

The causes of poor establishment of ginger (*Zingiber officinale*) in Queensland, Australia

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Abstract. In recent years, Queensland ginger growers have sometimes found it difficult to establish crops. Seed-pieces either rot within a few weeks of planting or young plants grow poorly, shoots become yellow and eventually die. Surveys aimed at determining the cause of the problem showed that rhizomes designated for planting material were invariably infected by *Fusarium oxysporum* f. sp. *zingiberi* (*Foz*). The pathogen was also isolated from fungicide-treated seed-pieces that were ready for planting, and from newly planted seed-pieces. The soft-rot bacterium *Erwinia chrysanthemi* was also recovered from rotting seed-pieces but nearly always in association with *Foz*. It was not involved in the disease under normal soil moisture conditions, but rotting often occurred when seed-pieces were inoculated with both *E. chrysanthemi* and *Foz* and planted in wet soil. Experiments with biocides confirmed that *Foz* was the main cause of poor emergence, as only fungicides effective against *Foz* (i.e. carbendazim and benomyl) reduced the percentage of seed-pieces that rotted in the ground. Poor crop establishment appears to be a more severe form of a disease than has been present in the Queensland ginger industry for many years. Changes in farming practices are probably responsible for the increased disease severity, as vegetative compatibility studies suggested that a more virulent form of *Foz* has not recently been introduced.

Additional keywords: Rhizome rot, *Fusarium oxysporum* f. sp. *zingiberi*, *Erwinia chrysanthemi*, biocides.

Introduction

Most of the ginger (*Zingiber officinale*) grown in Australia is produced within 50 km of Yandina in south-east Queensland (latitude 26°33'S, longitude 152°57'E). This region has a warm, subtropical climate and an annual rainfall of ~1680 mm, but supplementary irrigation is used in the ginger industry to reduce sunburn and delay fibre development. Ginger growing in Australia commenced in the 1920s when a cultivar now known as 'Queensland' was introduced from China (Whiley 1981). About 6000 tonnes of ginger are currently produced from this cultivar each year. The crop is planted in spring and harvested in March for confectionary ginger, or over the following 12 months for the fresh market. The remainder is left in the ground until winter and used for processing. Rhizomes required for the next season's planting-material remain in the ground until August or September, when 'seed-pieces' are cut from washed rhizomes, dipped in fungicide and stored until planting.

In recent years, some ginger growers have experienced difficulty establishing newly planted crops. Seed-pieces either rotted in the ground within a few weeks of planting, or young plants grew poorly, became yellow and unthrifty and eventually died. In the 1997 planting season, for example,

the problem occurred on about one third of farms, with more than 90% of plants failing to emerge from some batches of seed-pieces. Establishment was particularly poor with cv. Canton, a relatively new cultivar that is becoming more popular because of its larger sized 'knobs', which are preferred by producers of high quality confectionary ginger.

The cause of poor emergence was not immediately apparent, as none of the known soil-borne pathogens of ginger normally attack the crop during its establishment phase (Pegg *et al.* 1974). Symptoms caused by *Fusarium oxysporum* f.sp. *zingiberi* (*Foz*) and root-knot nematode (*Meloidogyne* spp.), for example, are not usually seen until late in the season, while bacterial soft rot caused by *Erwinia chrysanthemi* and *Pythium* rot are mainly problems in stored ginger (Pegg *et al.* 1974; Pegg and Stirling 1994). An unidentified species of *Fusarium* that was introduced from overseas in 1954 produced symptoms somewhat similar to those observed in 1997 and 1998 (a severe rhizome rot, leaf chlorosis and pseudostem collapse), but this disease was thought to have been eliminated soon after it was detected (Teakle 1965).

The objective of this work was to determine the cause of poor establishment in Queensland ginger crops. Potential

pathogens were isolated from affected ginger and tested for pathogenicity on seed-pieces. Biocides with different spectra of activity were also applied to seed-pieces to ascertain which pathogens were primarily responsible for poor emergence.

Methods

Potential pathogens associated with ginger planting material

Thirteen fields of ginger (cv. Canton) on seven farms that were to be used for 'seed' in the spring of 1998 were surveyed in April and May 1998. Depending on disease severity, between approximately 5 and 150 m of row were randomly selected in each field and individual plants (i.e. a single multi-branched rhizome with numerous aerial shoots known as pseudostems) were examined *in situ* for aboveground disease symptoms. The number of plants with prematurely yellow, wilted or dead shoots was recorded. When symptoms were observed, rhizomes were inspected for signs of discoloration and potential pathogens were isolated as described later.

Samples of seed-pieces from six farms where growers used normal commercial procedures (i.e. washed rhizomes cut by hand into seed-pieces and then dipped in a recommended fungicide) were collected from storage bins in August 1998, just prior to planting. Between 35 and 100 seed-pieces were then planted in soil that had never grown ginger previously. After 6 weeks, emergence was recorded and seed-pieces were assessed for symptoms of disease and pathogens were isolated.

During the period from September to November 1998, 40 seed-pieces were dug from newly planted crops on eight farms. Emergence was recorded, seed-pieces were examined for signs of deterioration and potential pathogens were isolated. Two assessments were done, the first approximately 1 month after planting (T1), and the second about a month later (T2).

Isolation of potential pathogens

Pathogens were isolated from ginger that was first thoroughly washed with tap water, dipped in 95% (v/v) ethanol and then flamed. For fungal isolation, each rhizome piece was cut with a sterile scalpel and internal tissue with obvious discoloration or disease was plated on potato-dextrose agar (PDA; potato 200 g, dextrose 20 g, agar 18 g/L of distilled water) containing streptomycin (S) (100 µg/mL) or corn-meal agar containing polymyxin, penicillin and pimarinic (Eckert and Tsao 1962). Bacteria were isolated by crushing pieces of discoloured rhizome in sterile distilled water and streaking the suspension on to sucrose-peptone agar (SPA) (Fahy and Hayward 1983). Bacteria and fungi from the isolation plates were purified and stored for subsequent identification and testing.

Pathogenicity tests with fungi and bacteria

The pathogenicity of bacterial and fungal isolates collected in 1998 was determined by either dipping seed-pieces in a suspension of the organism or inoculating soil with the organism. Controls consisted of seed-pieces grown in the absence of the bacteria or fungi. In each case, pathogens were re-isolated from any ginger that became diseased.

For all *Fusarium* isolates, cultures were grown on PDA for 7 days. Spore suspensions were prepared in sterile water, filtered through gauze and ginger seed-pieces (five per isolate) were dipped for 5 min in a suspension containing 10^6 spores/mL. The treated seed-pieces were then planted in steamed (60°C for 60 min) peat-sand mix. After growing in a constant environment glasshouse for 2 months (temperatures of 23–25°C at night and 26–29°C during the day), plants were checked for the characteristic wilting of shoots and rotting of rhizomes.

Five *Pythium* isolates were cultured in sterile cornmeal-sand mix (3 g cornmeal, 100 g washed river sand mixed with 15 mL water in glass jars and autoclaved for 20 min at 121°C on two consecutive days) for 3 weeks. The cornmeal-sand inoculum was then incorporated in steamed peat-sand mix at 1 or 10 g/L into which ginger seed-pieces were planted. Peat-sand mix amended with sterile cornmeal sand alone served as controls. After growing in a constant environment glasshouse as above for 2 months, rhizomes were assessed for disease.

For isolates of *Geotrichum*, seed-pieces (half with buds intact and half with buds that were damaged by rubbing the rhizome on a coarse carrot grater) were dipped for 10 min in a suspension containing 10^8 spores/mL. Seed-pieces were then air-dried for 30 min, sealed in plastic bags and incubated in the laboratory at ambient temperatures. After 1 month, seed-pieces were assessed for rotting.

The pathogenicity of *Erwinia chrysanthemi* was confirmed as previously described (Stirling 2002). Three isolates of a commonly occurring Gram-negative, fermentative bacterium identified as *Enterobacter* sp. were tested by dipping seed-pieces in a suspension (10^9 cfu/mL) of bacteria grown on SPA. Half the replicate seed-pieces were then planted in steamed peat-sand and kept in a glasshouse at 23–28°C, while the others were placed in plastic bags and incubated in the laboratory at ambient temperatures. Seed-pieces were visually assessed for rotting after 2, 4 and 6 weeks.

Sclerotia of *Sclerotium rolfsii* were harvested from diseased ginger and placed on ginger seed-pieces in plastic bags. Seed-pieces were examined for rotting after 6 weeks.

Experiments with biocides

In the 1998/99 season, the effect of various biocides on shoot emergence and disease development was determined in both field and pot experiments. Because both fungi and bacteria were associated with the disease, biocides were selected that had activity against one or both groups of organisms. The chemicals used and concentrations in water were as follows: benomyl 250 µg/mL (Benlate, Dupont), carbendazim 1000 µg/mL (Bavistin FL, BASF), copper hydroxide 1500 µg/mL (Dry Bordeaux, Chemspray), copper oxychloride 1500 µg/mL (Cuprox, AgChem), azoxystrobin 125 µg/mL (Amistar, Cropcare), metalaxyl 375 µg/mL (Ridomil 250EC, Ciba-Giegy) and calcium hypochlorite 100 µg/mL. In all experiments with biocides, seed-pieces dipped in water were used as controls. Pathogens were isolated from a random sample of diseased tissue at each assessment.

For the pot experiments, ginger rhizomes were harvested from two farms (Yandina 1 and Noosa), washed before cutting and then dipped in the various biocides for 5 min. Because some growers immerse seed-pieces in water at 48°C to eliminate root-knot nematodes (Colbran and Davis 1969), the water for some treatments was maintained at this temperature for 20 min rather than at ambient temperatures of ~23°C. After dipping, 12 replicate seed-pieces of each treatment were air-dried and stored for 3 days before being planted in 1 L pots containing steamed peat-sand mix. Pots were placed in a glasshouse at 23–27°C and after 3 months the original seed-pieces and the developing rhizomes were assessed for disease by cutting each longitudinally and noting the proportion of rotted tissue.

For the field experiments, seed-pieces collected from growers' cutting tables were dipped in various biocides and stored in brown paper bags until they were planted on the farm from which the ginger was obtained. The experiments were arranged as randomised blocks. In the experiment at Yandina 1, which consisted of five treatments, 21 seed-pieces were planted in each of six replicate 2.5 m plots. At Eumundi 1, 50 seed-pieces per 6 m plot were replicated five times in each of eight treatments. Plants were assessed for disease ~10 weeks

after planting by counting the number of healthy and diseased shoots in each plot. All seed-pieces in a 1 m length of row were then dug up and assessed for incidence of rotting.

In another field experiment, ginger rhizomes were washed and stored in paper bags at ambient temperature. Rhizomes were cut into seed-pieces after 1, 4 and 6 days, and at each of these times, half the pieces in each batch were then dipped in carbendazim. Dipped and not-dipped seed-pieces were used to establish a field trial on a farm near Noosa consisting of six treatments, replicated five times with 25 seed-pieces per 2.5 m plot. Disease assessment was done in the same way as previous experiments.

The effect of Foz, E. chrysanthemi, Enterobacter sp. and soil moisture on rotting of ginger seed-pieces

Ginger seed-pieces were dipped for 5 min in either water, separate suspensions of *Foz*, *E. chrysanthemi* or *Enterobacter* sp., or in combinations of the three organisms. The final concentration in the suspension was 10^6 spores/mL for *Foz* (BRIP 39298) and 10^8 cfu/mL for each bacterium. Treated seed-pieces were planted in steamed peat-sand mix in 1 L pots. The soil in half the replicates from each treatment was allowed to drain freely after watering (low moisture), whereas the other replicates were placed on saucers so that the base of each pot always stood in water (high moisture). Plants were grown in a shade-house for 10 weeks and then harvested. Each seed-piece was cut longitudinally and the percentage of discoloured or rotted tissue was visually estimated.

The susceptibility of two ginger cultivars to Foz

Seed-pieces of cultivars Canton and Queensland were dipped in suspensions (10^3 or 10^6 spores/mL) of *Foz* (BRIP 39298). They were then potted in steamed peat-sand mix. The pots were placed in a shade-house and watered normally. Seed-pieces dipped in water were used as controls. Fifty replicate seed-pieces were set up for each treatment. Half the replicates were harvested after 2 months and the other half after 4 months. At each harvest, the number of diseased and healthy shoots was counted, then each seed-piece and newly developing rhizome (at 4 months) was cut longitudinally to estimate the percentage of rotted tissue.

Vegetative compatibility of Foz isolates

Monoconidial isolates of *Foz* that had been tested for pathogenicity and subsequently stored on sterile filter paper were reconstituted on PDA + streptomycin and then transferred to carnation-leaf agar (CLA, water agar containing dried, gamma irradiated pieces of carnation leaf). *Nit* mutants were generated using the techniques of Puhalla (1985) and Correll *et al.* (1987). Cultures on CLA were then transferred to half-strength potato-sucrose agar containing 1.5% potassium chlorate (KPSA). The plates were incubated at 25°C and chlorate-resistant mutants that emerged as fast growing sectors from the restricted colonies on KPSA were sub-cultured on to minimal medium (MM) (Correll *et al.* 1987). *Nit* mutants were generated from 22 *Foz* isolates obtained from nine farms.

The *nit* mutants were assigned to phenotypic classes (*nit1*, *nit3* or NitM) on the basis of their growth on media containing one of three different nitrogen sources, nitrate, nitrite or hypoxanthine (Correll *et al.* 1987). All mutants were stored on MM or as dried cultures on filter paper.

Complementation testing was done by placing mycelium from *nit* mutants 15 mm apart on MM (Correll *et al.* 1987) and incubating the plates at 25°C in the dark for up to 10 days. Thus, *nit1*, *nit3* and NitM mutants generated from each isolate were paired among themselves or with *nit* mutants from all other isolates. Vegetatively compatible *nit*

mutants complemented one another by forming a heterokaryon. This was easily visible as a line of dense aerial growth of mycelium where hyphae of two sparsely growing colonies came into contact and anastomosed. When *nit* mutants from different isolates complemented each other, their parents were assigned to the same VCG.

Statistical Analysis

Data from the glasshouse and field experiments with biocides were analysed by analysis of variance (ANOVA) using Genstat 5 data analysis software. Means were compared using least significant difference (LSD) at $P = 0.05$. When necessary, percentage data were transformed (angular arcsine) prior to analysis by ANOVA.

Results

Pathogens associated with ginger planting material

The results in Table 1 show that *Foz* was commonly isolated from diseased rhizomes in crops designated for seed-piece production. The other *Fusarium* spp. were either *F. solani* or *F. oxysporum* (Burgess *et al.* 1994). *Pythium* spp. were less frequently isolated (four out of seven farms), while *Sclerotium rolfsii*, *Geotrichum* sp., and the bacterium *E. chrysanthemi* were each isolated from one farm.

A random sample of seed-pieces from storage bins on four farms had a relatively high incidence of *Foz*, whereas incidence was low on one farm (Table 2) and the fungus was not detected on a farm where long crop rotations are employed, and where rhizomes are harvested by hand and the seed-piece preparation process was not mechanised.

The severity of problems affecting seed-piece emergence varied from farm to farm (Table 3). On most farms, less than 20% of seed-pieces failed to produce shoots, but on a few farms this figure was greater than 50%. In most cases, disease incidence was greater 2 months after planting than after 1 month. Blackening of buds was the main symptom observed on seed-pieces that failed to produce shoots. The tissue in many of these seed-pieces remained firm and only the vascular strands were discoloured. However, in the advanced stages of disease, the tissue was sometimes soft and mushy and there was a strong and offensive odour, or seed-pieces were completely hollowed out.

Isolations from diseased seed-pieces revealed that *Foz* was associated with poor emergence at all monitoring sites. *E. chrysanthemi* was isolated less consistently, being detected on only five of eight farms. *S. rolfsii* was isolated at a low frequency from two farms, but *Pythium* was never recovered from newly planted seed-pieces. A Gram-negative, fermentative bacterium identified as *Enterobacter* sp. (GN Microplate BIOLOG, Hayward, CA) was commonly isolated from seed-pieces colonised by either *E. chrysanthemi* or *Foz*.

Pathogenicity tests

A total of 37 *Fusarium* isolates was tested and 28 were pathogenic to ginger. All the pathogens were identified as *Foz* (Burgess *et al.* 1994). The symptoms produced were a

Table 1. Disease levels in ginger and potential pathogens isolated in April–May 1998, in fields destined for seed-piece production

| Farm location | Site ID | No. of diseased plants/m of row ^A | Organisms isolated |
|---------------|-----------|--|--|
| Noosa | A | 0.2 | <i>Foz</i> ^B ; <i>Fusarium</i> sp. |
| | B | 0.9 | <i>Foz</i> ; <i>Fusarium</i> sp.; <i>Pythium</i> |
| Yandina 1 | A | 3.5 | <i>Foz</i> ; <i>Fusarium</i> sp.; <i>Pythium</i> |
| | B | 2.2 | <i>Foz</i> ; <i>Fusarium</i> sp.; <i>Pythium</i> |
| Eumundi 1 | A | 2.0 | <i>Foz</i> ; <i>Fusarium</i> sp.; <i>Geotrichum</i> |
| | B | 3.0 | <i>Foz</i> ; <i>Erwinia</i> ; <i>Fusarium</i> sp. |
| | C | 2.1 | <i>Erwinia</i> ; <i>Fusarium</i> sp. |
| Eumundi 2 | A | 1.7 | <i>Foz</i> ; <i>Fusarium</i> sp.; <i>Pythium</i> ; <i>Sclerotium rolfsii</i> |
| | North Arm | A | 2.2 |
| Beerwah | B | 2.4 | <i>Foz</i> |
| | A | 1.0 | <i>Foz</i> ; <i>Pythium</i> |
| Nambour | B | 4.0 | <i>Foz</i> |
| | A | 0.4 | <i>Foz</i> |

^AThere were 5–7 ginger plants/m row.^B*Fusarium oxysporum* f. sp. *zingiberi*.**Table 2. Level of disease in samples of stored ginger seed-pieces collected from different farms after fungicide treatment**

| Farm location | Cultivar | Fungicide | Storage time (weeks) | % diseased seed-pieces | Potential pathogens isolated |
|---------------|------------|-------------|----------------------|------------------------|---|
| Noosa | Canton | Carbendazim | 2 | 46 | <i>Geotrichum</i> , <i>Foz</i> ^A |
| Beerwah | Queensland | Carbendazim | 4 | 88 | <i>Foz</i> |
| Eumundi 2 | Canton | Carbendazim | 7 | 77 | <i>Foz</i> , <i>Erwinia</i> |
| Eumundi 1 | Canton | Carbendazim | 4 | 9 | <i>Foz</i> |
| Nambour | Queensland | Benomyl | 4 | 0 | – |
| Beerwah | Canton | Carbendazim | 3 | 35 | <i>Foz</i> |

^A*Fusarium oxysporum* f. sp. *zingiberi*.**Table 3. Disease status of fungicide-treated seed-pieces planted at several sites on various farms in 1998 and assessed approximately 1 month (T1) and 2 months (T2) after planting**

| Farm location | Per cent seed-pieces that did not emerge due to disease | | | | | | | |
|---------------|---|----|----------------|----|--------|----|--------|----|
| | Site 1 | | Site 2 | | Site 3 | | Site 4 | |
| | T1 | T2 | T1 | T2 | T1 | T2 | T1 | T2 |
| North Arm | 0 | 2 | – ^A | – | – | – | – | – |
| Yandina 1 | 0 | 5 | 15 | 14 | – | – | – | – |
| Yandina 2 | 12 | 10 | 0 | 7 | – | – | – | – |
| Yandina 3 | 2 | 0 | 0 | 19 | – | – | – | – |
| Eumundi 1 | 0 | 30 | 5 | 18 | 22 | – | 14 | 14 |
| Eumundi 2 | – | 62 | – | 62 | 12 | 41 | 23 | 74 |
| Noosa | 45 | – | 17 | 37 | – | – | – | – |
| Beerwah | 0 | 58 | 10 | 45 | 10 | 12 | – | – |

^A– = not done.

brown discoloration and rotting of seed-pieces. A few showed advanced symptoms where only a shell and some fibrous tissue persisted. Most seed-pieces did not produce a shoot, and if one was produced it soon turned yellow, wilted and eventually died. Two of the *Foz* isolates (BRIP39298 and BRIP 39299) were lodged in the Herbarium, Department of Primary Industries, Indooroopilly, Queensland.

The single isolate of *S. rolfsii* rotted seed-pieces within a month. Affected tissue had a faint pinkish-yellow tinge and the coarse mycelium of the fungus was clearly visible within the tissue. Abundant sclerotia were produced in 4–6 weeks. Isolates of *Geotrichum*, *Pythium* and *Enterobacter* did not rot ginger.

Table 4. Effects of various biocides on disease development in ginger seed-pieces from two farms

| Treatment | No. diseased seed-pieces (out of 12) | | Mean % rot in seed-pieces | |
|------------------------------|---|-------|---------------------------|-------|
| | Yandina 1 | Noosa | Yandina 1 | Noosa |
| Control (water) | 8 | 8 | 66.7 | 51.7 |
| Copper hydroxide | 5 | 4 | 34.3 | 11.3 |
| Azoxystrobin | 12 | 8 | 86.7 | 57.5 |
| Metalaxyl | 7 | 7 | 58.3 | 53.8 |
| Hot water | 10 | 8 | 74.3 | 41.3 |
| Carbendazim | 2 | 2 | 2.9 | 0.8 |
| Carbendazim (20 min at 48°C) | 2 | 5 | 1.7 | 10.8 |
| Benomyl | 4 | 2 | 13.3 | 2.5 |
| Benomyl (20 min at 48°C) | 6 | 4 | 25.8 | 2.5 |
| LSD ($P = 0.05$) d.f. = 98 | nd ^A | nd | 30.2 | 27.7 |

^And = analysis not done.

Table 5. Effect of biocides on disease in seed-pieces from Yandina 1

| Treatment | Mean shoots/plant at 9 weeks | Mean % diseased seed-pieces at 9 weeks |
|----------------------------------|---------------------------------|---|
| Control (water) | 0.14 | 78.5 |
| Carbendazim | 0.54 | 16.9 |
| Carbendazim (maintained at 48°C) | 0.75 | 23.3 |
| Metalaxyl | 0.09 | 71.4 |
| Carbendazim + metalaxyl | 0.63 | 19.4 |
| LSD ($P = 0.05$) d.f. = 29 | 0.27 | 22.31 |

Table 6. Effect of various biocides on disease in seed-pieces from Eumundi 1

| Treatment | Mean shoots /plant at 10 weeks | Mean % diseased seed-pieces at 10 weeks |
|------------------------------------|-----------------------------------|--|
| Control (water) | 0.39 | 84.4 |
| Carbendazim | 1.79 | 18.8 |
| Copper oxychloride | 1.04 | 37.5 |
| Copper oxychloride + carbendazim | 1.64 | 15.6 |
| Calcium hypochlorite | 0.48 | 75.0 |
| Calcium hypochlorite + carbendazim | 1.86 | 3.1 |
| Azoxystrobin | 0.67 | 53.1 |
| Azoxystrobin + carbendazim | 1.71 | 9.4 |
| LSD ($P = 0.05$) d.f. = 31 | 0.20 | 26.35 |

Susceptibility of two ginger cultivars to Foz

Both cv. Canton and cv. Queensland were equally susceptible to *Foz*, as there was no significant difference in the total number of seed-pieces that were rotted after 4 months for either cultivar (data not shown). However, after 2 months, cv. Queensland seed-pieces had produced more shoots than cv. Canton. There was also less rotting of rhizomes in cv. Queensland at 2 months (data not shown).

Tests with biocides

In two pot experiments, seed-pieces dipped in carbendazim, benomyl or copper hydroxide showed the

least amount of rotting (Table 4). Metalaxyl and azoxystrobin gave no disease control, as the incidence of rotting was similar to that in untreated ginger. In both experiments, the predominant pathogen was *Foz*. However, *E. chrysanthemi* was also present in ginger from the farm at Noosa.

The results of two field experiments (Yandina 1 and Eumundi 1) with a range of biocides confirmed that *Foz* was the main pathogen responsible for seed-piece rot. In both trials, all treatments containing fungicides shown to have efficacy against *Foz* in the previous experiments gave some disease control (Tables 5 and 6). *E. chrysanthemi* was

Table 7. Interaction table showing the effect of different post-washing storage times and carbendazim on the development of disease in ginger

| Effect of storage time | Mean % diseased seed-pieces | | | LSD ($P = 0.05$) |
|------------------------|-----------------------------|--------|--------|--------------------|
| | 1 day | 3 days | 6 days | |
| | 62.3 | 70.5 | 82.7 | 13.5 |
| Effect of fungicide | Carbendazim | Water | | 16.5 d.f. = 29 |
| | 49.7 | 93.9 | | |

Table 8. Interaction table showing the effect of different soil moisture contents on the development of rot in ginger when seed-pieces were treated with *Fusarium oxysporum* f. sp. *zingiberi*, *Erwinia chrysanthemi* or *Enterobacter* sp. alone or in various combinations

| Treatment | Mean % rotting in seed-pieces ^A | | | |
|--|--|--------|---------------|--------|
| | Low moisture | | High moisture | |
| Control (water) | 1.7 | (0.13) | 8.2 | (0.29) |
| <i>Enterobacter</i> sp. | 0.0 | (0.01) | 1.4 | (0.12) |
| <i>Foz</i> ^B | 55.4 | (0.84) | 64.3 | (0.93) |
| <i>E. chrysanthemi</i> | 0.0 | (0.00) | 22.2 | (0.49) |
| <i>Foz</i> + <i>Enterobacter</i> sp. | 35.7 | (0.64) | 64.3 | (0.92) |
| <i>Foz</i> + <i>E. chrysanthemi</i> | 26.4 | (0.54) | 96.4 | (1.38) |
| <i>Enterobacter</i> sp. + <i>E. chrysanthemi</i> | 2.9 | (0.17) | 36.6 | (0.65) |
| LSD ($P = 0.05$) d.f. = 196 | (0.37) | | | |

^AEquivalent means, with angular (arcsin) transformed means in parentheses.

^B*Fusarium oxysporum* f. sp. *zingiberi*.

detected in a few seed-pieces together with *Foz* in the experiment at Yandina 1.

In the experiment in which seed-pieces were stored before being dipped in carbendazim, fungicide-treated seed-pieces developed significantly less disease than untreated seed-pieces at all three storage times (Table 7). Overall, the amount of disease increased as the storage time prior to fungicide treatment increased. The pathogen most commonly isolated was *Foz*. *E. chrysanthemi* was also present, but at a much lower frequency, and was nearly always associated with *Foz*.

The effect of Foz, E. chrysanthemi, Enterobacter sp. and soil moisture on rotting of seed-pieces

Enterobacter sp. had no effect on rhizome rot at either high or low moisture levels (Table 8). However, significant rotting occurred at high but not low moisture levels when *E. chrysanthemi* was either present on its own or with *Enterobacter* sp. In contrast, high soil moisture did not exacerbate rotting when *Foz* was present on its own. However, the combination of *Foz*, *E. chrysanthemi* and high moisture resulted in almost complete rotting of all rhizomes (Table 8).

Vegetative compatibility groups

Three or four *nit* mutants were recovered for each of the 22 wild-type *Foz* isolates. The majority of mutants were *nit1*.

Based on complementation tests, all of the isolates were grouped in the single VCG group 0460.

Discussion

A survey of maturing ginger crops that were to be used for planting material in the following season showed that they were invariably infested with *Foz*. The pathogen was not eliminated during the seed preparation process, as *Foz* was still recovered after rhizomes had been washed, cut into seed-pieces and then dipped in a recommended fungicide. *Foz* was also isolated from rotting seed-pieces planted in soil that had never previously grown ginger, providing further evidence that the fungus was consistently present in planting material and was associated with batches of seed-pieces that did not establish well in the field.

Vegetative compatibility studies of a range of *Foz* isolates showed that they all belonged to group 0460, probably because the same fungus had been spread throughout the ginger industry on planting material. Pathogenicity tests clearly showed that this fungus was capable of rotting ginger seed-pieces within a few weeks of planting. Isolates identified as *F. solani* or *F. oxysporum* (Burgess *et al.* 1994) did not produce any symptoms.

The results of the biocide trials provided further evidence that *Foz* was the main cause of crop establishment problems. Carbendazim and benomyl, two fungicides recommended for control of *Foz*, increased emergence and reduced rotting of

seed-pieces in pots and in the field. Copper, which is a general biocide, also reduced levels of disease. In contrast, metalaxyl, which has efficacy against *Pythium* spp., was ineffective.

The role of other potential pathogens was investigated, but these organisms were either non-pathogenic, or were not consistently associated with the problem in the field. *Pythium*, a pathogen of ginger in saturated soils (Trujillo 1964; Dake and Edison 1989) and in storage (Grech and Swarts 1990; Pegg and Stirling 1994) was not isolated from newly planted seed-pieces and did not cause seed-piece rotting, even at relatively high inoculum levels. *Enterobacter* sp., a bacterium that was commonly isolated from rotted ginger, also did not damage ginger in pathogenicity tests. *E. chrysanthemi* and *S. rolfsii* were pathogenic, but *E. chrysanthemi* was not found in all affected fields and *S. rolfsii* was rarely encountered.

The observation that *Foz* was the main cause of poor emergence was unexpected. This pathogen has been present in the Queensland ginger industry since approximately 1930, but the symptoms it produces (yellowing, dieback and rhizome rotting) are usually not apparent until late in the season. Rotting of newly planted seed-pieces is known to occur, but such losses are usually insignificant. Therefore it appears that a more severe form of the disease has developed in recent years. One possible reason is that a government-sponsored disease-free planting material scheme was discontinued in the 1980s, and so infected plants in fields reserved for planting material are often not removed and destroyed. Secondly, inoculum densities of *Foz* have probably increased because more market ginger is being grown and these crops often stay in the ground for at least 15 months before harvest. In addition, land is being cropped more frequently because there is a limited amount of land available for ginger cultivation. Finally, harvesting, washing, fungicide-dipping and planting operations have become more mechanised in recent years and the resulting mechanical damage to knobs and buds probably provides a better infection court for pathogens such as *Foz*.

This study concentrated on cv. Canton, a large-knobbed selection favoured for high quality confection, as growers believed it was more susceptible to *Foz* than cv. Queensland. Data collected after 2 months from pot experiments suggested that this observation may have been true, as cv. Queensland produced more healthy shoots in *Foz*-infested soil than cv. Canton. However, after 4 months, both cultivars were equally susceptible, as most of the initial shoots had died and there was no difference in the number of infected rhizomes. The more rapid disease development in cv. Canton probably occurred because seed-pieces of similar weight to cv. Queensland have fewer knobs and buds and, therefore, produce fewer shoots early in the season. Any loss of shoots to disease will, therefore, have a greater impact on this cultivar. In addition, the large knob size of cv. Canton makes it more prone than cv. Queensland to mechanical damage,

and this may increase its susceptibility to pathogens. Blackened, non-viable buds infected with *Foz* were often observed in un-germinated seed-pieces of cv. Canton, but were rarely seen in cv. Queensland.

Survey data showed that the severity of emergence problems varied between fields and from farm to farm. It is impossible to draw firm conclusions about the reasons for this variation, but the most severe damage occurred when crops infected with *Foz* were used for planting material, and obviously diseased rhizomes were taken to the cutting table rather than being identified and discarded in the field.

Observations made since 1997 suggest that crop establishment problems in the ginger industry were also more severe in some years than others, possibly because environmental factors played a role. In 1998, for example, 309 mm of rain fell at Yandina during September and October, which meant that the soil was very wet for the first few weeks after ginger was planted. Under such conditions, *E. chrysanthemi* probably contributed to the emergence problem. This bacterium is commonly isolated from ginger that is infected with *Foz*, and on its own it is capable of rotting ginger seed-pieces, particularly in wet soil (Stirling 2002). This study confirmed the importance of soil moisture in bacterial soft rot and showed that when *Foz* and *E. chrysanthemi* occur together in wet soil, emergence is very poor.

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References

- Burgess LW, Summerell BA, Bullock S, Gott KP, Backhouse D (1994) 'Laboratory manual for Fusarium research.' (University of Sydney)
- Colbran RC, Davis JJ (1969) Studies of hot water treatment and soil fumigation for control of root-knot nematodes in ginger. *Queensland Journal of Agricultural and Animal Sciences* **26**, 339–445.
- Correll JC, Klittich CJR, Leslie JF (1987) Nitrate nonutilising mutants of *Fusarium oxysporum* and their use in vegetative compatibility tests. *Phytopathology* **77**, 1640–1646.
- Dake GN, Edison S (1989) Association of pathogens with rhizome rot of ginger in Kerala. *Indian Phytopathology* **42**, 116–119.
- Eckert JW, Tsao PH (1962) A selective antibiotic medium for isolation of *Pythophthora* and *Pythium* from plant roots. *Phytopathology* **52**, 771–777.
- Fahy PC, Hayward AC (1983) Media and methods for isolation and diagnostic tests. In 'Plant bacterial diseases: a diagnostic guide'. (Eds PC Fahy, GJ Persley) pp. 337–376. (Academic Press: Australia)

- Grech NM, Swarts DH (1990) Post-harvest application of fungicides for control of fungal decay of ginger rhizomes stored under simulated low-temperature shipping conditions. *Phytophylactica* **22**, 457–458.
- Pegg KG, Stirling GR (1994) Ginger. In 'Diseases of vegetable crops'. (Ed. DM Persley) pp. 55–57. (Department of Primary Industries: Queensland).
- Pegg KG, Moffet ML, Colbran RC (1974) 'Diseases of ginger in Queensland.' Advisory Leaflet No. 1284, Division of Plant Industry, Department of Primary Industries Queensland.
- Puhalla JE (1985) Classification of strains of *Fusarium oxysporum* on the basis of vegetative compatibility. *Canadian Journal of Botany* **63**, 179–183.
- Stirling AM (2002) *Erwinia chrysanthemi*, the cause of soft rot in ginger (*Zingiber officinale*) in Australia. *Australasian Plant Pathology* **31**, 419–420. doi:10.1071/AP02046
- Teakle DS (1965) Fusarium rhizome rot of ginger in Queensland. *Queensland Journal of Agricultural and Animal Sciences* **22**, 265–272.
- Trujillo EE (1964) 'Diseases of ginger (*Zingiber officinale*) in Hawaii.' Circular 62, Hawaii Agricultural Experiment Station, University of Hawaii.
- Whiley AW (1981) Effect of plant density on time to first harvest maturity, knob size and yield in two cultivars of ginger (*Zingiber officinale* Rosc.) grown in southeast Queensland. *Tropical Agriculture* **58**, 245–251.

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