7.1 and 7.2). Of the three fungi added 2 weeks after transplanting, *Pythium* isolate SW165 was the only pathogen, as it increased root rotting and decreased shoot dry weight compared with the un-inoculated control. However, it did not increase the level of damage caused by *P. myriotylum* (Tables 7.1 and 7.2). Root dry weight was significantly less at 36-30°C than at a constant 30°C, and there were also significant reductions in root dry weight due to *P. myriotylum* at transplanting and the two *Pythium* isolates applied 2 weeks after transplanting (data not shown).

Table 7.1: Effect of *P. myriotylum* on root-rot when capsicum plants growing at different temperatures were inoculated 2 weeks after transplanting with other fungi or left un-inoculated.

Preplant inoculum	Temperature	Post-plant inoculum				l.s.d. (P = 0.05)
		Nil	*Pythium* SW137	*Pythium* SW165	*Fusarium* SW35	
Control (CMS)	30°C	1.0	1.0	2.0	1.2	0.604
Control (CMS)	36-30°C	1.0	1.6	3.0	1.4	
P. myriotylum	30°C	2.4	2.4	3.0	2.2	
P. myriotylum	36-30°C	3.6	4.0	3.8	4.0	

Table 7.2: Effect of *P. myriotylum* on shoot dry weight when capsicum plants growing at different temperatures were inoculated 2 weeks after transplanting with other fungi or left un-inoculated.

Preplant inoculum	Temperature	Post-plant inoculum				l.s.d. (P = 0.05)
		Nil	*Pythium* SW137	*Pythium* SW165	*Fusarium* SW35	
Control (CMS)	30°C	2.5	2.6	2.0	2.3	0.36
Control (CMS)	36-30°C	2.3	1.8	1.7	2.1	
P. myriotylum	30°C	2.0	1.8	1.9	2.0	
P. myriotylum	36-30°C	1.7	1.6	1.4	1.4	

Experiment 2 (Interaction between *Pythium* and *Macrophomina*)

Materials and methods

Ten week old capsicum plants growing in 150 mL cells were transplanted into watertight pots and 300 mL of pasteurised potting mix was added. The potting mix contained CMS1 inoculated previously with *P. myriotylum* isolate SW249, CMS2 inoculated previously with *Macrophomina* isolate SW116,

or a mixture of both colonised substrates. In treatments with only one fungus, an additional 10 g L^{-1} un-inoculated CMS was included and the control consisted of 20 g L^{-1} of un-inoculated CMS. Pots were placed in waterbaths at 30 or 36°C for 12 days and then half the plants at 36°C were transferred to 30°C. Thus, the experiment consisted of four fungi (*P. myriotylum*, *Macrophomina*, a mixture of the two fungi and an un-inoculated control) x three temperature regimes (constant 36°C, constant 30°C or 36°C for 12 days followed by 30°C until harvest). There were five replicate pots of each treatment. A further 11 days later, dry weights of tops and roots were recorded and roots were rated for rotting as described for the previous experiment. Root tissue (10 pieces/treatment) was taken from the margins of lesions in all treatments and fungi were isolated as described previously.

Results

Two plants inoculated with *Macrophomina* had severely rotted roots, but since *P. myriotylum* was isolated from the affected roots, these replicates were discarded. Analysis of variance on data collected when plants were harvested (Table 7.3) showed that *P. myriotylum* reduced shoot weight and caused severe root rot, particularly when the temperature was 36°C for at least some of the time. *Macrophomina* did not cause root rotting. It was isolated at all temperatures and from all treatments in which it was included, but the frequency of isolation (only 4 occurrences in 30 root pieces) was relatively low. There was also a significant effect of temperature, with shoots and roots growing better at 30°C than at higher temperatures (Table 7.3).

Table 7.3: Effect of *Pythium myriotylum* and *Macrophomina* sp. alone and in combination, on root rotting and biomass of capsicum plants grown under three temperature regimes.

Parameter		Treatment				l.s.d. (P = 0.05)
		Fungal inoculum				
	Temp	Nil	*Macrophomina*	*P. myriotylum*	*P. myriotylum* and *Macrophomina*	
Root-rot rating	30°C	1.0	1.0	2.6	2.4	
	36 – 30°C	1.0	1.0	4.8	4.8	0.51
	36°C	1.0	1.4	5.0	5.0	
		Nil	***Macrophomina***	***P. myriotylum***	***P. myriotylum* and *Macrophomina***	
Mean shoot dry weight		3.1	3.2	2.0	2.0	0.30
Mean root dry weight		0.98	0.87	0.85	0.81	n.s.
		30°C	**30 - 36°C**	**36°C**		
Mean shoot dry weight		2.9	2.6	2.3		0.26
Mean root dry weight		1.00	0.79	0.85		0.131

Experiment 3 (Interaction of *Pythium* and *Rhizoctonia*)

Materials and methods

Methods were similar to the previous experiment, except that *Rhizoctonia* isolate SW 334 was substituted for *Macrophomina* and a temperature of 36°C was not included. . Plants were harvested after 11 days, roots were rated for rotting, dry weights of tops and roots were measured and pathogens were isolated as described previously.

Results

P. myriotylum increased root rotting in both temperature regimes, but the extent of root rotting was much greater at 36/30°C than at 30°C (Table 7.4). In contrast, *Rhizoctonia* did not rot roots when it was inoculated alone, and did not increase disease severity in the presence of *P. myriotylum*. None of the treatments affected root dry weight, but shoot dry weight was reduced by *P. myriotylum*. *Rhizoctonia* was isolated from all treatments (7 out of 20 root pieces), but recovery from the treatment with both *P. myriotylum* and *Rhizoctonia* was poor, possibly because *P. myriotylum* grew from root pieces sterilised with hypochlorite and may have masked the *Rhizoctonia*.

Table 7.4: Effect of *Pythium myriotylum* and *Rhizoctonia* sp. alone and in combination, on root rotting and biomass of capsicum plants grown under two temperature regimes.

Parameter		Nil	*Rhizoctonia*	*P. myriotylum*	*P. myriotylum* and *Rhizoctonia*	l.s.d. (P = 0.05)
Root-rot rating	30°C	1.0	1.0	2.2	2.2	0.68
	30 - 36°C	1.0	1.2	4.6	4.4	
Mean shoot dry weight		2.4	2.5	1.8	2.1	0.29
Mean root dry weight		0.70	0.72	0.67	0.60	n.s.

Discussion

These experiments aimed to determine whether fungi commonly isolated from capsicum roots cause root-rotting or exacerbate damage caused by *P. myriotylum*. In earlier tests (see chapter 4), *Rhizoctonia* and species of *Pythium* other than *P. myriotylum* and *P. aphanidermatum* rotted the roots of seedlings but *Fusarium* and *Macrophomina* did not.

Pythium SW165 was the only one of the fungi tested that was clearly pathogenic to capsicum plants under the experimental conditions. It reduced shoot weight and increased root-rot, but not as severely as *P. myriotylum*. Root rotting was relatively minor at a constant 30°C but damage was more severe when plants were stressed at 36°C prior to inoculation. This isolate was included in taxonomic studies (see chapter 5) and was tentatively identified as *P. dimorphum* or *P. undulatum*. It produces large chlamydospores but no sexual structures.

As in previous experiments, *P. myriotylum* was highly pathogenic to capsicum. Interestingly, plants damaged by *P. myriotylum* that were grown at 36°C did not recover quickly when transferred to 30°C. Two weeks after they were transferred, their roots were still badly rotted. This suggests that plants do not readily recover when heat-induced root rotting caused by *P. myriotylum* has occurred. Root systems attacked by *P. myriotylum* in the field during the first month or two after planting, when soil temperatures are high due to the absence of a crop canopy, may therefore not recover at night or when temperatures become more benign as the crop matures. The stress of fruit-fill or some other factor may then cause such plants to collapse.

Our results indicated that *Fusarium*, *Macrophomina*, *Rhizoctonia* and some isolates of *Pythium* were not pathogenic to mature capsicum plants, either by themselves or in combination with *P. myriotylum*. Although these fungi are often recovered from capsicum plants affected by sudden wilt, they are not part of the disease syndrome. They are competent saprophytes and probably colonise root tissue that is either debilitated due to excessively high temperatures or destroyed by *P. myriotylum* or *P. aphanidermatum*.

Chapter 8: Technology transfer and recommendations

Industry Meetings

The observations made in this project have been disseminated widely through the major capsicum growing regions in Queensland via a series of seminars. An information meeting was held in February 2002 at a grower's property in the Lockyer Valley. All major growers in the area were invited. At the meeting, we discussed sudden wilt of capsicum and the effects of temperature, and also conducted a farm walk. During this walk, we discovered that the grower's 'suddenly wilting' capsicum plants were actually suffering from Sclerotium basal stem rot and not root rot. This highlighted the fact that 'sudden wilt' is an above-ground symptom that may have various causes and reinforced the need for correct disease diagnosis.

In March 2002, we presented a seminar that was advertised widely by the Bowen District Growers' Association. About 25 growers, industry consultants and QDPI personnel from Bowen and Ayr attended this meeting. We received feedback from growers and agronomists and visited properties where we measured soil temperature, set up soil temperature recorders and discussed future collaboration.

A similar meeting was held at the headquarters of the Bundaberg Fruit and Vegetable Growers Association in April 2002. About 15 participants attended, including growers, consultants and extension officers from QDPI. Again, we received feedback from growers and some offered to participate in future work. We were also interviewed by Mr. Jim Kennedy for the morning farming program on ABC local radio.

Finally, we presented our results in May 2002 at the QDPI seminar series held in conjunction with a regular meeting of the Australasian Plant Pathology Society. The meeting was well attended by QDPI plant pathologists from Indooroopilly, the Lockyer Valley and the Darling Downs and also by staff and students from the University of Queensland and the Cooperative Research Centre for Tropical Plant Protection.

Publications

In July 2001, a report was prepared on work done during the first 18 months of this project. This report was posted to all growers whose properties were surveyed for sudden wilt during the first phase of the work. A press release indicating that the report was available was published in Queensland

Fruit and Vegetable News in August 2001. Since then, more than 35 copies have been distributed to interested parties, including growers, crop consultants, seed companies and researchers. In September 2002, a summary of this work was published in Good Fruit and Vegetables (Appendix 3).

The above publications and a Powerpoint copy of the seminar were forwarded to Vegetable Industry Development Officers Samantha Heritage and Julia Telford after they were completed.

Recommendations

This project was established to investigate the causes of sudden wilt of capsicum and not to examine control measures. However, work on control is the logical next step. A range of possible management options are therefore being investigated in a follow-up research project (VG02020) that commenced in January 2003.

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Appendix 1A: Bowen – Summary of Meteorological Information – Autumn

	Parameter	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	Average
February	Average maximum temperature (°C)	34.0	30.3	32.1	31.6	31.3	30.8	31.9	30.5	32.5	31.6	29.4	30.9	32.4	31.5
	Average minimum temperature (°C)	23.2	24.0	24.8	23.6	24.3	24.1	23.2	23.9	24.2	24.2	23.4	23.9	24.8	24.0
	# of days >28°C	28	25	27	28	25	23	28	27	28	26	22	28	27	26
	# of days >30°C	28	17	25	26	22	20	26	19	27	20	12	22	23	22
	Total rainfall (mm)	33	843	134	16	172	145	25	331	51	262	687	146	185	233
	Average daily cloud cover (%)	35	82	67	58	56	69	49	71	66	68	71	61	56	62
	# of days <30% cloud cover	10	0	2	3	6	3	11	4	3	1	2	2	6	4
March	Average maximum temperature (°C)	30.1	30.5	31.8	32.1	29.9	31.4	30.5	29.6	31.5	30.5	30.2	31.4	32.0	30.9
	Average minimum temperature (°C)	22.9	22.2	22.2	21.9	21.6	23.6	22.9	23.1	22.5	23.5	22.3	23.0	22.3	22.6
	# of days >28°C	23	30	31	31	27	31	25	27	30	29	30	29	31	29
	# of days >30°C	19	26	28	30	13	30	19	8	26	21	18	26	31	23
	Total rainfall (mm)	258	4	9	0	170	32	21	331	11	121	58	30	2	81
	Average daily cloud cover (%)	68	49	37	35	61	50	58	69	51	68	44	46	33	51
	# of days <30% cloud cover	6	12	15	17	3	9	10	2	9	1	10	7	18	9
April	Average maximum temperature (°C)	28.4	29.1	29.7	29.2	28.7	30.8	30.4	28.8	31.0	27.9	27.9	29.3	30.3	29.3
	Average minimum temperature (°C)	20.4	20.6	21.1	20.3	19.4	21.0	22.2	19.3	22.8	21.0	21.5	21.4	21.0	20.9
	# of days >28°C	19	24	29	23	20	29	26	23	30	13	16	26	27	23
	# of days >30°C	4	10	8	8	2	19	22	1	24	3	3	4	17	10
	Total rainfall (mm)	253	4	16	16	5	8	47	11	13	112	141	24	8	50
	Average daily cloud cover (%)	52	58	45	46	45	45	50	36	56	61	62	44	38	49
	# of days <30% cloud cover	11	6	13	10	9	10	11	15	5	5	7	8	13	9
May	Average maximum temperature (°C)	26.0	27.0	27.7	27.6	26.8	27.4	27.5	26.4	27.2	26.9	26.2	27.8	28.2	27.1
	Average minimum temperature (°C)	19.4	18.4	20.2	18.9	17.6	19.6	18.2	15.7	19.3	18.5	18.2	14.1	17.6	18.1
	# of days >28°C	3	6	15	13	3	10	9	4	9	3	6	16	14	9
	# of days >30°C	0	0	0	0	0	0	0	0	1	0	1	0	1	0
	Total rainfall (mm)	273	17	100	5	7	28	43	39	165	28	8	1	1	55
	Average daily cloud cover (%)	63	50	54	37	62	56	43	38	51	39	50	21	41	47
	# of days <30% cloud cover	4	4	5	13	8	7	13	15	8	13	5	23	11	10

Appendix 1B: Bowen – Summary of Meteorological Information – Spring

	Parameter	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	Average
August	Average maximum temperature (°C)	25.0	26.0	26.2	25.8	24.7	23.5	25.5	25.2	26.5	25.0	25.7	26.4	25.7	25.5
	Average minimum temperature (°C)	10.6	14.1	14.9	15.1	13.6	16.5	14.9	14.5	16.6	15.2	14.3	13.8	15.2	14.6
	# of days >28°C	2	3	4	0	0	0	0	0	5	0	2	1	0	1
	# of days >30°C	1	0	1	0	0	0	0	0	0	0	1	0	0	0
	Total rainfall (mm)	3	2	0	51	0	106	17	22	135	2	4	2	7	27
	Average daily cloud cover (%)	12	26	34	32	27	64	29	40	40	30	29	21	29	32
	# of days <30% cloud cover	28	19	15	21	19	4	21	13	15	19	16	23	19	18
September	Average maximum temperature (°C)	26.8	28.0	28.5	26.9	26.7	26.8	27.2	27.4	27.6	27.2	28.0	27.8	27.6	27.4
	Average minimum temperature (°C)	15.1	14.6	19.0	18.1	15.4	16.6	14.8	17.5	19.6	16.8	15.6	17.8	16.0	16.7
	# of days >28°C	3	13	24	3	3	3	5	6	11	4	8	10	11	8
	# of days >30°C	0	2	2	0	0	0	1	1	0	1	2	1	0	1
	Total rainfall (mm)	0	0	15	30	8	4	13	5	15	3	0	21	1	9
	Average daily cloud cover (%)	34	14	40	31	30	24	21	34	39	28	28	22	24	28
	# of days <30% cloud cover	17	28	10	16	18	21	19	13	14	20	17	20	19	18
October	Average maximum temperature (°C)	29.0	29.4	29.0	28.7	29.0	28.8	28.9	28.8	29.5	29.5	29.2	29.7	29.3	29.1
	Average minimum temperature (°C)	18.8	19.6	18.6	19.7	19.8	19.6	20.4	18.6	21.7	20.2	20.1	20.0	20.4	19.8
	# of days >28°C	26	30	29	19	27	19	23	23	28	28	28	25	25	25
	# of days >30°C	4	6	1	2	2	5	2	0	8	9	2	9	5	4
	Total rainfall (mm)	2	0	1	5	0	20	23	4	64	3	88	17	0	18
	Average daily cloud cover (%)	36	25	23	34	33	36	44	32	60	32	43	29	24	35
	# of days <30% cloud cover	14	22	23	13	17	17	12	18	8	15	11	18	23	16

Appendix 1C: Bundaberg – Summary of Meteorological Information – Autumn

	Parameter	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	Average
February	Average maximum temperature (°C)	-	29.7	29.6	30.1	28.8	28.7	30.7	30.3	31.9	30.0	28.8	30.0	32.3	30.1
	Average minimum temperature (°C)	-	22.4	22.0	21.6	21.0	21.4	21.0	21.9	22.8	21.2	20.1	20.9	22.5	21.6
	# of days >28°C	-	27	24	27	23	17	25	26	28	25	25	27	26	25
	# of days >30°C	-	10	13	17	2	9	19	17	24	15	5	12	24	14
	Total rainfall (mm)	-	62	458	54	83	351	54	112	189	137	95	65	29	141
	Average daily cloud cover (%)	-	59	66	53	66	62	45	56	-	-	-	-	-	58
	# of days <30% cloud cover	-	1	3	3	2	4	7	2	-	-	-	-	-	3
March	Average maximum temperature (°C)	-	29.4	28.0	29.5	28.0	30.1	29.3	28.3	31.5	29.6	30.0	31.1	29.9	29.6
	Average minimum temperature (°C)	-	19.9	19.8	19.4	19.5	20.2	19.7	19.9	20.2	20.5	19.6	21.6	19.8	20.0
	# of days >28°C	-	28	15	26	13	29	27	22	29	27	26	30	29	25
	# of days >30°C	-	7	1	6	4	11	7	4	28	12	11	23	13	11
	Total rainfall (mm)	-	32	330	24	101	17	13	104	7	69	19	76	55	71
	Average daily cloud cover (%)	-	40	56	41	51	33	47	54	-	-	-	-	-	46
	# of days <30% cloud cover	-	9	5	12	8	15	8	10	-	-	-	-	-	10
April	Average maximum temperature (°C)	-	27.7	26.4	27.5	25.9	27.4	28.2	27.2	28.4	27.4	26.8	27.6	29.0	27.5
	Average minimum temperature (°C)	-	17.4	18.2	17.1	16.8	17.3	18.3	16.9	18.5	16.4	18.1	18.1	17.9	17.6
	# of days >28°C	-	10	3	8	2	10	19	7	20	6	11	13	21	11
	# of days >30°C	-	4	0	1	0	1	9	2	6	1	6	0	7	3
	Total rainfall (mm)	-	3	80	15	47	26	163	29	78	6	41	27	40	46
	Average daily cloud cover (%)	-	42	55	40	43	38	52	33	-	-	-	-	-	43
	# of days <30% cloud cover	-	10	4	13	10	13	10	15	-	-	-	-	-	11
May	Average maximum temperature (°C)	-	25.4	23.7	25.4	25.0	24.6	24.8	24.4	25.4	25.7	24.6	25.6	25.3	25.0
	Average minimum temperature (°C)	-	16.0	15.6	15.2	13.2	16.3	16.1	15.0	14.9	15.0	15.1	13.3	14.3	15.0
	# of days >28°C	-	1	0	0	0	0	0	0	1	2	1	1	2	1
	# of days >30°C	-	0	0	0	0	0	0	0	0	0	0	0	0	0
	Total rainfall (mm)	-	27	127	17	42	103	257	97	130	116	74	22	41	88
	Average daily cloud cover (%)	-	46	62	44	32	57	54	43	-	-	-	-	-	48
	# of days <30% cloud cover	-	5	4	9	18	7	9	11	-	-	-	-	-	9

Appendix 1D: Bundaberg – Summary of Meteorological Information – Spring

	Parameter	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	Average
August	Average maximum temperature (°C)	22.7	24.8	23	23.7	22.9	22.8	23.1	23.3	24.3	23.1	24.4	24.2	22.6	23.5
	Average minimum temperature (°C)	9.5	11.1	10.9	11.9	10.8	11.8	11.8	10.9	13.5	11.2	10.5	9.9	11.9	11.2
	# of days >28°C	0	4	0	0	0	0	0	0	0	0	1	2	0	1
	# of days >30°C	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Total rainfall (mm)	18	1	59	82	10	22	24	5	71	35	12	5	122	36
	Average daily cloud cover (%)	19	20	33	25	35	42	32	26	-	-	-	-	-	29
	# of days <30% cloud cover	23	23	14	23	16	11	20	20	-	-	-	-	-	19
September	Average maximum temperature (°C)	24.1	26.9	24.9	24.3	25.8	25.6	26.0	26.1	26.0	25.2	26.7	25.6	26.1	25.6
	Average minimum temperature (°C)	12.3	13.1	13.6	14.6	13.4	14.4	13.1	15.1	15.9	13.7	13.3	14.7	14.8	14.0
	# of days >28°C	0	9	2	3	4	4	4	6	5	0	5	1	5	4
	# of days >30°C	0	3	0	1	1	0	1	1	1	0	1	0	0	1
	Total rainfall (mm)	6	0	97	83	2	8	146	35	134	15	0	49	7	45
	Average daily cloud cover (%)	37	15	31	43	22	32	20	41	-	-	-	-	-	30
	# of days <30% cloud cover	13	25	16	11	21	15	22	11	-	-	-	-	-	17
October	Average maximum temperature (°C)	27.1	27.3	26.0	25.9	26.7	26.1	26.0	26.4	27.3	27.4	27.2	28.1	27.7	26.9
	Average minimum temperature (°C)	17.1	17.3	15.1	16.8	16.7	16.6	16.4	16.7	16.8	17.3	17	17	17.4	16.8
	# of days >28°C	4	8	3	6	7	5	1	3	9	9	10	12	11	7
	# of days >30°C	1	1	0	1	3	1	0	1	3	1	2	3	4	2
	Total rainfall (mm)	11	46	12	75	43	139	96	12	109	243	200	39	9	79
	Average daily cloud cover (%)	42	38	35	39	40	49	46	40	-	-	-	-	-	41
	# of days <30% cloud cover	10	12	13	14	14	12	7	12	-	-	-	-	-	12
Total rainfall Aug – Oct (mm)		35	48	167	241	56	170	266	51	315	293	211	93	138	160

Sudden wilt of capsicum was severe during spring of 1991 and 1997. In an attempt to relate occurrence of sudden wilt to weather conditions, extreme events during the period from 1990 to 2002 are highlighted if the average maximum temperature in August was greater than 24.5°C; there were more than 2 days in August with maximum temperatures greater than 28°; the average maximum temperature in September was greater than 26°C; there were more than 5 days in September with maximum temperatures greater than 28°C; average daily cloud cover in September was less than 25%; there were more than 20 days in September with less than 30% cloud cover; total rainfall from August to October was less than 55 mm.

Appendix 2A: Capsicum fields sampled several times during the growing season

Grower	Previous two crops	District	Soil treatment	Cultivar	Planting date	Sampling dates
Hull	Capsicum, zucchini × 2	Bundaberg	Untreated	Target	23 Mar 00	
Zaina	Capsicum, capsicum	Bundaberg	Untreated	Toledo	24 Mar 00	26 Apr 00 16 May 00
Curino	Capsicum, capsicum	Bundaberg	MB (250 kg/ha^{-1})	Bombardier	17 Mar 00	12 Jul 00
Manera	Capsicum, rock melon	Bundaberg	MB (250 kg/ha^{-1})	Toledo	24 Mar 00	
Prossliner	Zucchini, cucumber	Bundaberg	Untreated	Bombardier	12 Sept 00	
Zaina	Capsicum, capsicum	Bundaberg	Untreated	Raptor	30 Aug 00	17 Oct 00 13 Nov 00
Barbera	Capsicum, sweet corn	Bundaberg	MB (250 kg/ha^{-1})	Merlin	26 Sept 00	13 Dec 00
Manera	Sorghum, tomato	Bundaberg	MB (250 kg/ha^{-1})	Toledo	18 Sept 00	
Nane	Cotton, water melon	Bowen	Untreated	Merlin	14 Aug 00	26 Sep 00 31 Oct 00
Collyer	Capsicum, fallow	Bowen	MS (500 L/ha^{-1})	Merlin	1 July 00	17 Aug 00 31 Oct 00

Appendix 2B: Details of fields where symptoms of sudden wilt were observed

Grower	Previous two crops	District	Soil treatment	Cultivar	Planting date	Sampling date
Skilton	Sugar cane, sweet potato	Bundaberg	Untreated	Target	20 Sep 99	27 Jan 00
Zaina	Sugar cane	Bundaberg	Untreated	Sundance	2 Nov 99	
SP Exports	Wheat	Murgon	Untreated	Unknown (Red)	4 Jan 00	28 Apr 00
SP Exports	Oat	Murgon	Untreated	Unknown (Yellow)	4 Jan 00	
Manera	Capsicum, forage sorghum	Bundaberg	MB (250 kg/ha^{-1})	Raptor	12 Jan 00	22 Mar 00
Curino	Capsicum, capsicum	Bundaberg	MB (31 kg/ha^{-1})	Ninja	19 Jan 00	
Tortorica	Capsicum, pumpkin	Gumlu	Untreated	Toledo	25 Feb 00	30 Jun 00
Land	Fallow, capsicum	Gumlu	Untreated	Merlin	29 Feb 00	
Tudehope	Capsicum, zucchini	Gumlu	Untreated	Merlin	5 Mar 00	
Zabala	Rock melon, capsicum	Gumlu	Untreated	Ninja Yellow	7 Mar 00	
Collyer	Capsicum, capsicum	Bowen	Untreated	Merlin	10 Mar 00	3 Jul 00
De Domenico	Rock melon × 2	Gumlu	Untreated	Hot Spot	16 Mar 00	30 Jun 00
Chapman	Capsicum, rock melon	Gumlu	Untreated	Legend	28 Mar 00	1 Jul 00
Nane	Cotton, capsicum	Bowen	Untreated	Merlin	7 Apr 00	3 Jul 00
Stackelroth	Rock melon, capsicum	Bowen	Untreated	Unknown (Red)	12 Apr 00	29 Jun 00

Appendix 2C: Incidence of root-rotting, and crown rot of capsicum plants sampled from Bundaberg and Bowen at three sampling times during the growing season

Grower	District	Soil treatment	Sudden wilt present	Root-rot rating [d] (average of 5 plants)			% large roots rotted			Crown rot (no. plants / 5)		
				E[a]	M[b]	L[c]	E	M	L	E	M	L
Hull	Bundaberg	Untreated	No	1	1	1	0	0	0	0	0	0
Zaina	Bundaberg	Untreated	No	1	1.2	1.4	0	0	0	1	0	0
Curino	Bundaberg	MB	No	1	1	1.4	0	2	10	0	0	0
Manera	Bundaberg	MB	No	1	1	1	0	0	0	0	0	0
Prossliner	Bundaberg	Untreated	No	1	1.6	2.2	0	19	15	0	0	0
Zaina	Bundaberg	Untreated	No	1	1	1.2	0	5	0	0	0	0
Barbera	Bundaberg	MB	No	1	1	1	0	5	4	0	0	0
Manera	Bundaberg	MB	No	1	1.2	1	0	7	4	0	0	0
Nane	Bowen	Untreated	No	1.2	-	1.8	12	-	20	0	-	0
Nane	Bowen	Untreated	Yes	2.6	-	4.4	78	-	79	0	-	3
Collyer	Bowen	MS	No	1	-	2	0	-	38	0	-	0
Collyer	Bowen	MS	Yes	-	-	4	-	-	78	-	-	4

[a] E = early-crop sampling; [b] M = mid-crop sampling; [c] L = late-crop sampling.
[d] Root-rot rating: 1 = 0-5%, 2 = 6-25%, 3 = 26-50%, 4 = 51-75% and 5 = 76-100% of small roots rotted.

Appendix 2D: Fungi isolated from capsicum plants sampled from Bundaberg and Bowen at three sampling times during the growing season

Grower	District	Soil treatment	Sudden wilt present	Presence of fungi in rotted root, crown or vascular tissue														
				Pythium			*Fusarium*			*Macrophomina*			*Rhizoctonia*			Unknown		
				E[a]	M[b]	L[c]	E	M	L	E	M	L	E	M	L	E	M	L
Hull	Bundaberg	Untreated	No	-	+	+	+	+	+	-	-	-	-	-	-	-	+	-
Zaina	Bundaberg	Untreated	No	-	+	-	-	-	-	-	-	-	-	-	-	+	+	+
Curino	Bundaberg	MB	No	-	-	+	+	+	+	-	-	-	-	-	-	+	+	+
Manera	Bundaberg	MB	No	-	-	-	+	+	+	-	-	-	-	-	-	-	+	+
Prossliner	Bundaberg	Untreated	No	-	+		+	+		-	-		-	-		+	-	
Zaina	Bundaberg	Untreated	No	+	+		+	+		-	-		-	-		-	-	
Barbera	Bundaberg	MB	No	-	-		+	+		-	-		-	-		-	-	
Manera	Bundaberg	MB	No	+	+		+	-		-	-		-	-		-	+	
Nane	Bowen	Untreated	No	+		+	+		+	-		+	-		-	-		+
Collyer	Bowen	MS	No	-		-	+		+	-		-	-		-	-		+
Nane	Bowen	Untreated	Yes	+		+	+		+	+		+	+		+	+		+
Collyer	Bowen	MS	Yes			+			+			-			-			+

[a] E = early-crop sampling; [b] M = mid-crop sampling; [c] L = late-crop sampling

\+ = Fungus isolated from site

\- = Fungus not isolated from site

Appendix 2E: Degree of root-rotting of non-wilted and sudden wilt-affected capsicum plants at Bundaberg and Bowen

Grower	District	Soil Treatment	Root-rot rating [a] (average 5 plants)		% large roots rotted	
			Non-wilting plants	Sudden wilt-affected plants	Non-wilting plants	Sudden wilt-affected plants
Skilton	Bundaberg	Untreated	1.6	4.2	0	97
Zaina	Bundaberg	Untreated	1.6	2.4	0	44
SP Exports	Murgon	Untreated	2.2	4.8	4	95
SP Exports	Murgon	Untreated	2.8	4.8	36	95
Manera	Bundaberg	MB	2.4	3.6	12	79
Curino	Bundaberg	MB	1.0	3.4	2	79
Nane	Bowen	Untreated	1.0	3.8	0	93
Stackelroth	Bowen	Untreated	1.0	3.4	0	75
Tortorica	Gumlu	Untreated	-	5.0	-	80
Land	Gumlu	Untreated	-	4.4	-	87
Tudehope	Gumlu	Untreated	-	4.2	-	96
Zabala	Gumlu	Untreated	-	4.0	-	81
Collyer	Bowen	Untreated	-	4.6	-	91
De Domenico	Gumlu	Untreated	-	4.2	-	89
Chapman	Gumlu	Untreated	-	3.8	-	87
Mean			1.7	4.0	7	84

[a] Root-rot rating: 1 = 0-5%, 2 = 6-25%, 3 = 26-50%, 4 = 51-75% and 5 = 76-100% of small roots rotted.
[d] - = Observations not done.

Appendix 2F: Fungi isolated from roots of sudden wilt-affected and non-wilting capsicum plants from Bundaberg and Bowen

Grower	District	Soil Treatment	*Pythium*		*Fusarium*		*Macrophomina*		*Rhizoctonia*	
			Non-wilting plants	Sudden wilt-affected plants	Non-wilting plants	Sudden wilt-affected plants	Non-wilting plants	Sudden wilt-affected plants	Non-wilting plants	Sudden wilt-affected plants
Skilton	Bundaberg	Untreated	-	-	+	+	-	+	-	+
Zaina	Bundaberg	Untreated	-	-	+	+	-	-	-	-
SP Exports	Murgon	Untreated	+	+	+	+	+	+	-	-
SP Exports	Murgon	Untreated	+	+	+	+	+	+	+	+
Manera	Bundaberg	MB	+	+	+	+	-	-	-	-
Curino	Bundaberg	MB	+	+	+	+	-	-	-	-
Nane	Bowen	Untreated		+		+		+		-
Stackelroth	Bowen	Untreated		+		+		-		+
Tortorica	Gumlu	Untreated		-		+		+		+
Land	Gumlu	Untreated		+		+		+		+
Tudehope	Gumlu	Untreated		-		+		-		+
Zabala	Gumlu	Untreated		-		-		-		-
Collyer	Bowen	Untreated		+		+		+		-
De Domenico	Gumlu	Untreated		+		+		-		+
Chapman	Gumlu	Untreated		+		+		+		-

+ = Fungus isolated from site
- = Fungus not isolated from site

Appendix 3: Summary report published in Good Fruit and Vegetables (Sept 2002) Vol 13 (4): 53-54.

SUDDEN WILT OF CAPSICUM – A HOT TOPIC

Introduction

Sudden wilt is the major soil-borne disease problem facing the Australian capsicum industry. Plants appear healthy until fruit set, when they suddenly begin to wilt (Figure 1). Affected plants die or produce unmarketable fruit. The problem occurs in all capsicum production areas and is particularly severe in some years. The last major outbreaks occurred in Bundaberg in 1997 and in Bowen in 2001 when plant losses of 25% were common, even after soil fumigation with methyl bromide.

Although there had been speculation about potential causes and root rotting pathogens were thought to be involved, the cause of sudden wilt was not known when we commenced our research in January 2000. Consequently, we set out to determine whether root pathogens were the primary causal factors and whether the soil physical or biological environment influenced disease development. This article summarises progress made during the last 2 ½ years.

Development of sudden wilt

Observations during the 2000 season showed that the first symptoms of sudden wilt are a slight yellowing of leaves and inconspicuous wilting and shrivelling of fruit. As the disease progresses, affected plants wilt completely and then defoliate. If the root system is examined carefully before above-ground symptoms are visible, rotted fine roots can be found and these primary infections later spread rapidly to large roots. The end result is extensive rotting of most of the root system. Between 70 and 90% of large roots are generally rotted in plants with incipient sudden wilt symptoms, compared with fewer than 5% in healthy plants.

Potential pathogens associated with sudden wilt

Several fungi were consistently isolated from diseased plants during the 2000 season. *Fusarium* was recovered from both small and large roots and was very common in some fields. *Pythium*, *Rhizoctonia* and *Macrophomina* were also recovered consistently but not as frequently. Some fields had all four fungi, but more commonly only two or three fungi were present. Other potential pathogens (e.g. nematodes, bacteria or viruses) did not appear to be associated with the disease.

Pathogenicity studies

Initial laboratory and glasshouse tests with more than 200 fungal isolates from the field showed that the four potential pathogens had different effects on small capsicum seedlings:
Pythium. Most isolates caused root rotting but disease severity varied between isolates.
Fusarium. Less than 15% of isolates were pathogenic in laboratory tests.
Rhizoctonia. Most isolates had little or no effect on seedlings.
Macrophomina. Isolates did not cause disease in the glasshouse.

To determine whether the above fungi were capable of damaging plants at the fruit-fill stage, each was inoculated onto 6-week-old capsicum plants. *Pythium* was the only fungus to cause severe damage. Plants began to wilt within a few days of inoculation (Figure 2) and within 1 week, root systems were severely rotted (Figure 3). Both of the isolates tested were pathogenic, but one species (*P. myriotylum*) was more damaging than the other (*P. aphanidermatum*). A further experiment confirmed the pathogenicity of both species of *Pythium* and showed that severe root rotting could occur when the pathogens were added to soil at low inoculum densities.

Effect of temperature on capsicum plants and *Pythium*

Since growers had noticed that sudden wilt usually occurred when temperatures were high, we examined the effect of temperature on capsicum plants and on *Pythium*. We found that in the absence of pathogens, soil temperatures of more than 30°C were detrimental to capsicum. Shoot growth was reduced at both 35 and 40 °C and there was also a dramatic effect on root size and root health (Figure 4). We also showed that both *P. myriotylum* and *P. aphanidermatum* have optimum growth temperatures of about 36°C and that they still grow well at 40°C (Figure 5). Therefore, at temperatures where the capsicum plant is under stress, the two *Pythium* species are growing very quickly. This is an ideal situation for disease to occur. We have now demonstrated experimentally that severe root rotting can occur if plants are grown at 35°C in the presence of either *P. myriotylum* or *P. aphanidermatum* (Figure 6).

Capsicums, like many other intensively-grown vegetable crops, are grown on plastic mulch in raised beds with trickle irrigation. During the last two years, we have measured temperatures under plastic in Bowen and Bundaberg and have found that soil temperatures can rise above 40°C, even when the air temperature is less than 30°C. Thus, temperature conditions that are ideal for *Pythium* root rot sometimes occur during autumn and spring in the main capsicum growing areas of Queensland. Studies of temperature, rainfall and cloud cover records from the Bureau of Meteorology and observations by growers have also confirmed that the incidence of sudden wilt in the years from 1990 to 2001 was highest in the hottest and driest years.

Current research

We have shown that severe root rotting of capsicum occurs when heat-stressed plants are attacked by *Pythium* species that have optimum temperatures of about 36°C. It is therefore likely that temperature stress and *Pythium* are important factors in the sudden wilt syndrome. However, it is also possible that other factors are involved. For example, root rot caused by *Pythium* may be only one component of the problem, with other pathogens attacking damaged root systems and preventing plants from recovering when soil temperatures decline at night. It is also possible that *Pythium* is not involved in all instances, as we have found fields where we have been unable to isolate *Pythium* from diseased plants. Current research is focussed on clarifying these issues.

Disease management

It is clear from our studies to date that sudden wilt will not be eliminated by simply controlling *Pythium*. We may also need to modify environmental factors such as temperature, as they exacerbate the disease, and correct biological imbalances that result in soil conditions conducive to pathogen activity. Such issues are to be investigated in a new research project that is to commence in January 2003.

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Figure 1. Capsicum plants affected by sudden wilt.

Figure 2. Above-ground symptoms of *Pythium* root rot.
The plant on the right was inoculated with *P. myriotylum*.

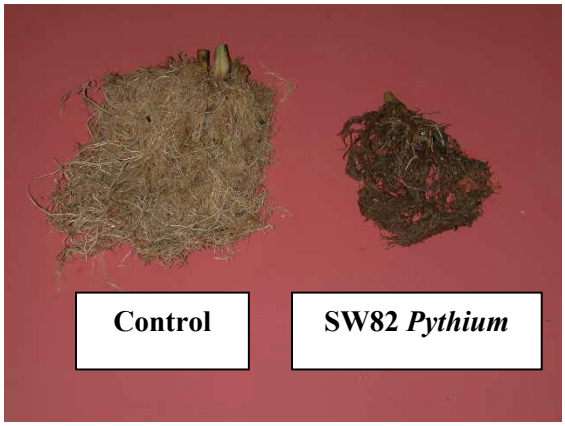

Figure 3. Root rotting caused by *Pythium myriotylum* .

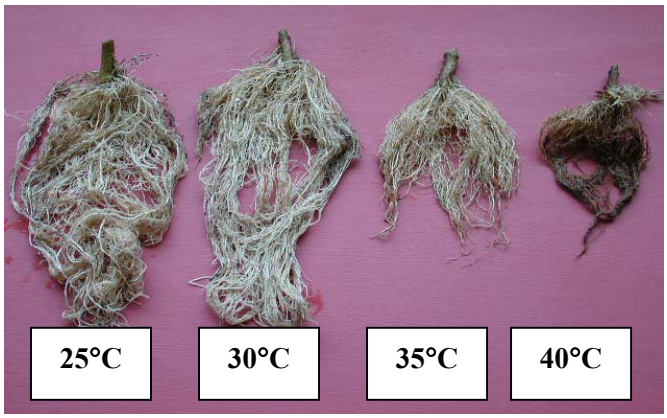

Figure 4. Capsicum roots grown at different soil temperatures in the absence of pathogens.

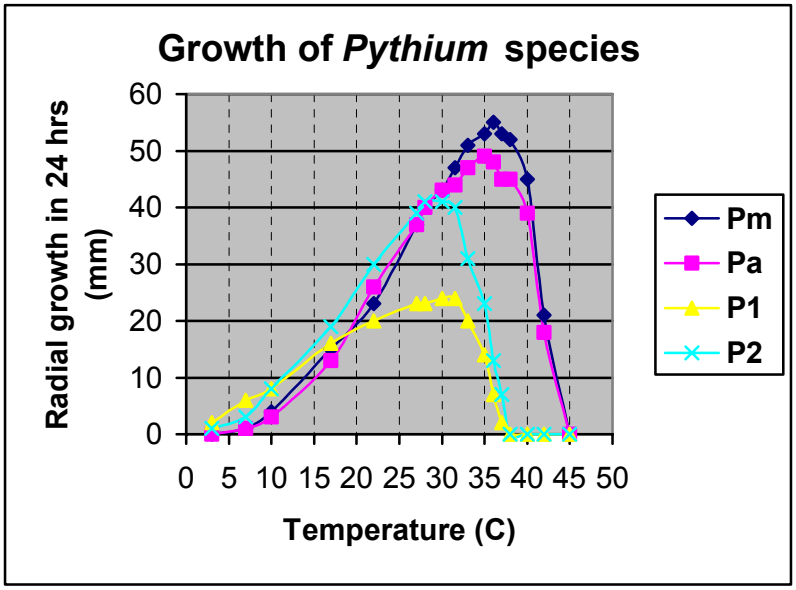

Figure 5. Growth of several species of *Pythium* at different temperatures. Note that *P. myriotylum* (Pm) and *P. aphanidermatum* (Pa) grow well at 35 -40°C, but that other species (P1 and P2) do not.

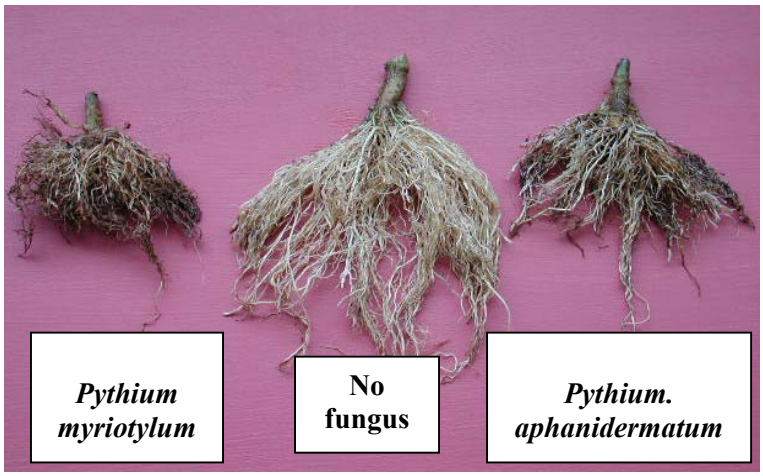

Figure 6. Roots of capsicum plants grown at a soil temperature of 35°C.