# VG327
# Cause and control of new lettuce diseases

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## SUMMARY

Black root rot of lettuce caused by the fungus *Chalara elegans* was found in crops on the eastern Darling Downs, Granite Belt, Brisbane metropolitan region and Bundaberg but not the Lockyer Valley. In addition, it caused problems in hydroponically grown crops at Gatton and Mareeba. Losses were usually related to the use of infected seedlings. Introduction of the organism to nurseries and hydroponic production areas was due to the use of contaminated peat. Studies showed some samples of imported peat contained *Chalara elegans*. Once introduced, the disease was maintained on contaminated trays. Benomyl was much more effective than chlorine as an eradicant treatment for trays.

Lettuce varieties ranged from very susceptible to very resistant, e.g. Fame, Classic, Winguard are susceptible and Monaro Target and Centenary are resistant. Rotations with non-host crops reduced disease severity in later lettuce plantings.

*Chalara elegans* is a variable pathogen and different isolates showed different host ranges. Isolates from lettuce were often pathogenic to beans, some cucurbits, tobacco, sweet peas, pansy and viola. Severe outbreaks in pansy and viola have now also been traced to infested peat. Isolates from cotton (ex. Narrabri) were generally not highly virulent on lettuce.

Corky root caused by the bacterium *Rhizomonas suberifaciens* was found in crops on the Eastern Darling Downs and the Lockyer Valley. A soil sampling technique and bioassay was developed which indicates probable disease severity. The causal bacterium survives naturally in the soil and infects young plants. Field experiments showed disease severity could be reduced by using transplants rather than direct seeding. Field experiments also showed some new varieties bred for resistance in the USA have strong resistance to local strains of *R. suberifaciens*.

## GENERAL INTRODUCTION

Lettuce plants with black roots and severe stunting were first found in 1983 in the Brisbane metropolitan district. The cause of the disorder was identified as a fungus *Chalara elegans* Nag Raj and Kendrick (syn. *Thielaviopsis bassicola* (Berk and Br.) Ferraris). The disease declined, possibly due to the use of benomyl, until 1990 when recordings were made in crops on the Eastern Darling Downs. The severe stunting/slow growth associated with the disease caused concern for many growers.

Plants suspected of being affected by corky root were first seen in the Lockyer Valley in 1991. The identification was confirmed by van Bruggen and Jochimsen (1993) and further recordings were made on the Eastern Darling Downs and there were reports of it in southern lettuce growing districts.

The first crops found to be affected were direct seeded and we considered this may have contributed to the high incidence. In view of the series of recordings of corky root in the USA (Florida, New York, Wisconsin, California) and Europe, the disease appeared to have the potential to cause serious on-going losses.

This project was undertaken to observe the incidence of these two diseases, factors contributing to outbreaks and practical control methods. Since the two diseases are caused by unrelated organisms, this report will deal with each one individually.

# A. LETTUCE BLACK ROOT ROT (*CHALARA ELEGANS*)

## INTRODUCTION

*Chalara elegans* (Nag Raj and Kendrick) is a soil-borne fungus. It was first recorded in Queensland as a pathogen of sweet pea (*Lathyrus odoratus* L.) in 1930 (Simmonds 1966) and since then, the host range has been extended to include tobacco (*Nicotiana tabacum* L.) and *Lupinus angustifolius* L. Elsewhere, *Chalara elegans* is recognised as having a wide host range affecting species in over 15 plant families (Subramanian 1968). In several economically important crops, it is a serious pathogen. Black root rot diseases of cotton, tobacco, chicory, endive, soybean, peanut are some of these. Although lettuce has not been recorded as a natural host anywhere in the world, endive (*Chicorium endivia* L.) and chicory (*C.. intybus L.*) are in the same family (Compositae) and black root rot is considered their major disease in South Africa (Prinsloo *et al.* 1993).

*Chalara elegans* has no perfect stage but reproduces by two asexual spore forms — the endoconidia which are produced in large numbers and the thick-walled chlamydospores. The former are thought to be easily dispersed by wind and water, and germinate readily, thus providing the ability for rapid multiplication of infection sites. The chlamydospores are resistant to desiccation (Stover 1950) and provide a means of long-term survival. Infection occurs by direct penetration of tobacco roots (Stover, 1950), bean roots and hypocotyl (Christou, 1962), citrus roots (Tsao & Van Gundy, 1962), cotton roots (Mathre *et al.*, 1966) and Japanese holly roots (Wick and Moore, 1983). In some other species, e.g. chicory and peanut, penetration occurs after the formation of an appressorium (Prinsloo *et al.* 1992; Jones 1991). The fungus then invades all tissues of the root with the exception of the xylem. Affected tissues become rotted and eventually slough off leaving the stringy xylem elements. The typical dark colouration is, in part, due to the formation of the black chlamydospores in large numbers.

Although *C. elegans* has a wide host range, there is evidence that the fungus exists as strains which show different host specificities. Keller and Shanks (1955) found that tobacco strains did not attack poinsettia and vice versa. Lloyd and Lockwood (1963) reported that *T.*

*basicola* strains from poinsettia, orange and pea were moderately to highly virulent on bean and pea but non-pathogenic to tobacco. In contrast, strains from tobacco were highly virulent on tobacco but non-pathogenic on bean plants .

Inoculum concentration influenced the expression of host specificity in work reported by Tabachnick *et al.*,1979. In a comparison of nine isolates from various geographic and host origins, host specificity was more apparent at an inoculum concentration of $10^4$ endoconidia per gram of soil than at $10^3$ or $10^2$ per gram of soil. The nine isolates were grouped in four categories based on their virulence to cotton and bean. One isolate was non-pathogenic; one was weakly pathogenic to cotton but not bean; four were highly virulent to both cotton and bean and three were highly virulent to cotton but non-pathogenic to bean.

Control measures for black root rot have been directed at nursery hygiene, varietal resistance and fungicidal control. Graham and Timmer (1991) found Canadian peat was a source of infection for citrus nurseries and once introduced, the fungus remained active in the nursery and surrounds. They suggested peat consignments be tested for the presence of *C. elegans* before use. Other hygiene measures include general cleanliness of the nursery and discarding used potting media. Varietal resistance has been sought and is used in some crops, notably tobacco (Lucas 1975) . Suitable resistant parents for a breeding program have been found among commercial cultivars of chicory and endive (Prinsloo *et al.* 1993).

Many fungicides have high activity against *Chalara elegans* and may have a role, especially in controlling the disease in nurseries. In fields, fumigants containing chloropicrin are commonly used to reduce disease severity, however, the fungicides propiconazole and etaconazole applied over the row at planting gave 46% and 64% control in peanuts (Labuschagne and Kotze, 1996). The disease was well controlled in tobacco seedbeds (Prinsloo *et al.*, 1989) by fluzilazole 0.4 g a.i./$m^2$ and triadimenol (0.046 g a.i./$m^2$). Benomyl (0.4 g a.i./$m^2$) was less effective. In cotton, triadimefon has been registered as a seed dressing. The severity of disease is related to the inoculum density in the soil. With tobacco, *Chalara elegans* was found less frequently and in lower densities in fields where rotations had been practised as compared with those in continuous tobacco (Meyer *et al.* 1989).

Limited work has been done to investigate the effect of various rotations but it is generally assumed that non-host plants or immune varieties of a crop plant will lead to lower disease if two-year rotations are practised. Ideally rotations should commence before high soil inoculum levels are reached. In a study which involved the addition of plant tissues of lucerne, carrot, rye and onion all reduced the survival of phialospores but onion was the most effective (Chittaranjan and Pinja, 1994).

## 1. DISEASE DISTRIBUTION AND CONTRIBUTING FACTORS

The first record of *C. elegans* in lettuce was made in 1983 from Rochedale, Brisbane. A specimen, BRIP 13916 was deposited in the mycological herbarium at Indooroopilly. Further records were made commencing in 1990 from plant specimens which were either submitted by growers or found during field surveys.

### METHODS

**Pathogen isolation**. Several methods were used to obtain isolates to confirm the presence of the pathogen in samples: (a) Root pieces were surface sterilized in sodium hypochlorite (1000 ppm available chlorine) for 30 seconds then rinsed in sterile water, blotted dry and transferred to potato dextrose agar (PDA); (b) Root sections with young lesions were brushed free of soil under running tap water and incubated on water agar (2%) in Petri dishes for 24 h. Chains of phialoconidia that developed were transferred by sterile needle to PDA; (c) Variations of the carrot disc baiting method first described by Yarwood (1946) were tested to isolate the fungus from soil potting mix and peat samples. These included sandwiching a layer of soil between two carrot slices, placing carrot discs on top of moist soil, placing two drops of a soil suspension on a carrot disc and incubating for two days (soil suspension: 10 mL soil shaken with 30 mL water then allow heavier particles to settle before taking the sample); (d) Soil samples were placed in 10 cm diameter pots and sown with 20 seed cv. Yatesdale. Seed was covered with a thin layer of Vermiculite and roots examined for lesions after four weeks.

**Isolate maintenance.** Isolates were preserved by freeze-drying and on agar cubes in sterile distilled water. During transferring, white sectors in the cultures were avoided.

**Host range studies.** Two experiments were conducted.

Experiment A. Seed of 13 species reported as hosts of *C. elegans* by Klimova and Koshkelova (1981) and cultivated in south-east Queensland were sown in pots of infested mix (millet seed soaked 24 h, autoclaved, inoculated, incubated 7 d then mixed into UC potting mix 1% v/v) and rated for disease severity after 4 weeks.

Experiment B. Speedling trays were filled with a 1:1 peat/vermiculite seedling mix infested by adding 1% infected millet grain and seed of several crop and weed species sown (Table 1). Ten cells were used for each species. *Portuclaca oleracea* was propagated by nodal cuttings.

Six weeks later, plants were removed, roots washed and inspected for symptoms of black root rot. The degree of damage was rated on a 0-5 scale where 0, - no disease; 1, slight browning of small roots, tap root slightly affected; 2, necrotic secondary roots, tap root slightly affected; 3, tap root with large lesions, most lateral roots affected; 4, tap root severely affected, most lateral roots diseased or missing; 5, root system wholly affected.

**TABLE 1**

**Weeds and crop plants grown in a potting medium infested with *Chalara elegans***

| **Botanical Name** | **Common Name** |
|---|---|
| *Ageratum houstonianum* Miller | Blue billygoat weed |
| *Avena sativa* L. | Common oats |
| *Bidens pilosa* L. | Cobblers peg |
| *Cajanus cajan* (L.) Mill sp. Dunn | Pigeon pea |
| *Cardamine hirsutum* (L.) | Flickweed |
| *Cartharanthus roseus* (L.) | Periwinkle |

| Botanical Name | Common Name |
|---|---|
| *Curcurbita maxima* L. cv. Ken's Special | Pumpkin |
| *Echinochloa utilis* Ohwi and Yabuno | Japanese millet |
| *Euphorbia prostrata* | Caustic weed |
| *Gomphrena celosioides* Matt. | Gomphrena weed |
| *Gossypium hirsutum* L. | Upland cotton |
| *Helianthus annus* L. | Sunflower |
| *Hypochoeris radicata* L. | Flatweed |
| *Lablab purpureus* | Lablab bean |
| *Latuca sativa* L. cv. Yatesdale | Lettuce |
| *Macroptilium atropurpureum* DC Urban. | Siratro |
| *Panicum laevifolium* Hack. | Sweet panic |
| *Pisum sativum* L. cv. Sugarsnap | Pea |
| *Portulaca oleracea* L. | Pigweed |
| *Saccharum officinarum* L. | Sugarcane |
| *Solanum nigrum* L. | Blackberry nightshade |
| *Sonchus oleraceus.* L | Common sowthistle |
| *Sorghum halepense* (L.) Pers. | Johnson grass |
| *Trifolium repens* L.cv. Big Bee | White clover |

## RESULTS

**Distribution.** In the Brisbane metropolitan area, crops at Runcorn and Rochedale showed the disease (Fig. 1). Further west in the Lockyer Valley no recordings were made from field grown crops, although lettuce were affected in a hydroponic production area. No recordings were made in Toowoomba near-metropolitan crops but the disease was widespread in crops on the Eastern Downs. It was also recorded on two properties on the Granite Belt. Black root rot was present on one of three farms growing lettuce in the Bundaberg district and was also recorded in a hydroponic lettuce production unit at Mareeba. Infected lettuce seedlings were found in a seedling production area on the eastern Downs.

In addition to lettuce, we found *C. elegans* affecting seedlings of ornamentals, particularly viola and pansy, in several nurseries. Unthrifty seedlings in retail outlets were often affected by black root rot (see Appendix — Black root rot, a new concern for nurseries and Fig. 2).

**Source of inoculum.** Infestation of seedling nurseries and hydroponic production units suggested the origin of inoculum need not be soil. Peat samples collected from unopened bags were tested using the carrot baiting technique and positive recordings made in a low proportion. In all, 42 bales of peat were sampled from farms and nurseries. Peat from Canada, New Zealand, Ireland, Germany and Holland were sampled. *Chalara elegans* was found in two bales of New Zealand peat (Warrior and Kiwi) while a related species *C. thielavioides* was isolated from two bales of Canadian peat. A third, unknown species (*Chalara* sp.) was also isolated from Canadian peat.

A positive recording of *Chalara elegans* was made in freshly prepared peat-vermiculite medium sampled from the hydroponic lettuce production unit in the Lockyer Valley.

A small glasshouse experiment showed *C. elegans* to be retained in polystyrene Speedling trays. Three trays were selected randomly from the lettuce seedling nursery where *C. elegans* was found. A sample of potting mix was also taken. Similar, sterile speedling trays and UC mix from DPI Indooroopilly were used in a comparative growth study where the treatments were:

1. Farmer's tray — DPI mix
2. Farmer's tray — farmer's mix
3. DPI tray — DPI mix
4. DPI tray - farmer's mix

Seed (cv. Yatesdale) was sown and seedlings examined after four weeks. The results (Fig 3) clearly show the Chalara was carried over in the farmer's tray.

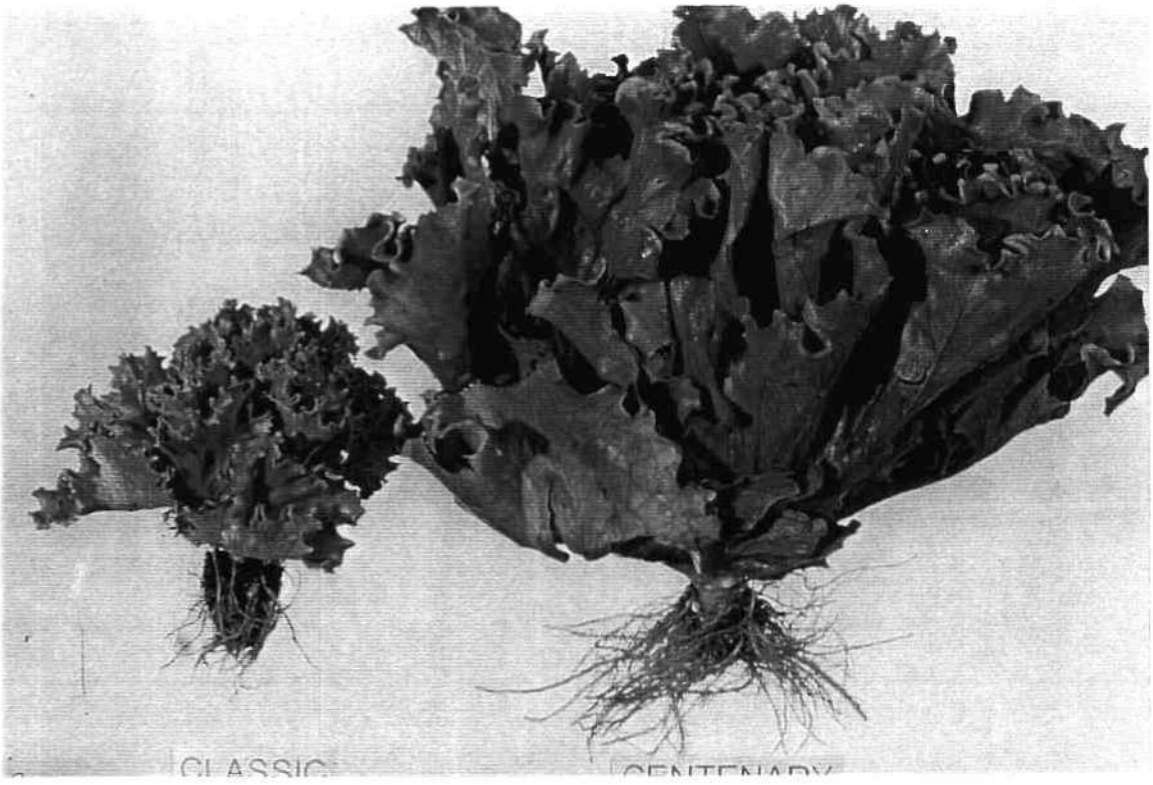



**Fig. 1.** Black root rot affects the growth of transplants. Both varieties (Classic and Centenary) are the same age and show differences in susceptibility to the disease.

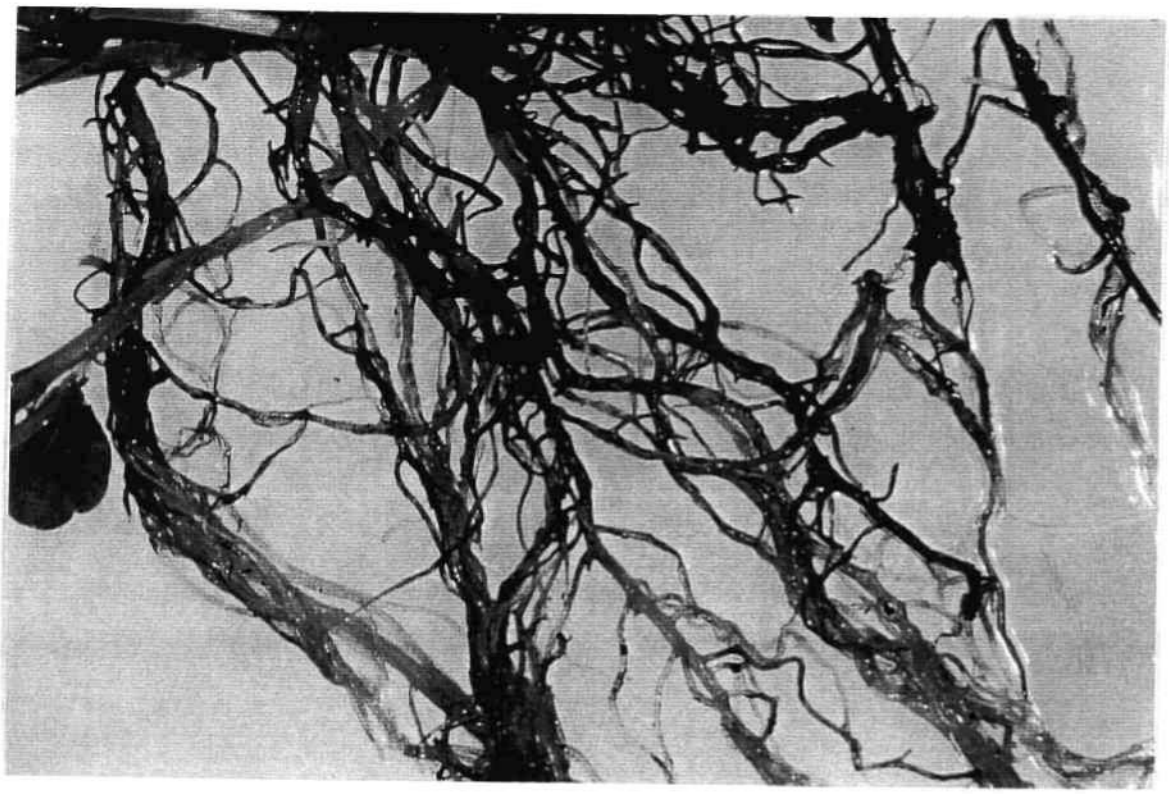


Fig. 2 Black root rot (*Chalara elegans*) affecting pansy.


Fig. 3 Inoculum carryover in seedling trays: Symptoms of black root rot developed in plants grown in trays from a farmer's seedling nursery. There was no evidence of inoculum in the batch of farmer's seedling potting mix sampled.

Roots of the weed *Sonchus oleraceus* L. were collected from farms on the eastern Downs, washed free of soil and sections with lesions incubated in Petri dishes. Chains of phialoconidia developed from the lesions.

In a test of five soils collected from farms on the Eastern Downs (4) and Toowoomba (1), the presence of *C. elegans* was confirmed in 40 samples by both carrot baiting and growing lettuce seedlings in soil samples. Neither technique showed *C. elegans* to be present in the Toowoomba soil sample.

**Fungal growth studies.** The growth rate of the fungus was determined in a multi-range incubator (10 chambers, max. 39.2°C; min. 9.0°C). The optimum temperature range for growth of the fungus was 23-26°C while growth was negligible below 12°C and above 32°C.

**Host range.** Experiment A. Lettuce and bean were most affected (Table 2). The cucurbits watermelon, cucumber and rockmelon were less severely affected (roots healthy, crown discoloured) while other species were unaffected. Both isolates behaved similarly.

Experiment B. Several species showed symptoms of infection (Table 3). Lettuce was most severely affected but Pigeon pea, cotton, lab-lab, pea, sunflower and Johnson grass were also noticeably infected. Minor root lesions occurred with weed species such as nightshade and common sowthistle and in cultivated species such as white clover and Siratro.

## TABLE 2

**Severity of root or crown infection of several crop species by two lettuce isolates (2864 and 3143) of *Chalara elegans***

| Crop species | Disease severity[A] | |
|---|---|---|
| | **Isolate 2864** | **Isolate 3143** |
| Bean (*Phaseolus vulgaris* L.) cv. Covey | +++ | +++ |
| Capsicum (*Capsicum frutescens* L.) cv. Northern Bell | - | - |
| Celery (*Apium graveolens* L.) cv. Tendercrisp | - | - |
| Cotton (*Gossypium hirsutum* L.) cv. Siokra 1-4 | - | - |
| Cucumber (*Cucumis sativus* L.) cv. Crystal Apple | + | - |
| Eggplant (*Solanum melongena*) L. cv. N.Y. Purple | - | - |
| Lettuce (*Lactuca sativa* L.) cv. Classic | +++ | +++ |
| Lettuce cv. Great Lakes | +++ | +++ |
| Parsley (*Petroselinum crispum* (Mill.) Nym.) | - | - |
| Radish (*Raphanus sativus* V.) cv. French Breakfast | - | - |
| Rockmelon (*Cucumus melo* L.) cv. Laguna | + | + |
| Tomato (*Lycopersicon esculentum* Miller) cv. Floradade | - | - |
| Watercress (*Rorippa nasturtium-aquaticum* (L.) Hayek) | - | - |
| Watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai) cv. Crimson Sweet | ++ | ++ |

**TABLE 3**

**Severity of black root rot symptoms on plants grown in a peat/vermiculite medium infested with isolate 4150 of *Chalara elegans***

| Plant species | Mean disease severity (0-5) |
|---|---|
| *A. lonstonianum* | 0 |
| *A. sativa* | 0 |
| *B. pilosa* | no germination (N.G.) |
| *C. cajun* | 3.0 |
| *C. hirsuta* | 0 |
| *C. roseus* | 1.10 |
| *C. maxima* | 1.6 |
| *E. prostrata* | N.G. |
| *G. celosiodes* | N.G. |
| *G. hirsutum* | 3.0 |
| *H. annuus* | 2.0 |
| *H. radicata* | N.G. |
| *L. purpureus* | 3.1 |
| *L. sativa* | 3.6 |
| *M. atropurpureum* | 1.0 |
| *P. laevifolium* | 0 |
| *P. sativum* | 2.8 |
| *P. oleracea* | 0 |
| *S. officinarum* | N.G. |
| *S. nigrum* | 1.0 |
| *S. oleraceus* | 1.0 |
| *S. halepense* | 2.0 |
| *T. repens* | 1.7 |

## DISCUSSION

Lettuce production in Queensland has always been predominantly (90%) from the south-east corner. Twenty-five years ago, areas close to metropolitan Brisbane (Runcorn, Sunnybank, Nudgee, Rochedale, Cleveland-Redland Bay) were the centres of production. With urbanisation these areas have contracted and the industry has developed in the Lockyer Valley, Toowoomba, eastern Darling Downs and Granite Belt. Coinciding with this has been the cultural change from direct seeding to transplanting seedlings grown in soil-less mixes . There has also been the development of hydroponic lettuce production, especially of fancy types for the restaurant trade.

Although first recorded in Queensland in 1930, *Chalara elegans* has not become a serious or even common disease of its recorded hosts, sweet pea and tobacco. It was surprising, therefore, to find it widespread and causing economic damage to lettuce in 1990/92.

The results of our observations suggest that this increase in distribution and severity is due initially to peat infested with *Chalara elegans.* Seedlings which become infected in the seedling stage introduce the disease to the field, while infested seedling trays serve as a source of inoculum for later sowings. This scenario is supported by the following facts:

1. *Chalara elegans* was positively confirmed in two bales of previously unopened peat.

2. The disease was established in two hydroponic lettuce units which used soil-less (i.e. peat based) media for seedling production. Both of these were sited in areas where field lettuce has not become affected (Lockyer Valley and Atherton Tableland), thus making aerial contamination from soil unlikely.

3. The disease was identified in a lettuce seedling production area where a peat-vermiculite medium was used. It has also been found affecting ornamental seedlings (pansy, viola) from nurseries in the Lockyer Valley and Redlands districts.

4. The experiment in which trays and potting mix from a commercial nursery were compared with sterile trays and steamed UC mix from the DPI at Indooroopilly showed that contaminated trays alone were sufficient to cause severe symptoms in seedlings. In the absence of efficient tray sterilization, the disease would be self perpetuating.

Once established in the field, *C. elegans* could remain for several years due to the longevity of its chlamydospore and the reservoir of infection provided by weed hosts such as *Sonchus oleraceus.* The host range studies demonstrated that *Chalara elegans* was capable of infecting (without predisposing injury) crop species such as legumes, cucurbits, sunflower and cotton, some grasses, e.g. Johnson grass as well as weeds such as Sowthistle and nightshade. Even though symptoms were slight on some species, they could serve as alternative hosts in the survival of *C. elegans* between lettuce crops. Several crop species were unaffected by the isolates used in these studies, e.g. capsicum, celery, eggplant and tomato. Suitable grass rotation crops would be oats or Sweet Panic.

The levels of *C. elegans* in four out of five soil samples collected from fallow lettuce fields were sufficient to cause disease symptoms in lettuce seedlings grown in them.

Soil temperature will probably play a part in determining the longevity of the organism in Queensland production areas. The optimum temperature for growth of *C. elegans* was 23-26°C but the maximum was a relatively low 32°C. In this regard, it is similar to *Verticillium dahliae* which has a maximum temperature for growth of 33°C. Verticillium is commonly present in Redlands and Lockyer Valley in potatoes and tomatoes, has limited distribution in the Bundaberg district but is rarely recorded north of Bundaberg except in cooler elevated tablelands Both organisms have long-lived, survival spores. It is probable, therefore, that the field distribution of *C. elegans* will also be limited to about Bundaberg .

The importance of peat as a source of *C. elegans* was first recognised by Graham and Timmer (1991) who showed that after introduction to a nursery area, the organism survived in peat debris and was distributed within the nursery by air-borne phialoconidia. Our observations support this since we were able to show *C. elegans* was present in the prepared potting mix of a contaminated nursery but were unable to show its presence in unopened bags of peat being

used for its preparation. It seems highly probable therefore that the occurrence of *C. elegans* in lettuce crops is due to the occasional presence of the organism in peat rather than the development of local strains of this previously rare pathogen.

## 2. PATHOGENIC VARIABILITY BETWEEN ISOLATES OF *CHALARA ELEGANS* AND *C. THIELAVIOIDES* OBTAINED FROM VARIOUS SOURCES.

As noted in the Introduction, many authors have found the host range of *C. elegans* to be extremely variable . A degree of pathogenic specificity is common i.e. isolates obtained from a certain host species are generally highly pathogenic to that host but variable in pathogenicity to other hosts.

During this project, *C. elegans* was isolated from peat suggesting this could be the source of *Chalara elegans* causing black root rot in lettuce. It was essential this be proven. *Chalara elegans* had also been reported as the cause of a new root disease of cotton in NSW (Allen 1990) and we had noted its presence in punnets of pansy and viola. The following series of experiments was conducted to seek similarities between isolates from various sources to these 3 hosts - lettuce, cotton and pansy/viola. The pathogenicity of isolates of *C. thielavioides* from peat was also examined.

### METHODS

Six separate glasshouse experiments were conducted between July 1994 - August 1996. Isolates were obtained from peat, lettuce, viola, cotton and soil from lettuce farms.

A root dip technique was used in the first experiment and infested potting mix in the others.

**Root dip inoculation (Expt 1).** Spore suspensions were prepared by flooding PDA cultures with sterile water and dislodging conidia. All suspensions were adjusted to 1 x $10^6$ conidia/mL. Seedlings of lettuce, viola and cotton were raised individually in seedling trays for different periods to enable each species to develop to transplanting size:- viola 7 wk,

lettuce 3 wk and cotton 10d. Seedling roots were washed then dipped in spore suspensions and transplanted to individual pots filled with UC mix. A water treatment was included for comparison. After 20d, plants were removed from pots and the severity of root symptoms rated on a scale from 0-5.

**Infested mix as inoculum (Experiments 2-6).** Infested white millet seed was added to UC mix at rates of 1-2% by volume. Inoculum was prepared by soaking millet overnight in distilled water, draining off excess water and autoclaving in small containers. These were later seeded with agar cubes of Chalara culture. The millet cultures were allowed to grow for 2-3 weeks then used to infest UC potting mix at rates between 1-2% by volume. The inoculum was distributed evenly through the potting mix by shaking in a plastic bag. In experiments 2-4, seed was sown on the top of the infested mix then covered with a layer of vermiculite. In some cases, this led to an uncertainty over the cause of pre-emergence losses which could have been due to disease or just poor germination. The method used in expts. 5 and 6 was one which allowed seed to germinate in a layer of sterile mix then for roots to grow through to the infested mix. This allowed an early plant count to be made and compared with a final plant count. Missing plants, particularly small pansy and lettuce seedlings could then be included as deaths due to the disease. For lettuce, viola and pansy, 10cm diameter pots were filled with 400ml infested mix, then a layer of 50ml sterile UC medium. Seed (approx. 20 per pot) were sprinkled on this layer then covered with 50 mL vermiculite. For the larger seeded cotton,12.5 cm diameter pots were used and filled with 600 mL infested mix, 200 mL sterile medium, 5 seed sown and covered with 200 mL vermiculite.

**Disease ratings.** Black root rot causes death and disintegration of the smaller roots. While healthy plants have a white fibrous root system, diseased plants have a root system which at first shows diseased sections on secondary feeder roots progressing to loss of all feeder roots and disease lesions on the tap root. Severely diseased plants may survive for some time by continually producing adventitious roots. The rating system for disease severity was based on the proportion of the root system affected. It was used in conjunction with an examination of healthy control plants. After washing free of potting mix, plant root systems were assigned a value from 0-5:

0 — no disease; similar to control plants.

1 — trace presence; small lesions on feeder roots

2 — low; a few small feeder roots totally affected, most roots healthy. Overall <25% roots diseased or lost

3 — moderate; most roots with lesions, many( >25<50%) severely affected

4 — severe; most roots (50-<90%) severely affected

5 — very severe; >90% of all roots severely affected or lost or plant dead.

**Experimental design**. The experiments were laid out in randomized blocks with split plots in either 3 or 4 replications.

Experiment 1. 6 isolates of *C. elegans* (C.e.)and 4 isolates of *C. thielavioides* (C.t.) were compared using 2 lettuce cultivars (Classic and Centenary), viola (Johnny - Jump-Up) and cotton (Siokra -1-4). 4 replications. Inoculation was by root dip.

Experiment 2. The same isolates were used in this experiment. Potting mix was infested with millet grain inoculum (1.5% by volume) and seed of viola (30/pot), lettuce cv. Classic (15/pot) and cotton (5/pot) sown. 4 replications.

Experiment 3. 6 isolates of C.e. and 4 isolates of C.t. were compared. Grain inoculum (1.5% by volume ) infested the potting medium. Lettuce cv. Classic( 25/pot), viola cv. Johnny-Jump-Up (25/pot) and cotton Siokra-1-4 (5/pot) sown on the surface of the infested potting medium. 4 replications.

Experiment 4. 6 isolates of C.e., 4 of C.t. and 3 of *Chalara* sp. Were tested for pathogenicity using infested UC mix (1.5% by volume) and cultivars Classic, Johnny-Jump-Up and Siokra-1-4.

Experiment 5. 17 isolates of C.e. and 1 of C.t. were included in this test. Grain inoculum (2% by volume) infested the potting medium. Seed of lettuce cv. Yatesdale, viola cv. Johnny-Jump-Up and cotton c.v. Siokra-1-4 were sown into a layer of sterile medium. 3 replications.

Experiment 6. 17 isolates of C.e. and 1 of Ct. Grain inoculum 2% by volume infested the medium. Test species were lettuce cv. Yatesdale, pansy cv. Oranges & Lemons cotton cv. Siokra-1-4 and tobacco cv. Hicks Q46.

**RESULTS**

Experiment 1. The two lettuce isolates of C.e. (4122, 2864) caused severe root rot on lettuce cv. Classic but not on cv. Centenary. All isolates caused severe symptoms on viola. Isolates of C.e. from peat, viola and cotton soil caused symptoms on cotton. (Table 4 ).

Experiment 2. In this experiment the same isolates were compared using infested mix instead of root dipping. The only isolates which caused symptoms on lettuce were the lettuce isolates (4122, 2864). All C.e. isolates caused moderate - severe symptoms on viola as well as one of the C.t. isolates (4124-1) All C.e. isolates except 4122 caused severe symptoms on cotton (Table 5 ).

Experiment 3. Lettuce isolates (4122, 4150) were the only ones which caused severe symptoms on that host. All C.e. isolates were highly pathogenic to viola while C.t. isolates were not. Isolate 4117 from cotton soil caused most severe symptoms on cotton but C.e. isolates from lettuce (4150), peat (4121, 4120) and viola (24316) caused moderate - severe symptoms. C.t. isolates were of low virulence. ( Table 6 )

Experiment 4: Three isolates from lettuce (2864, 4122, 4150) and one from peat (4121) caused severe symptoms on lettuce. All C.e. isolates except 24316 were pathogenic to viola. One C.t. isolate (4139) also caused moderate damage to this host. Only 2 isolates of C.e. (4150, 4121) caused moderate damage to cotton. Table 7 )

Experiment 5. All 9 isolates of C.e. from lettuce or lettuce soil as well as one peat isolate (4297) were highly virulent on lettuce. Isolate 4121 ex peat caused moderate symptoms. The remaining 6 isolates of C.e. from viola, cotton and peat caused only slight damage. Isolates 4150, 4121, 4327, 4330 and 4234 caused severe symptoms on viola. These isolates were from lettuce, peat, viola and cotton. Other lettuce isolates caused low-moderate disease on

viola. On cotton, severe symptoms were caused by isolates 4249 (lettuce soil) 4237 (lettuce) 4234, 4117 (cotton). Moderate symptoms were due to 4150 (lettuce), 4121 (peat) and 4330, 4327 (viola). ( Table 8 )

Experiment 6. Seven isolates (6 ex lettuce, 1 ex peat) were significantly more virulent to lettuce than the other isolates (Table 9 ). These caused severe disease symptoms. In pansy, disease levels were only low-moderate with a maximum disease rating of 3.0 caused by isolate 4121 (ex peat). Two other isolates, 4150 and 4489 (ex lettuce and pansy) were not significantly different in their disease severities. Six isolates caused severe disease (>4.0) on cotton while only minor symptoms were noticed on tobacco.

## DISCUSSION

The six experiments were spread over a two year period and for some of the isolates, virulence declined. For example isolate 24316, initially highly virulent to viola and cotton became of low virulence in experiments 4-6. This change is probably due to accidental selection of a low virulent type during routine transferring of isolates. A similar change occurred with isolate 2864 in experiment 6. In 5 experiments this isolate was strongly virulent to lettuce and pansy but was greatly reduced in experiment 6.

Overall, disease ratings in experiment 6 were lower than in previous tests.

Pathogenicity of *C. thielavioides.* Several isolates of C.t. and undetermined Chalara species were included in the experiments. In experiment 1 where root dip inoculation was used, severe symptoms were induced on viola and moderate symptoms on lettuce. In later experiments where root wounding was not a factor, disease levels were quite low. It can be concluded that *C. thielavioides* is unlikely to be a problem in these hosts. It may, however, be of concern to rose growers since C.t. has been recorded as a root pathogen of roses.

Virulence of lettuce isolates. The 9 isolates originating from diseased lettuce plants or baited from lettuce soil were all highly virulent to that host. Three (2864, 4122, 4150) consistently caused severe disease in viola while 4 (4277, 4289, 4247, 4250) caused moderate disease

severity. In cotton , results were variable between experiments, although isolate 4150 caused moderate- severe symptoms in the 4 experiments in which it was included. Isolates such as 2864, 4277 and 4289 were of low virulence to cotton.

Virulence of peat isolates. Four isolates of C.e. were collected from peat samples and included in at least 2 of the experiments. Isolates 4120 and 4123 were of low virulence to lettuce, high virulence to viola and moderate virulence to cotton. Isolates 4121 caused moderate symptoms on lettuce, severe symptoms on viola and moderate - severe symptoms on cotton. Isolate 4279 showed high virulence to lettuce, low virulence to viola and few symptoms on cotton. Its low virulence to viola separates it from the 3 New Zealand isolates (4120,4121,4123).

The isolate from Irish peat (4279) thus showed a different pattern to the 3 isolates from New Zealand peat which had higher virulence to lettuce and lower virulence to viola.

Virulence of isolates from viola/pansy. The 4 isolates were similar with a pattern of low virulence on lettuce, high virulence on viola and moderate virulence on cotton. As mentioned previously, isolate 24316 showed reduced virulence in the final 3 tests.

Virulence of isolates from cotton/cotton soil. The 2 isolates 4117 and 4234 were highly pathogenic to cotton, of low virulence to lettuce and were moderate - severe on viola.

To summarise the data from 6 experiments is difficult but it appears that the only isolates which consistently caused severe damage to lettuce were those which originated from this crop or from peat.

Most isolates were capable of causing at least moderate damage to viola.

The degree of damage to cotton plants caused by isolates of low virulence was often difficult to judge, since roots of cotton plants are naturally discoloured. Isolates from cotton (4177, 4234) consistently caused severe root symptoms and stunted growth. Some other isolates e.g. 24316, 4121 and 4150 caused moderate - high disease severity in the experiments. There was

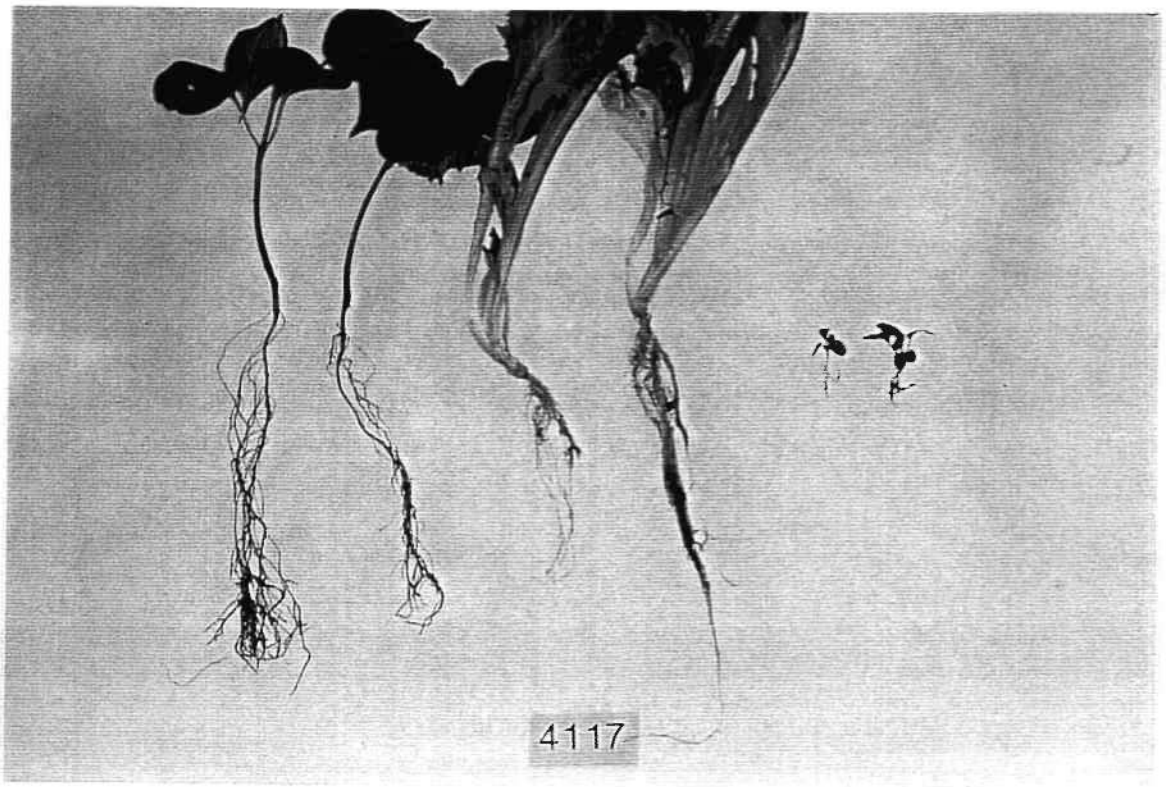
no consistent relationship between isolate origin and pathogenicity to cotton except for the 2 cotton isolates.

The link between the pathogenicity of peat isolates to lettuce and/or viola suggests peat could be the source of black root rot in these crops. Selection of strains adapted to particular hosts would then lead to isolates with the host specificity observed in these experiments. Isolates were generally most highly virulent to their host of origin.

**TABLE 4**

**Expt 1: Root rot severity in lettuce viola and cotton following root dip inoculation in isolates of *Chalara elegans* and *C. thielavioides***

| | | | Black root rot severity | | | |
|---|---|---|---|---|---|---|
| **Isolate** | **Origin** | **Species** | **lettuce** | | **viola** | **cotton** |
| | | | **cv. Classic** | **cv. Centenary** | | |
| 4122 | lettuce | C. e. | 4.3 | 1.8 | 5.0 | 1.5 |
| 2864 | lettuce | C. e. | 4.2 | 1.7 | 4.5 | 0 |
| 24316 | viola | C. e. | 2.3 | 1.2 | 5.0 | 4.5 |
| 4121 | NZ peat | C. e. | 2.0 | 1.2 | 5.0 | 4.3 |
| 4123 | NZ peat | C. e. | 1.8 | 1.0 | 5.0 | 3.6 |
| 4124-2 | lettuce | C. t. | 1.8 | 1.2 | 4.8 | 0 |
| 4124-1 | potting mix | C. t. | 1.8 | 0.7 | 4.0 | 0 |
| 4117 | cotton soil | C. e. | 1.8 | 1.3 | 5.0 | 5.0 |
| 4119 | C. peat | C. t. | 1.5 | 0.5 | 4.7 | 0 |
| 4118 | C. peat | C. t. | 1.0 | 0.8 | 4.2 | 0 |
| LSD P - 0.05 | | | 0.74 | 0.65 | 0.58 | 0.67 |



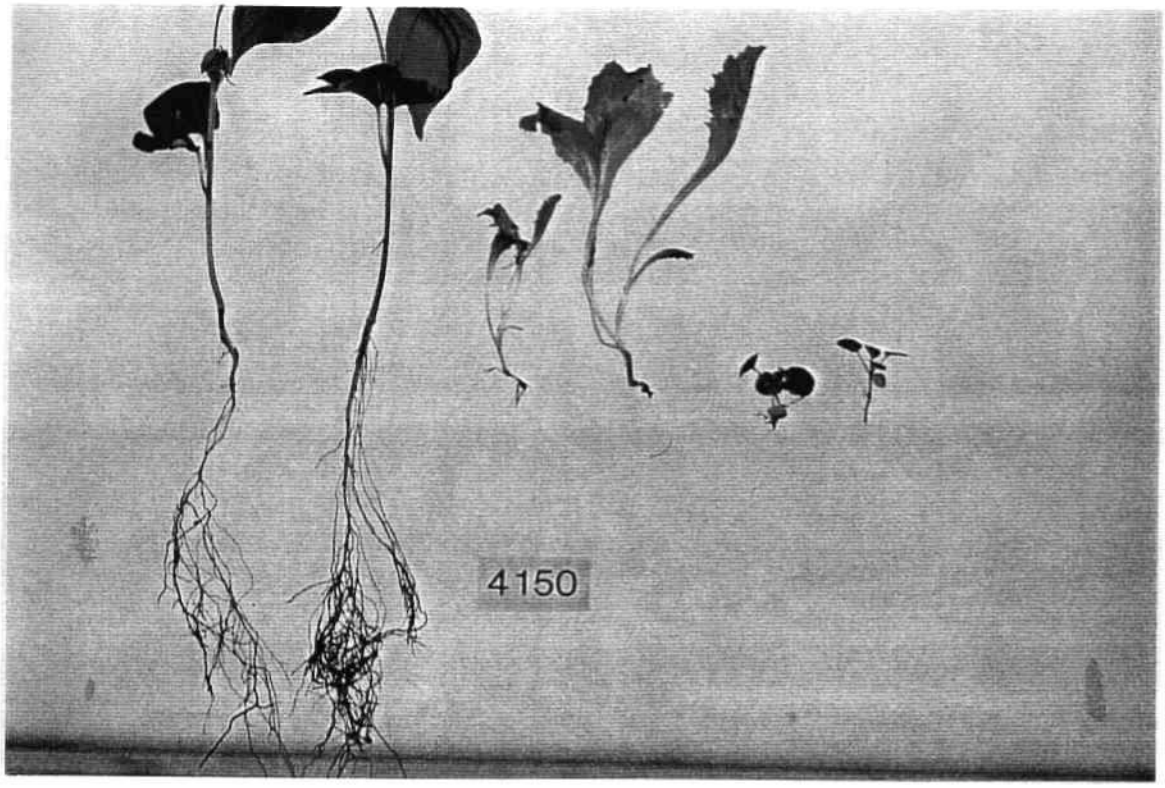




Fig. 4 Differences between isolates in pathogenicity to cotton, lettuce and viola: Isolate 4117 (ex cotton) reduced the growth of cotton plants by its severe root infection. Isolate 4150 was not pathogenic to cotton but was virulent on lettuce and viola.

## TABLE 5

**Expt 2: Root rot severity in lettuce, viola and cotton following growth in potting medium infested with 10 isolates of *Chalara elegans/C. thielavioides***

| Isolate | Origin | Species | Root rot severity (0-5) | | |
|---|---|---|---|---|---|
| | | | lettuce | viola | cotton |
| 4122 | lettuce | C. e. | 3.9 | 3.1 | 2.6 |
| 2864 | lettuce | C. e. | 3.8 | 3.4 | 4.3 |
| 4121 | N.Z. peat | C. e. | 0.7 | 4.7 | 5.0 |
| 24316 | viola | C. e. | 0.7 | 5.0 | 4.9 |
| 4117 | cotton soil | C. e. | 0.6 | 3.5 | 5.0 |
| 4123 | N.Z. peat | C. e. | 0.6 | 4.7 | 4.5 |
| 4119 | Can. peat | C. t. | 0.4 | 1.4 | 3.0 |
| 4124-1 | potting mix | C. t. | 0.3 | 3.0 | 2.9 |
| 4118 | Can. peat | C. t. | 0.3 | 0.9 | 3.1 |
| 4124-2 | lettuce | C. t. | 0.2 | 1.4 | 1.9 |
| LSD P = 0.05 | | | 0.27 | 1.15 | 1.06 |

## TABLE 6

**Expt 3: Root rot severity in lettuce, viola and cotton following growth in potting medium infested with 10 isolates of *Chalara elegans/C. thielavioides***

| Isolate | Origin | Species | Root rot severity (0-5) | | |
|---|---|---|---|---|---|
| | | | lettuce | viola | cotton |
| 4122 | lettuce | C. e. | 5.0 | 3.8 | 2.3 |
| 4150 | lettuce | C. e. | 4.9 | 4.7 | 4.0 |
| 4121 | N.Z. peat | C. e. | 3.3 | 5.0 | 4.0 |
| 24316 | viola | C. e. | 1.7 | 4.9 | 3.6 |
| 4124-2 | lettuce | C. t. | 1.3 | 1.8 | 1.0 |
| 4120 | N.Z. peat | C. e. | 0.9 | 4.6 | 4.3 |
| 4117 | cotton soil | C. e. | 0.8 | 5.0 | 4.9 |
| 4124-1 | potting mix | C. t. | 0.4 | 0.8 | 1.4 |
| 4118 | Can. peat | C. t. | 0.4 | 0.9 | 0.7 |
| 4139 | Can. peat | C. t. | 0.2 | 1.3 | 1.4 |
| LSD P = 0.05 | | | 0.51 | 0.86 | 0.85 |

## TABLE 7

**Expt 4: Root rot severity in lettuce viola and cotton following growth in potting medium infested with 10 isolates of *Chalara elegans/C. thielavioides***

| Isolate | Origin | Species | Root rot severity (0-5) | | |
|---|---|---|---|---|---|
| | | | lettuce | viola | cotton |
| 2864 | lettuce | C. e. | 5.0 | 4.9 | 1.1 |
| 4122 | lettuce | C. e. | 5.0 | 4.2 | 1.1 |
| 4150 | lettuce | C. e. | 5.0 | 5.0 | 2.9 |
| 4121 | N.Z. peat | C. e. | 4.3 | 4.9 | 2.7 |
| 4118 | Can. peat | C. t | 1.5 | 1.3 | 1.1 |
| 4139 | Can. peat | C. t | 1.3 | 3.9 | 0.2 |
| 24316 | viola | C. e | 0.8 | 0.7 | 1.4 |
| 4124-1 | potting mix | C. t. | 0.8 | 1.9 | 0.8 |
| 4230-1 | peat | C. sp. | 0.7 | 1.2 | 0.7 |
| 4120 | NZ peat | C. e. | 0.6 | 4.9 | 1.3 |
| 4124-2 | lettuce | C. t. | 0.6 | 2.4 | 0.9 |
| 4230-2 | peat | C. sp. | 0.6 | 0 | 0.4 |
| 4230-3 | peat | C. sp. | 0.6 | 0.6 | 0.5 |
| LSD P = 0.05 | | | 1.1 | 1.3 | 1.0 |

## TABLE 8

**Expt 5. Root rot severity in lettuce viola and cotton due to 17 isolates of *Chalara* elegans and one isolate of C. *thielavioides***

| Isolate | Origin | Species | Root rot severity (0-5) | | |
|---|---|---|---|---|---|
| | | | lettuce | viola | cotton |
| 4150 | lettuce | C. e. | 5.0 | 5.0 | 3.3 |
| 4122 | lettuce | C. e. | 5.0 | 3.1 | 1.2 |
| 4250 | lettuce soil | C. e. | 5.0 | 2.9 | 1.3 |
| 4249 | lettuce soil | C. e. | 5.0 | 0.6 | 4.8 |
| 4277 | lettuce | C. e. | 5.0 | 3.6 | 0.7 |
| 2864 | lettuce | C. e. | 4.8 | 3.2 | 0.5 |
| 4279 | peat | C. e. | 4.8 | 1.5 | 0.7 |
| 4247 | lettuce soil | C. e. | 4.6 | 2.8 | 1.7 |
| 4289 | lettuce | C. e. | 4.6 | 2.8 | 0.6 |
| 4277 | lettuce | C. e. | 4.5 | 1.7 | 4.7 |
| 4121 | N.Z. peat | C. e. | 2.3 | 5.0 | 3.9 |
| 4327 | viola | C. e. | 1.9 | 5.0 | 3.2 |
| 4330 | viola | C. c. | 1.1 | 4.8 | 3.2 |
| 4120 | N.Z. peat | C. e. | 1.1 | 2.4 | 1.2 |
| 4234 | cotton | C. e. | 0.8 | 4.3 | 4 5 |
| 4117 | cotton soil | C. e. | 0.2 | 2.9 | 4.1 |
| 4118 | Can. peat | C. t. | 0.2 | 0.2 | 0 |
| 24316 | viola | C. e. | 0.2 | 0.2 | 0 |
| LSD P = 0.05 | | | 0.47 | 1.28 | 1.32 |

## TABLE 9

**Composite of results of experiments 1-6 in which the virulence of several isolates of *C. elegans* and *C. thielavioides* to lettuce viola and cotton were compared**

| | | | LETTUCE | | | | | | VIOLA | | | | | | COTTON | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | 4 | 5 | 6 | 1 | 2 | 3 | 4 | 5 | 6 | 1 | 2 | 3 | 4 | 5 | 6 |
| 2864 | lettuce | C. e. | 4.2 | 3.8 | | 5.0 | 4.8 | 1.9 | 4.5 | 3.4 | | 4.9 | 3.2 | 0.3 | 0 | 2.6 | | 1.1 | 0.5 | 0.2 |
| 4122 | lettuce | C. e. | 4.3 | 3.9 | 5.0 | 5.0 | 5.0 | 4.7 | 5.0 | 3.1 | 3.8 | 4.2 | 3.1 | 2.2 | 1.5 | 2.6 | 2.3 | 1.1 | 1.2 | 0.6 |
| 4150 | lettuce | C. e. | | | 4.9 | 5.0 | 5.0 | 3.6 | | | 4.7 | 5.0 | 5.0 | 2.3 | | | 4.0 | 2.9 | 3.3 | 4.2 |
| 4237 | lettuce | C. e. | | | | | 4.5 | 1.7 | | | | | 1.7 | 0.3 | | | | | 4.7 | 0 |
| 4277 | lettuce | C. e. | | | | | 5.0 | 4.7 | | | | | 3.6 | 0.9 | | | | | 0.7 | 0.4 |
| 4289 | lettuce | C. e. | | | | | 4.6 | 2.7 | | | | | 2.8 | 1.1 | | | | | 0.6 | 0.3 |
| 4247 | lettuce soil | C. e. | | | | | 4.6 | 4.6 | | | | | 2.8 | 1.8 | | | | | 1.7 | 2.6 |
| 4249 | lettuce soil | C. e. | | | | | 5.0 | 4.4 | | | | | 0.6 | 1.6 | | | | | 4.8 | 1.5 |
| 4250 | lettuce soil | C. e. | | | | | 5.0 | 4.3 | | | | | 2.9 | 2.0 | | | | | 1.3 | 2.5 |
| 4120 | NZ peat | C. e. | | | 0.9 | 0.6 | 1.1 | 1.7 | | | 4.6 | 4.9 | 2.4 | 0.4 | | | 4.3 | 1.3 | 1.2 | 4.3 |
| 4121 | NZ peat | C. e. | 2.0 | 0.7 | 3.3 | 4.3 | 2.3 | 0.8 | 5.0 | 4.7 | 5.0 | 4.9 | 5.0 | 3.0 | 4.3 | 5.0 | 4.0 | 2.7 | 3.9 | 4.5 |
| 4123 | NZ peat | C. e. | 1.8 | 0.6 | | | | | 5.0 | 4.7 | | | | | 3.6 | 4.5 | | | | |
| 4279 | Irish peat | C. e. | | | | | 4.8 | 4.7 | | | | | 1.5 | 1.2 | | | | | 0.7 | 2.5 |
| 4118 | Can. peat | C. t. | 1.0 | 0.3 | 0.4 | 1.5 | 0.2 | 0.4 | 4.2 | 0.9 | 0.9 | 1.3 | 0.2 | 0.7 | 0 | 3.1 | 0.7 | 1.1 | 0 | 0.2 |
| 4119 | Can peat | C. t. | 1.5 | 0.4 | | | | | 4.7 | 1.4 | | | | | 0 | 3.0 | | | | |
| 4139 | Can peat | C. t. | | | 0.2 | 1.3 | | | | | 1.3 | 3.9 | | | | | 1.4 | 0.2 | | |
| 4230-1 | peat | C. sp. | | | | 0.7 | | | | | | 1.2 | | | | | | 0.7 | | |
| 4230-2 | peat | C. sp. | | | | 0.6 | | | | | | 0 | | | | | | 0.4 | | |
| 4230-3 | peat | C. sp. | | | | 0.6 | | | | | | 0.6 | | | | | | 0.5 | | |
| 4124-1 | potting mix | C. t. | 1.8 | 0.3 | 0.4 | 0.8 | | | 4.0 | 3.0 | 0.8 | 1.9 | | | 0 | 2.9 | 1.4 | 0.8 | | |
| 4124-2 | lettuce | C. t. | 1.8 | 0.2 | 1.3 | 0.6 | | | 4.8 | 1.4 | 1.8 | 2.4 | | | 0 | 1.9 | 1.0 | 0.9 | | |
| 4237 | viola | C. e. | | | | | 1.9 | | | | | | 5.0 | | | | | | 3.2 | |
| 4489 | pansy | C.e. | | | | | | 0.4 | | | | | | 2.9 | | | | | | 4.5 |
| 4330 | viola | C. e. | | | | | 1.1 | | | | | | 4.8 | | | | | | 3.2 | |
| 24316 | viola | C. e. | 2.3 | 0.7 | 1.7 | 0.8 | 0.2 | 0.2 | 5.0 | 5.0 | 4.9 | 0.7 | 0.2 | 0.2 | 4.5 | 4.9 | 3.6 | 1.4 | 0 | 0 |
| 4117 | cotton soil | C. e. | 1.8 | 0.6 | 0.8 | | 0.2 | 0.8 | 5.0 | 3.5 | 5.0 | | 2.9 | 0.6 | 5.0 | 5.0 | 4.9 | | 4.1 | 5.0 |
| 4234 | cotton | C. e. | | | | | 0.8 | 1.8 | | | | | 4.3 | 2.2 | | | | | 4.5 | 5.0 |
| LSD P = 0.0 | | | 0.74 | 0.27 | 0.51 | 1.1 | 0.47 | 1.54 | 0.58 | 1.15 | 0.86 | 1.30 | 1.28 | 0.74 | 0.67 | 1.06 | 0.85 | 1.0 | 1.32 | 1.28 |

# 3. RELATIVE SUSCEPTIBILITY OF LETTUCE CULTIVARS TO *CHALARA ELEGANS*

Field observations suggested there were differences in susceptibility between lettuce cultivars. In particular, differences between the popular varieties Centenary and Classic were noticed (Fig. 1 ). Several experiments were carried out in the glasshouse to test the susceptibility of a wide range of lettuce cultivars.

## METHODS

Inoculation techniques and some other methodology varied between experiments as outlined below:-

Experiment 1. Forty-four cultivars were grown in speedling trays and five seedlings of each (at transplanting stage) were washed free of potting mix and root dip inoculated in a 1 x $10^6$/mL conidial suspension. Each seedling was transplanted to a 10 cm diameter pot, grown for four weeks then rated for disease severity on a 0-5 scale.( p. 19 )

Experiment 2. Eleven cultivars were compared using root dip and infested potting mix inoculation techniques. Plants were raised individually in speedling trays to transplanting size then inoculated by either

(a) root dip in a conidial suspension ( 1 x $10^6$/mL); then transplanted to U.C. mix.

(b) infested U.C. mix — incorporation of 1% (v/v) infested white millet grain in the U.C. transplanting medium.

These treatments were compared with

(c) control — transplanting to U.C. mix.

The experiment was laid out in a randomized blocks design with 11 treatments (varieties), three sub-treatments (inoculation methods) and 10 replications.

Each seedling was transplanted to a 10 cm diameter pot and grown for three weeks. Roots were washed, rated for disease severity then the 10 replicate root systems of each cultivar were bulked and oven-dried weights determined.

Experiment 3. Thirty-five cultivars were grown in speedling trays and transplanted to 10 cm diameter pots filled with infested U.C. mix (1% v/v). There were five replications of inoculated plants and two of control plants. After three weeks growth, plants were removed, roots gently washed and rated for disease severity (0-5). It was apparent that, with some resistant cultivars, root growth was stimulated by the presence of *C. elegans* (Fig. 5 ). Plants were, therefore, also rated for root bulk in comparison with the control plants of each variety: 1, <25% root volume *c.f.* control plants; 2, >25%-50%; >50%-75%; 4, >75%-100%; 5, >100%.

Experiment 4. Potting mix was infested with millet seed inoculum (2% v/v) and dispensed to 10 cm diameter pots. Lettuce seed (20 per pot) of the seven test cultivars were placed on the surface and covered with a thin layer of vermiculite. There were replicates in a randomized blocks design. Plants were grown for six weeks then roots rated for disease severity (0-5).

Experiment 5. Nine fancy lettuce cultivars were compared for susceptibility. An isolate, 2864, was grown on sterile millet then added to U.C. mix at 2% v/v. Lettuce seed (20/10 cm pot) were sown on the surface and covered with vermiculite. There were four replicates of each treatment. The susceptible cultivar, Oxley, was included as a standard. Five weeks after sowing, roots were rated for disease severity (0-5).

**TABLE 10**

**Expt 1: The severity of root disease caused by *Chalara elegans* to 44 lettuce cultivars using a root dip technique**

| Cultivar | Disease severity (0-5) | Cultivar | Disease Severity (0-5) |
|---|---|---|---|
| Winterset | 0.5 | 9668 | 4.2 |
| Kirralee | 0.6 | G.L. 3 | 4.2 |
| Centenary | 1.2 | 9674 | 4.4 |
| Galaxy | 1.4 | 5477A | 4.4 |
| Summer Salinas | 1.8 | Wintersalad | 4.4 |
| NKX 030 | 1.8 | Yatesdale | 4.4 |
| Nambucco | 2.0 | EXP 54 | 4.6 |
| Monaro | 2.2 | Supagreen | 4.6 |
| NW 132 | 2.2 | Oxley | 4.6 |
| S479A | 2.4 | MD1 | 4.8 |
| SPS 3 | 2.4 | S472A | 4.8 |
| SPS 2 | 2.5 | G.C. 2 | 4.8 |
| Salinas | 2.8 | NKX031 | 5.0 |
| Target | 2.8 | SPS 5 | 5.0 |
| S469A | 3.0 | Classic | 5.0 |
| SPS4 | 3.6 | Palo Verde | 5.0 |
| Black Velvet | 3.7 | Fame | 5.0 |
| SPS 1 | 3.8 | MT2 | 5.0 |
| 9669 | 3.8 | Superior | 5.0 |
| SPS CR1 | 3.8 | NKX029 | 5.0 |
| SPS CR2 | 4.0 | | |
| La Jolla | 4.0 | | |
| Buffalo | 4.2 | | |
| Sea Green | 4.2 | | |

## RESULTS

Experiment 1. The 44 cultivars and lines showed a range of susceptibility to black root rot (Table 10 ). While some popular varieties (Fame, Classic, Oxley, Yatesdale) were very susceptible, others (Kirralee, Centenary, Galaxy, Nambucco) were resistant.

Experiment 2. There were marked differences in the susceptibility of lettuce cultivars to the disease (Table 11). Some (Classic, NKX 029 and Yatesdale) were highly susceptible while others (Monaro, Kirralee, NKX 030 and Centenary) were resistant. Inoculation by root dip was more severe than growing in infested potting medium, although the ranking of cultivars was similar with both techniques. Differences in cultivar susceptibility were also observed on commercial properties (Figure 1). Root dipping caused all varieties to have lower dry weights than the check but there was evidence of growth stimulation in resistant lines grown in the infested mix (Table 12).

### TABLE 11

**Expt. 2. The severity of black root rot symptoms in 11 lettuce cultivars following growth in infested potting medium or root dip inoculation with *Chalara elegans***

| Cultivar | Disease severity (0-5)[A+] | |
|---|---|---|
| | Infested medium | Root dip inoculation |
| Monaro | 0.40 | 1.60[B] |
| Kirralee | 0.50 | 1.50[B] |
| NKX030 | 0.80 | 1.40[B] |
| Centenary | 0.80 | 1.47[B] |
| Nambucco | 0.80 | 2.20[B] |
| Target | 1.10 | 2.30[B] |
| Galaxy | 1.40 | 3.70[B] |
| Buffalo | 3.90 | 4.70[B] |
| Yatesdale | 4.60 | 4.80 |
| NKX029 | 4.60 | 4.90 |
| Classic | 4.90 | 5.00 |
| LSD (P = 0.05) | 0.51 | 0.54 |

[A]0, no disease; 5, >90% of roots affected. [B]Value is significantly greater (P = 0.05) than the corresponding value in the other column.

Experiment 3. Varieties ranged from completely resistant to highly susceptible (Table 13 ). Of the more popular varieties, Elliot, Patriot, Target Centenary and Fraser were resistant while Yatesdale, Sea Green, Winguard, Classic and Fame were susceptible. There was a trend for highly resistant cultivars (<1.0) to have larger root bulk in plants challenged by *C. elegans*. In severely diseased lines, root bulk was greatly diminished (Table 13, Fig.5 ).

**TABLE 12**

**Expt. 2. The effect of black root rot (2 inoculation methods) on dry weight of lettuce seedlings**

| Cultivar | Dry weight g (% difference from control) | | |
|---|---|---|---|
| | infested medium | root dip | control |
| Monaro | 2.086 (+ 11.4%) | 1.249 (- 32.7%) | 1.872 |
| Kirralee | 1.608 (+ 0.8%) | 1.181 (-26.0%) | 1.596 |
| NKX-030 | 1.546 (-8.5%) | 1.158 (- 31.5%) | 1.690 |
| Centenary | 1.701 (+ 30.3%) | 1.086 (-16.8%) | 1.305 |
| Nambucco | 2.058 (+ 26.7%) | 1.387 (- 14.6%) | 1.624 |
| Target | 2.188 (+ 37.7%) | 1.313 (-17.4%) | 1.589 |
| Galaxy | 1.011 (- 36.0%) | 0.715 (- 54.6%) | 1.583 |
| Buffalo | 0.925 (- 15.8%) | 0.402 (- 63.4%) | 1.098 |
| Yatesdale | 0.940 (- 62.7%) | 0.495 (- 74.0%) | 1.498 |
| NKX-029 | 1.197 (- 37.9%) | 0.788 (- 59.1%) | 1.929 |
| Classic | 0.965 (- 48.5%) | 0.511 (- 72.0%) | 1.875 |

## TABLE 13

**Expt. 3. The severity of black root rot and its effect on root development in 35 lettuce cultivars**

| Variety | Black root rot (0-5) | Root bulk (1-5) | Variety | Black root rot (0-5) | Root bulk (1-5) |
|---|---|---|---|---|---|
| NW 8005 | 0 | 5.0 | Fame | 3.4 | 3.2 |
| NW 8006 | 0 | 4.6 | NW 8008 | 3.8 | 3.2 |
| NW 8009 | 0.2 | 4.8 | NW 8013 | 3.8 | 2.2 |
| SPS761 | 0.2 | 4.0 | Classic | 4.0 | 2.0 |
| Elliot | 0.2 | 4.8 | NW 8010 | 4.2 | 1.4 |
| Patriot | 0.4 | 5.0 | NW 8016 | 4.4 | 1.2 |
| Salinas | 0.6 | 4.8 | NW 8018 | 4.4 | 1.4 |
| Warrior | 0.6 | 4.4 | Black Velvet | 4.4 | 2.2 |
| Target | 0.6 | 4.6 | Winguard | 4.4 | 3.2 |
| Centenary | 0.8 | 4.2 | Bamboo | 4.6 | 1.8 |
| Fraser | 0.8 | 4.2 | NW 8015 | 4.6 | 1.0 |
| Sally | 0.8 | 4.6 | NW 8014 | 4.8 | 1.4 |
| 8007 | 0.8 | 4.4 | NW 8017 | 4.8 | 1.4 |
| Monaro | 1.0 | 5.0 | NW 8019 | 4.8 | 1.6 |
| Top Gun | 2.2 | 3.4 | Sea Green | 5.0 | 1.4 |
| NW 8012 | 3.0 | 3.2 | Misty Day | 5.0 | 1.0 |
| NW 8011 | 3.2 | 2.4 | Yatesdale | 5.0 | 1.4 |
| El Toro | 3.2 | 3.4 | | | |

Experiment 4. Three of the seven cultivars (Magnum, NW 8005 and Rodeo) were highly resistant while the remaining four were highly susceptible (Table 14 ).

**TABLE 14**

**Expt. 4. Susceptibility of seven lettuce cultivars to black root rot**

| Cultivar | Disease Severity (0-5) |
|---|---|
| Magnum | 0.74 |
| NW 8005 | 0.83 |
| Rodeo | 0.87 |
| Sea Green | 4.00 |
| 159 | 4.03 |
| Summer Gold | 4.27 |
| 160 | 4.35 |

Experiment 5. None of the test varieties were as susceptible as the standard cv. Oxley. Cultivars Regency and Cos Red were significantly more susceptible than Myzuna, Lollo Bionda, Lollo Bello, Greenfield and Summer Red ( Table 15).

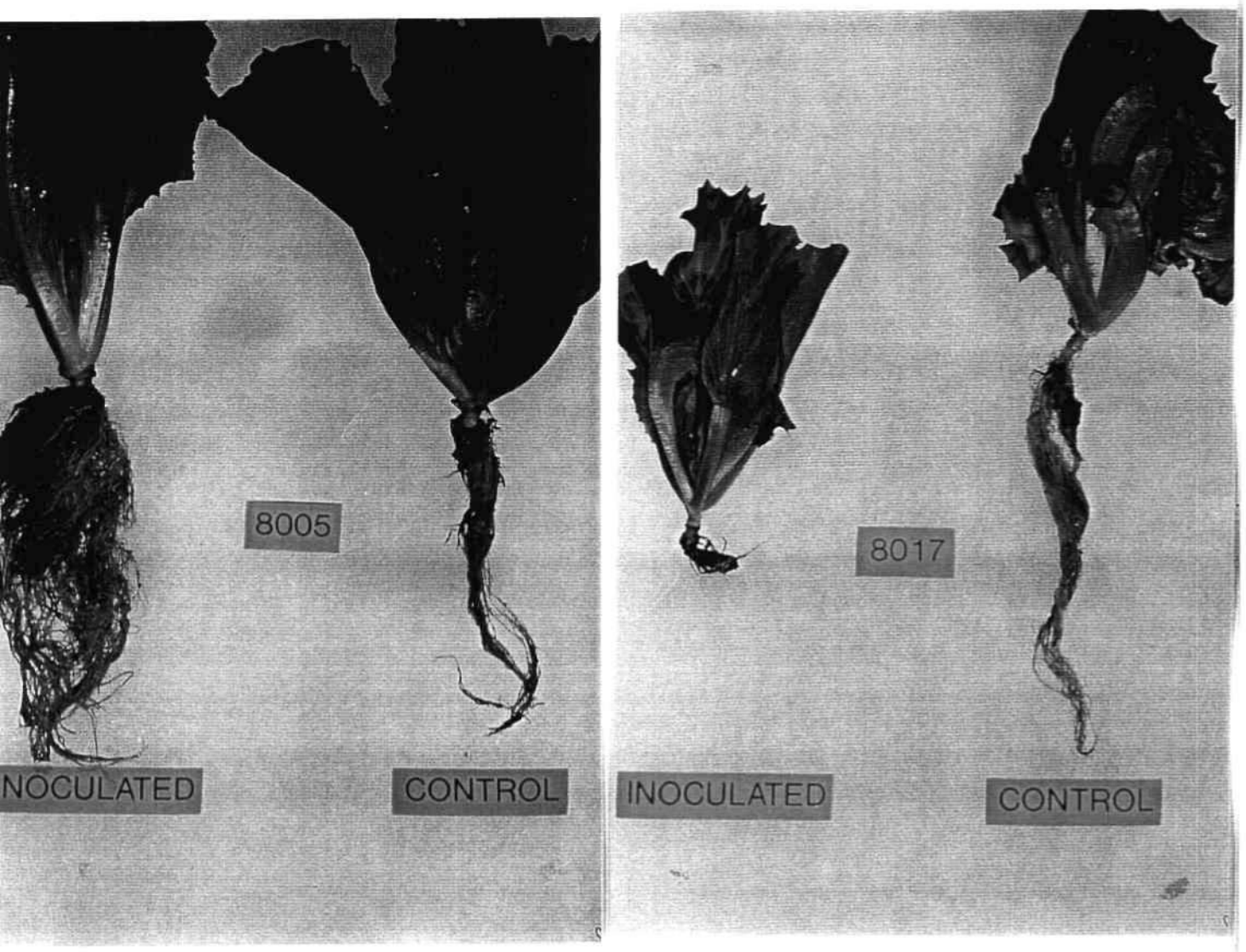



Fig. 5 Root growth stimulation by *Chalara elegans*: Lettuce line N.W. 8005 is resistant to black root rot and shows increased root bulk due to inoculation. Line NW8017 is susceptible and shows the typical effect of root rot with greatly reduced root bulk.

## TABLE 15

**Expt. 5. Severity of black root rot in fancy lettuce varieties sown into potting mix infested with *Chalara elegans*.**

| Lettuce Cultivar | Disease Severity |
|---|---|
| Magnum | 0.2 |
| Lollo Bionda | 0.40 |
| Lollo Bello | 0.47 |
| Greenfield | 0.87 |
| Summer Red | 0.90 |
| Red Velvet | 1.55 |
| Red Mignonette | 1.65 |
| Cos Red | 2.00 |
| Regency | 2.00 |
| Oxley | 4.12 |
| LSD (P <0.05) | 0.92 |

## DISCUSSION

This series of inoculation experiments shows that there are major differences in susceptibility to *Chalara elegans* in commercial varieties and breeding lines of lettuce. The differences between cultivars was consistent regardless of inoculation technique although root dip inoculation yielded higher ratings (Table ). Ranking of varieties in different experiments was also consistent, e.g. Yatesdale, Classic and Sea Green were always susceptible while Centenary, Kirralee and Monaro were resistant.

The very high levels of resistance and susceptibility in the cultivars suggests resistance is controlled by a single gene. Intermediate resistance levels in cultivars such as Target Galaxy and Buffalo may be due to the presence of modifying genes.

An interesting observation was the apparent root growth stimulation in resistant varieties (expts 2 & 3). This can also be seen in Fig. 5. This phenomenon has been observed by other workers. Yarwood (1974) reported increased growth of carrot, cucumber and the weed, cat's ear (*Hypochaeris radicata*) by non-pathogenic inoculation with *C. elegans*. Similarly, Shanks and Link (1958) reported that Thielaviopsis from elm increased the growth of poinsettia and tobacco. Yarwood found a greater stimulation of growth in a poor soil and speculated it could be a symbiotic association. With lettuce the stimulation of root growth suggests an increase in growth hormone giving increased root length rather than a nutritional effect. The stimulatory substances are most likely by products of the plant's biochemical defence mechanisms as they respond to infection challenges. The stimulation of root growth could be viewed as secondary defence mechanism allowing the plant to maintain an effective root system.

## 4. THE USE OF FUNGICIDES FOR CONTROL OF BLACK ROOT ROT

The fungicides benomyl (Benlate, WP 500 g/kg Du Pont) and propiconazole (Tilt, EC 250 g/L Ciba-Geigy) are registered in Queensland for control of water blister in pineapple and fruit rot in bananas caused by *Ceratocystis paradoxa* (Dade) *C. Moreau*, the conidial state of which is *Chalara paradoxa* (de Seyn.) Sacc. These fungicides were initially screened in culture plate tests against isolates of *C. elegans* and *C. paradoxa* and then tested in a glasshouse experiment for control of lettuce black root rot.

Fungicides could also have a place in controlling the carry-over of inoculum in nursery containers as described in section 1. The possible use of benomyl, chlorine and aldespray as the active ingredients for a speedling tray dip was examined in an experiment.

### METHODS

**Culture plate tests**. benomyl and propiconazole were added to molten PDA before plate pouring to give concentrations of 0, 0.001, 0.01, 0.1 and 1 μg/mL. Plates were inoculated with 5 mm discs taken from the margin of colonies of the four test isolates. Two isolates

(2864 and 3499) were *C. elegans* from lettuce and two (2959 and 3505) were *C. paradoxa* originating from pineapple and banana. The growth of *C. paradoxa* was much faster than that of *C. elegans*, hence growth measurements were made at 3 and 6 days, respectively.

**Plant tests.** The object of this study was to determine whether pre-transplanting fungicide treatments will protect against early field infection. Lettuce seedlings (cv. Yatesdale) were germinated in flats then pricked out to multi-celled seedling trays when cotyledons were expanded. Drenches (5 mL/cell) benomyl (250 and 500 μg/mL) and propiconazole (62 and 125 μg/mL) were made to some seedlings 1 and 3 weeks after pricking out. Other seedlings received a single application at 4 weeks or no fungicide treatment in the seedling trays.

Plants were root dip inoculated and transplanted to infested UC mix [root dip — $2 \times 10^6$ phialoconidia/mL; mix infested with 1% by volume of a perlite cornmeal culture as described by Miles and Wilcoxin (1984)] 4 weeks after pricking out. Post planting drenches (50 mL/10 cm pot) of benomyl (500 μg/mL) and propiconazole (125 μg/mL) were made to inoculated but previously untreated seedlings. Ten plants received each of the above treatments. In addition, ten uninoculated plants received each of the fungicide treatments to determine the effect on plant growth. Four weeks after inoculation, the roots of seedlings were assessed for the severity of black root rot using the described scale.

**Decontamination of polystyrene Speedling trays.** Speedling trays were first contaminated by growing infested seedlings, then dipped in solutions of the test chemicals then re-filled with clean UC mix and resown. Infestation of the central 20 cells of 60 cell polystyrene trays was done by root inoculating Yatesdale seedlings (isolate 2864, $1 \times 10^5$ conidia/mL) and planting one per speedling cell. Control trays were planted with clean seedlings. The infected plants were grown in the trays for 14 weeks then removed and rated for disease severity (0-5). Mean severity figures were derived for each of the 21 trays. Blocking for the subsequent experiment was by subdividing the 21 trays into 3 groups of similar disease severity. Replicate 1 contained the 7 trays with the lowest severity figures, while replicate 3 contained the 7 trays with the highest ratings.

Trays were hosed free of loose potting mix then dipped for 10 m in the appropriate treatment. A commercial preparation of sodium hypochlorite (125 g/L) was the source of chlorine. Aldespray 150 containing glutaraldehyde was used in conjunction with the "accelerator" at rates of 5 mL/L and 10 mL/L. Benlate (500 g/kg benomyl) was used at the rate of 1 g/L of dip.

After dipping, trays were lightly hosed and allowed to dry before refilling with steam sterilized UC mix. Lettuce seed (Yatesdale) were sown and later thinned to one plant per cell. Plants were grown in a glasshouse for seven weeks then roots were rated for disease severity.

## RESULTS

**Culture plate tests.** The culture plate tests showed the growth of isolates of *C. elegans* and *C. paradoxa* was inhibited (25% and 70%, respectively) by benomyl 0.01 µg/mL and there was complete inhibition on media amended with benomyl 0.1 µg/mL. Isolates of *C. paradoxa* were relatively more sensitive to propiconazole than isolates of *C. elegans* and showed complete inhibition at 0.01 µg/mL. The growth of *C. elegans* was reduced by approximately 50% at this concentration and completely inhibited by 1 µg/mL.

**Plant tests.** Two pre-planting drenches with benomyl 500 µg/mL gave some control of black root rot ($P < 0.01$) when plants were inoculated one week after the second application (Table 16). There was a negligible response to preplant drenches of benomyl at 250 µg/mL and all preplanting treatments of propiconazole. Drenches of both fungicides after transplanting gave a high level of control ($P < 0.01$) although propiconazole caused slightly abnormal growth.

## TABLE 16

**The effect of benomyl and propiconazole drenches on development of black root rot in lettuce**

| Drench | Number of applications | Disease severity[A] (0-5) |
|---|---|---|
| Benomyl | | |
| 500 mg/L | 2[B] | 3.8 |
| | 1[C] | 4.5 |
| | 1[D] | 0.9 |
| 250 mg/L | 2[B] | 4.2 |
| Propiconazole | | |
| 125 mg/L | 2[B] | 4.4 |
| | 1[C] | 4.8 |
| | 1[D] | 1.7 |
| 62 mg/L | 2[B] | 4.1 |
| No drench | | 5.0 |
| LSD (P = 0.05) | | 1.0 |

[A] 0, no disease; 5, >90% of roots affected

[B] Drenches (5 mL/plant) were applied to seedling trays 3 weeks and 1 week prior to inoculation

[C] Drench (5 mL/ plant) applied to seedling trays 2h prior to inoculation

[D] Drench (50 mL/ plant) applied 2h after inoculation and transplanting to 10 cm pots.

**Decontamination of Speedling trays.** The disease severity in plants at the completion of the contamination phase of the experiment is shown in Table 17. This also indicates the allocation of particular trays to dipping treatments and blocks. At the end of the contamination phase, there was some disease even in the trays planted to uninoculated plants (Treatment 7). During the growth of plants in the second phase of the experiment, i.e. after dipping, the vigour of plants in the benomyl treatment was noticeably higher than in other treatments. Results in Table 18 show disease severity was much less in plants in benomyl treated trays than in other treatments.

## DISCUSSION

Fungicidal control of the disease appears to be practical. Both benomyl and propiconazole showed high activity in plate tests and again in pot experiments. Seedling drenches may provide a very effective way of controlling the disease in the nursery where infection is due to contaminated peat or seedling trays. The production of clean seedlings is essential to minimizing field problems with the disease.

The results of the experiment aimed at tray decontamination indicate that besides direct contamination from trays, seedlings in a nursery environment can be readily contaminated by *C. elegans*. In this experiment, seedlings growing in sterile mix in sterile trays became infected. The source of inoculum is unknown but could possibly be due to insect transmission of conidia. Small flies were active in the root zone of seedlings and may have spread the inoculum from infected plants to the healthy controls. Aerial dispersal of the phialoconidia is also possible.

The use of benomyl as a tray disinfestation chemical was successful in controlling both types of infection. It is probable there was sufficient residual chemical to provide root uptake and systemic protection against both tray-borne inoculum and incidental inoculum via aerial or insect dispersal. The other chemicals having no such systemic properties did not provide the same degree of control.

**TABLE 17**

**Disease severity (0-5) in trays at the end of contamination period and their allocation to treatments**

| Trays allocated to treatment | Disease severity (0-5) | | | |
|---|---|---|---|---|
| | I | II | III | AV. |
| Chlorine 100 ppm Av. Cl | 2.8 | 3.8 | 4.7 | 3.8 |
| Chlorine 1000 ppm Av. Cl | 2.8 | 3.9 | 4.8 | 3.8 |
| Aldespray 5 ppm a.i. | 3.6 | 4.1 | 4.3 | 4.0 |
| Aldespray 10 ppm a.i. | 3.2 | 4.3 | 4.9 | 4.1 |
| Benomyl 500 ppm a.i. | 3.7 | 4.2 | 4.5 | 4.1 |
| Control (water dip) | 3.6 | 4.1 | 4.5 | 4.1 |
| Control (uninfested tray) | 1.4 | 1.5 | 3.0 | 2.0 |

**TABLE 18**

**The efficacy of chemical decontamination (10 min soak) treatments in controlling *Chalara elegans* in polystyrene Speedling trays. Treatment efficacy is inversely related to disease severity in lettuce grown in the trays**

| Trays allocated to treatment | Disease severity (0-5) | | | |
|---|---|---|---|---|
| | I | II | III | AV. |
| 1. NaOCl 100 ppm Av. Cl. | 2.7 | 2.7 | 3.5 | 2.97a |
| 2. NaOCl 1000 ppm Av. Cl. | 2.0 | 2.6 | 3.0 | 2.53a |
| 3. Aldespray 0.5% | 2.8 | 1.9 | 2.8 | 2.50a |
| 4. Aldespray 1.0% | 2.3 | 2.1 | 3.2 | 2.53a |
| 5. Benomyl 500 ppm a.i. | 0.3 | 0 | 1.3 | 0.53b |
| 6. Control (water dip -infested tray) | 1.8 | 2.4 | 3.0 | 2.40a |
| 7. Control (uninfested tray) | 1.8 | 1.8 | 3.5 | 2.37a |
| LSD P = 0.05 | | | | 1.37 |
| P = 0.01 | | | | 1.91 |

# 5. CROPPING SEQUENCE AND ITS EFFECT ON INOCULUM DENSITY

Continuous cropping of susceptible hosts is generally recognised as conducive to increased disease levels. Rotating with non-susceptible hosts or fallowing provides an opportunity for a reduction in inoculum density through a lowering of the viability of propagules due to age or fungistasis.

Crop rotation has been recommended as a control practice for black root rot in tobacco (Lucas, 1975). Evidence in support was provided in a survey of tobacco farms in western North Carolina by Meyer *et al.* (1989). The causal organism was found more frequently (70%) and at higher inoculum densities (46 cfu/g soil) in fields planted to continuous tobacco than those following an alternative crop (23%; 21 c.f.u./g soil).

An experiment was conducted over an 18 month period at Bundaberg Research Station to determine the effect on disease potential of various cropping cycles.

## METHODS

The experiment was carried out in 24 white plastic buckets (0.3 m diameter; 0.4 m height; 24L capacity). These were filled with a medium textured red earth adjusted to pH 6 by dolomite ,then left for 6 months in an open environment so soil could stabilize.

Inoculum was added to each bucket by removing the top 10 cm of soil from each and combining in a cement mixer with macerated agar cultures to give 1 x $10^4$ c.f.u./mL of soil. The soil was then replaced in the buckets.

Over an 18 month period, containers were planted with a susceptible lettuce cultivar (Yatesdale) then with 4 cycles of tolerant and susceptible plants arranged in 4 completely randomized blocks. Treatments were:-

(a) a tolerant lettuce cultivar (Warrior)

(b) a susceptible lettuce cultivar (Yatesdale, transplanted)

(c) a susceptible lettuce cultivar (Yatesdale, direct seeded)

(d) a susceptible weed species (*Solanum nigrum*)

(e) a susceptible weed species (*Sonchus oleraceus)*

(f) bare fallow

Lettuce plants were either directly seeded and thinned to 5 plants per container, or grown separately in a peat/vermiculite mixture and transplanted to the containers at the same rate. Because weed seeds are dispersed randomly in nature and usually occur at higher densities than lettuce plants, a thicker growth was also allowed to develop in treatments (d) and (e) than in the lettuce treatments. The container grown lettuce seedlings (treatment (b)) were transplanted to the pots 21 d after the direct seeded treatments.

Micro irrigation of pots was governed by tensiometers installed above and below the root zone.

When the plants were mature, they were assessed for root damage. For weed plots with higher plant populations, five plants were chosen at random for testing. When plants were mature, they were dug from the containers, soil was gently washed from the roots, and root condition was rated according to the following scale.

0 = no disease

1 = Slight disease on secondary roots only: < 10% damage

2 = Moderate disease on secondary roots: 10-25%, none on tap root

3 = Severe disease on secondary roots: >25-50%, none on tap root

4 = Very severe on secondary roots and some on tap root : >50-75%

5 = Very severe on secondary roots and severe on tap root :>75-90%

6 = No tap root functioning: >90%

At the completion of the 4$^{th}$ cropping cycle, soil samples were taken from each pot and propagule numbers estimated using a semi-quantitative technique: ( Baxter, 1990).

- A soil sample was dried and passed through a 2 mm sieve and a representative sub-sample mixed with a 0.05% streptomycin sulphate solution to make a paste.

- Whole carrots were surface washed with 70% ethanol and sliced 4-5 mm thick. The soil paste was applied to one side to cover the carrot disc.

- Petri dishes were lined with blotting paper previously dipped in 2.0% dicloran solution. Carrot slices were placed on the blotting paper and incubated at 24°C for 24 h.

- Soil was removed from the carrot discs with a jet of sterile water and carrot discs incubated for a further 48 h.

- Carrot discs were examined under a dissecting microscope and the number of individual *C. elegans* colonies per disc recorded.

**RESULTS**

Root disease ratings (Table 19 ) were variable although in any crop cycle they were higher for the Yatesdale treatments than for Warrior or the weed species. Only low levels of disease were seen in the weed species after the 2$^{nd}$ cycle and none subsequently.

## TABLE 19

**Cropping cycle experiment: Severity (0-5) of disease symptoms on susceptible and tolerant lettuce cultivars and 2 weed species from 4 crop cycles**

| Crop Cycle | Disease severity (0-5) | | | | |
|---|---|---|---|---|---|
| | Yatesdale d.s. | Yatesdale t.p. | Warrior d.s. | *S. nigrum* d.s. | *S. oleraceus* d.s. |
| July-Oct 1995 | 2.75 | 2.95 | — | — | — |
| Nov. - Jan 1996 | 3.70 | 4.70 | 2.0 | 0.5 | 0.2 |
| Feb. - April 1996 | 2.38 | 3.90 | 0.7 | 0 | 0 |
| May - Oct 1996 | 3.31 | 2.95 | 2.5 | 0 | 0 |

At the end of the 4th cycle, propagule numbers (Table ) were lowest for the fallow and weed treatments and progressively higher for the Warrior, Yatesdale direct seeded and Yatesdale transplant treatments.

## TABLE 20

**Relative density of propagules in soil after 4 crop cycles of lettuce, weed species and bare fallow**

| Treatment | Relative propagule density (0-100) |
|---|---|
| 1. Yatesdale direct seeded | 90 |
| 2. Yatesdale transplanted | 80 |
| 3. Warrior direct seeded | 45 |
| 4. *S. nigrum* direct seeded | 25 |
| 5. *S. oleraceus* directed seed | 21 |
| 6. Fallow | 8 |

## DISCUSSION

The disease severity in the Yatesdale plots showed fluctuations but always remained high. Overall disease severity was higher in the transplanted treatment, possibly due to root damage

during transplanting. The fluctuations in disease severity for cv. Warrior between crops are difficult to explain but indicate that the use of this variety does not lead to progressively lower disease levels. On the other hand, the absence of disease in the weed species in the last two crop cycles shows that they are relatively poor hosts and require high inoculum to produce disease symptoms.

The observations on disease symptoms are reflected in the results of the semi-quantitative test for propagule density (Table 20 ). In the 18 months duration of the experiment, *Chalara* was detected in low numbers in the fallow treatment, and progressively higher in the weed species, tolerant lettuce cultivar and susceptible lettuce cultivar. It can be concluded that rotations with non-host crops in excess of 18 months will reduce but probably not completely eliminate the pathogen from infested soil. A slower increase of the pathogen would occur if a tolerant or resistant lettuce cultivar were used instead of a susceptible.

# B. CORKY ROOT (*RHIZOMONAS SUBERIFACIENS*)

## INTRODUCTION

A corky root rot of lettuce was first described in New York State, USA by Hoff and Newhall (1960). Symptoms of "corky ridges on the tap root, dark brown lesions, rotting -off of side shoots and vascular discolouration of the stele" were attributed to NH3 or NO2 released from organic fertilizers. In south-east Queensland this problem is seen quite regularly. It occurs during Autumn and Spring when day temperatures are warm and night temperatures are cool. Internal tissues are also affected. The central xylem of the root is often discoloured red, yellow or brown. The tissue in the central part of the butt may be translucent, soft or partially decomposed.

A second corky root disease was later described by Busch and Barron (1963) and Amin and Sequeira (1966a) which was not related to the use of fertilizers.. After being attributed to toxic substances released from decomposing lettuce debris (Amin and Sequeira (1966b) the cause was finally shown to be a gram negative bacterium (*Rhizomonas suberifaciens*) (van Bruggen *et al.* 1988). The symptoms of this disease (called Infectious Corky Root, ICR) were similar in some respects to the non-infectious fertilizer induced disease but a distinguishing feature was the production of "longitudinal ridges on the tap root and main laterals". Van Bruggen *et al.* (1990) conducted a series of experiments to differentiate between the two corky root diseases. They found that at intermediate N levels (350-525 kg N/ha) N toxicity was expressed as a reddish discolouration of the tap root and main laterals, sometimes accompanied by thin longitudinal ridges on the tap root. At high N concentrations the stele became reddish brown and the whole tap root was brown and rotten. In contrast, ICR was not accompanied by pink or red lesions on the root but by yellow superficial discolouration of the roots. The thin corky ridges of non-infectious corky root were replaced by broad corky areas interspersed by longitudinal grooves.

Infectious corky root caused by *R. suberifaciens* was confirmed in the USA by van Bruggen *et al.* (1988) and the Netherlands, England, Spain and Greece (on sowthistle) by van Bruggen and Jochimsen (1992).

In Australia, lettuce from the Lockyer Valley showed similar symptoms in 1992. Samples sent to Dr van Bruggen were positively identified as I.C.R. caused by strains of *Rhizomonas suberifaciens* (van Bruggen and Jochimsen 1993). Several isolates baited from soil samples were shown to have moderate to high levels of DNA homology with the Californian reference strain CAI. They were also more virulent on the susceptible cv. Salinas than on breeding line 440-8 known to be moderately resistant to *R. suberifaciens* (O'Brien and van Bruggen 1992). The presence of *R. suberifaciens* in the Lockyer Valley was thus confirmed.

Although the identification of the causal organism of ICR is comparatively recent, there have been over 20 years of observations of the disease in the USA, including breeding for disease resistance.

Three lines were identified by Dickson (1963) as resistant to corky root- P.I. 175739, P.I. 174229 and P.I. 171669. Sequeira (1970) used the cos lettuce P.I. 171669 to produce the resistant cultivars Montello and Green Lakes (Sequeira 1978). The resistance base of Lactuca species was extensively screened by Brown and Michelmore (1988). High levels of resistance were identified in 19 accessions of *L. sativa, L. serriola, L. saligna, L. dentata, L. virosa* and *Lactuca* spp. The basis of the resistance was similar and due to a recessive allele at a single locus.

Resistance breeding programs using lines such as Montello and Green Lake have resulted in CR resistant crisphead lettuce varieties Misty Day and Glacier developed by Ryder and Waycott (1994) suitable for Californian conditions. In Florida, breeding for resistance commenced in 1976 to develop CR resistant lettuce cultivars (Guzman, 1981). Subsequently, releases of three crisphead types (Guzman 1984, Guzman *et al.*, 1990), two cos types (Guzman 1986) and three butterhead types (Guzman *et al.* 1992; Nagata *et al.*, 1992) have been made. Datman and Nagata (1992) claimed these CR resistant types have now replaced susceptible types in commercial lettuce production in Florida.

The gains in commercial production by the use of resistant varieties have been illustrated by studies in California and Florida. Marketable yield losses of 46-53% were recorded in Florida by Datnoff and Nagata (1992) while losses of 34-92% were recorded in California (O'Brien and van Bruggen, 1992). Highest losses occurred with warm growing conditions.

Although the causal organism of ICR was not identified until 1988, it was previously recognised that it was due to an infectious agent which was well adapted to survival in the soils of Florida and California. With its identification and growth in pure culture, experiments to determine host range and the effects of rotations could be conducted. Van Bruggen *et al.* (1990) found *R. suberifaciens* had a narrow host range restricted to members of the Compositae closely related to lettuce, e.g. endive, common sowthistle, and prickly lettuce. Inoculation of a range of vegetable, cereal, legume and grass species which could be grown in rotation or as cover crops failed to produce symptoms of the disease. Further work (O'Brien and van Bruggen, 1991) showed lower populations of the bacterium on the roots of resistant lettuce, and rye (a non-host) than on roots of a susceptible lettuce cultivar. The dilution of inoculum by sowing a non-host cover crop did not, however, result in significant benefits in the succeeding lettuce crop (van Bruggen *et al.* 1990). Longer term rotations with sugarcane did have a beneficial effect in Florida (Alvarey *et al.*, 1992) which persisted to allow profitable lettuce cropping for three to four cycles.

Corky root can be controlled by the common soil fumigant methyl bromide and by products such as metham sodium and dazomet which yield methyl isothiocyanate as their active ingredient (O'Brien and van Bruggen, 1990). Although high cost and negative environment considerations may mitigate against their use, dazomet and methyl bromide applied at 400 kg/ha gave head weight increases of 39 and 52% respectively.

Under Californian conditions, lettuce are direct seeded which has a cost advantage of $1 500 - $1 700 per hectare over a transplanted lettuce crop. Observations, however, showed that transplants are often not as severely affected by corky root as direct seeded plants. Van Bruggen and Rubatzky (1992) found that the use of transplants in severely infested fields gave significant yield increases. They recommended the use of 4-5 weeks old transplants to obtain maximum benefit from the apparent mature plant resistance responsible for the effect.

In the studies conducted in Queensland, we sought to confirm the segments of the studies described above which we considered essential for growers to combat corky root. We investigated methods of judging disease potential in fields, evaluated resistant cultivars and the use of transplanting for the reduction of corky root severity.

## 1. FIELD DETECTION AND SEVERITY PREDICTION

The symptoms of corky root are distinctive and are usually easily distinguished from black root rot, Rhizoctonia base rot and nitrite toxicity. There may sometimes be confusion when two diseases are present on the same plant, e.g. black root rot and corky root.

Symptoms. Young plants show yellow bands about 5 mm wide encircling the tap root (Fig. 6). These enlarge and become darker. A corky effect is seen as the epidermis becomes rough and eventually cracks. On older plants the vertical cracks extend deep into the tap root and are dark brown in colour. The root is weakened at these points and easily broken.

Assaying soil samples. Since Rhizomonas is difficult to isolate from plant tissue, it is easier to demonstrate its presence by baiting from soil with lettuce seedlings. The method may also be used to indicate probable disease potential in field sites.

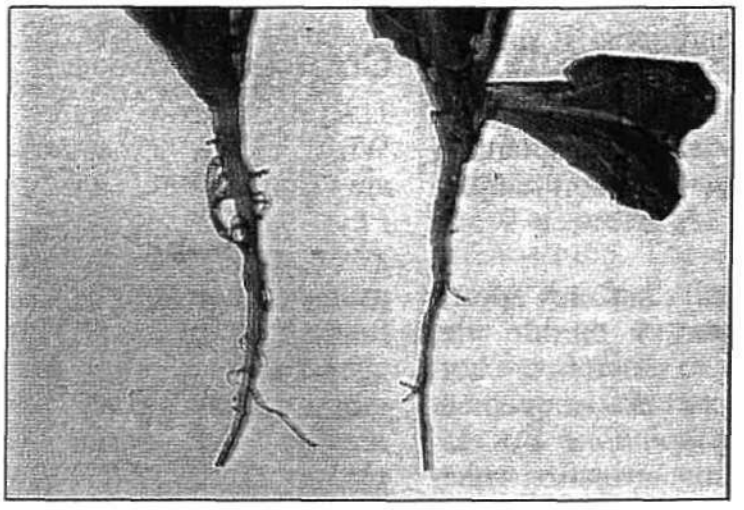
### METHODS

Representative soil samples are collected from each field site ,mixed thoroughly and passed through a coarse sieve. A soil suspension is made by mixing a 50g sub-sample in 75 mL distilled water plus three drops of Tween 80. Stir for 15 min before filtering through six layers of cheesecloth.

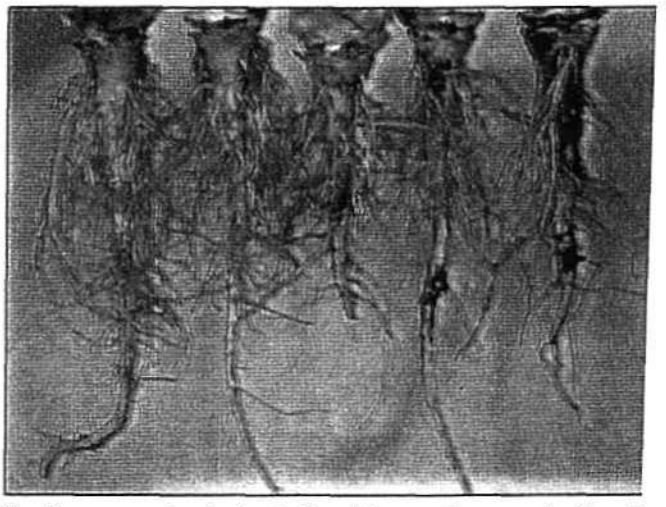
Test plants (cv. Yatesdale) are raised in flats until14 days old then transplanted individually to 10cm diameter pots filled with vermiculite. After watering in, 5 mL of soil suspension is poured around the base of each plant (15 plants per soil sample). Pots are kept in a controlled environment cabinet 28°C for four weeks before rating for disease severity using the 0-9 system of Brown and Michaelmore (1988).

Plants are fertilized with Hoaglands solution and CaNO3 solution once per week. The CaNO3 solution is applied three days after Hoaglands.

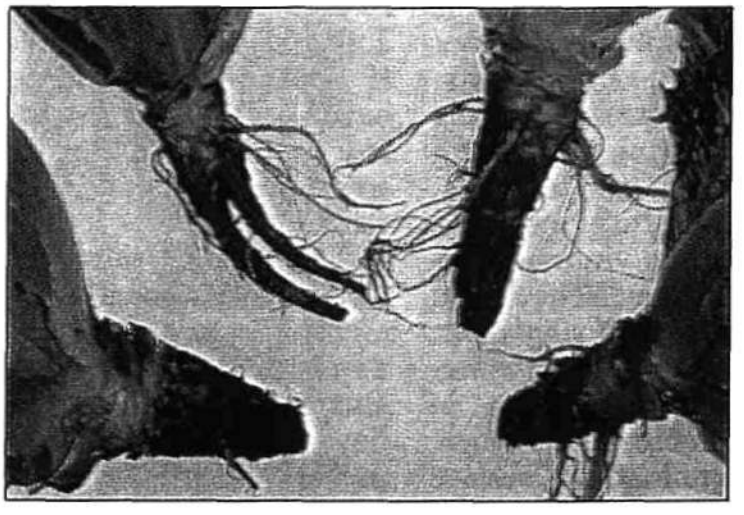
Preliminary experiments established that more severe disease symptoms developed at 28°C compared with 21°C and when the nutrient solutions were added 1/wk compared with 2/wk and 3/wk.



**Early signs of corky root in young lettuce plants.**



**Lettuce roots infected with corky root devel yellowish-brown bands on the tap root.**



**Longitudinal tissues eventually open up in lettuces infected with corky root.**

**Fig. 6** Stages in the development of corky root symptoms

Experiment 1. Ten field sites were sampled (5 from the eastern Downs and 5 from the Lockyer Valley) where the disease was known to occur. The soils were assayed for *R. suberifaciens* using the technique described above.

Experiment 2. Five more sites in the Lockyer Valley were sampled and baited for the presence of *R. suberifaciens.*

## RESULTS

Experiment 1. The results in Table 21 show higher disease levels in the Lockyer Valley than on the eastern Downs. *C. elegans* was also observed in the test plants from 4 sites.

Experiment 2. The disease had not been observed in the field at site 5 and this is indicated by its low rating. The test indicated corky root was present at much higher levels in the other samples which ranged in value from 2.6-4.7. (Table 22 ).

## DISCUSSION

The assay for the presence of corky root in soil was successful and gave a correct indication of the field severity of the disease. The main problems with the system are the long time (6 weeks) for conduct of the test and the limited number of samples (10) which can be assayed at one time in one growth cabinet. The test was also useful in indicating the presence of Chalara . Positive results for *Chalara* presence were obtained in samples from eastern Downs properties but not the Lockyer which agrees with field observations.

Site 6 in the Lockyer Valley was selected for field trial work to evaluate lettuce varieties for resistance to corky root and compare the effects of different planting methods on disease severity.

**TABLE 21**

**Severity of corky root and presence/absence of *C. elegans* in lettuce plants inoculated with soil suspensions from 10 farms on the eastern Downs and Lockyer Valley**

| Site | District | Corky root (0-9) | *C. elegans* (+/-) | Dry weight of test plants (g) |
|---|---|---|---|---|
| 1 | Eastern Downs | 1.53 | - | 32.7 |
| 2 | Eastern Downs | 1.26 | + | 18.9 |
| 3 | Eastern Downs | 1.26 | + | 26.3 |
| 4 | Eastern Downs | 1.20 | + | 19.5 |
| 5 | Eastern Downs | 1.53 | + | 27.7 |
| 6 | Lockyer Valley | 6.13 | - | 12.1 |
| 7 | Lockyer Valley | 4.06 | - | 12.4 |
| 8 | Lockyer Valley | 4.53 | - | 13.7 |
| 9 | Lockyer Valley | 4.93 | - | 12.8 |
| 10 | Lockyer Valley | 2.00 | - | 15.7 |
| Check 1 | | 0 | - | 20.2 |
| Check 2 | | 0 | - | 11.7 |

**TABLE 22**

**Severity of corky root in lettuce plants inoculated with a soil suspension from five Lockyer Valley sites**

| Site | Corky root (0-9) |
|---|---|
| 1 | 4.73 |
| 2 | 3.22 |
| 3 | 2.92 |
| 4 | 2.56 |
| 5 | 0.60 |
| Check | 0.00 |

Fig. 7 Baiting soils for the presence and severity of corky root (*Rhizomonas suberifaciens*): Comparison of control plant (on left) with three plants inoculated with soil extract from site 2.

## 2. CONTROL BY RESISTANT VARIETIES AND PLANTING METHOD

Advanced resistant breeding lines are coming forward from programs principally in the United States of America. We tested some of these from New World, Yates and S.P.S. in two field experiments. During the course of these experiments, we noticed that when transplants were used to fill gaps between the direct seeded plants, they were less affected by corky root. An experiment was later carried out to compare the effect of planting method on corky root severity.

### METHODS

Experiment 1. A range of commercial varieties were compared with the corky root resistant lines SPS761 and Misty Day. The trial was laid out as a triple replicated randomized block experiment with direct seeded plots 3 m long. Germination was very erratic and it was necessary to fill gaps with glasshouse grown seedlings. At maturity, only low numbers of the direct seeded plants were available for evaluation and this precluded statistical evaluation. The results in Table 23 are therefore offered only as a guide to varietal resistance.

Experiment 2. Twenty-two breeding lines and standard cultivars were direct seeded into single row plots, 3 m long. There were three replicate blocks. Seedlings were thinned to give 12-14 per plot. Growth was slow due to cool temperatures (sown 5 July 1994). At 10 weeks, five root systems from each plot were washed and rated for disease severity on a 0-6 scale:-

0, no symptoms except faint yellow smudges on root over very small area (1%).

1, distinct yellow discolouration on root surface — not extensive ( <10%.)

2, distinct yellow colouration (>10%) with or without small surface cracks.

3, extensive yellow/green/brown discolouration with surface cracks. Affecting <25% root area.

4, dark lesions with deep cracks covering 25-<50%.

5, dark lesions with deep cracks >50%. Secondary roots still present.

6, dark lesions with deep cracks >50% secondary roots almost absent, or ;tap root stubby due to corky root.

Experiment 3. This experiment was designed to compare the effects of direct seeding and transplanting on subsequent corky root severity and also observe how this can be modified by

## TABLE 23

**Expt 1. Evaluation of lettuce cultivars for susceptibility to corky root**

| Variety | Severity 0-6[A] | No. of plants rated |
|---|---|---|
| 1. Classic | 4.8 | 5 |
| 2. Fraser | 5 | 1 |
| 3. Top Gun | 5 | 1 |
| 4. Patriot | 5 | 4 |
| 5. Warrior | 5 | 2 |
| 6. Sea Green | 4.5 | 2 |
| 7. Elliot | 5 | 3 |
| 8. Fame | 4.2 | 4 |
| 9. Monaro | 4.8 | 5 |
| 10. Centenary | 6 | 1 |
| 11. SPS 761 | 1.5 | 2 |
| 12. Mercury | 4.3 | 12 |
| 13. Charger | 4.2 | 6 |
| 14. Sally | 4.0 | 10 |
| 15. Buffalo | 4.8 | 6 |
| 16. Winguard | 4.8 | 6 |
| 17. Black Velvet | 4.3 | 3 |
| 18. Misty Day | 3 | 1 |
| 19. El Toro | 4 | 1 |
| 20. Salinas | 5 | 1 |
| 21. Target | — | — |
| 22. Yatesdale | 5 | 1 |

[A] 0 - no symptoms; 1, yellow-brown areas (no corking); 2, small areas of corking <25% on tap root; 3, large areas of corking (>25%) on tap root (not stubby) + good 2° root growth; 4, tap root stubby with good 2° root growth; 5, tap root stubby with few 2° roots; 6, tap root stubby with no 2° rots.

plant resistance. The experimental design was of three treatments (planting methods) x three sub treatments (lettuce cultivars) replicated four times in a randomized block, split plot design. Sub plot size was 1 row x 3 metres (12-14 plants). The three planting methods were:-

(i) direct sowing — seed was hand sown, lightly covered and irrigated. Seedlings were later thinned to 12-14 per plot.

(ii) transplanted seedlings — seed was sown in a glasshouse then seedlings were transplanted to individual cells in Speedling trays. Field transplanting was carried out 30 days after sowing.

(iii) transplanted, inoculated seedlings — 8 days before field transplanting, glasshouse raised seedlings were inoculated with a suspension of *R. suberifaciens*. This treatment was included to enable a comparison between transplanted seedlings with different lengths of exposure to the causal organism.

The 3 varieties were Fraser (susceptible), NW8015 (moderately resistant) and NW8005 (highly resistant)

Rating for disease severity was made 10 weeks after the direct seeded plots were sown. Five roots were sampled from each plot, washed and rated on the 0-6 scale described previously.

**RESULTS**

Experiment 1. The corky root resistant lines SPS761 and Misty Day showed the fewest symptoms. Disease severity was very high in susceptible lines..(Table 23 )

Experiment 2. The two standard cultivars, Target and Fraser were severely affected. The collection of corky root resistant breeding lines showed a range of symptoms from practically disease-free to moderately affected. All, except two, showed significantly ($P = 0.05$) less disease than both standard lines (Table 24).

## TABLE 24

**Severity of corky root symptoms in 22 lettuce cultivars in a field trial at Gatton 1994**

| Cultivar/line | Disease Severity (0-6) |
|---|---|
| 8006 (NW) | 0.80 |
| 8007 (NW) | 0.93 |
| 8008 (NW) | 0.93 |
| LE 081 (9/1) (Yates) | 1.00 |
| 8005 (NW) | 1.33 |
| LE 081 (3/3) (Yates) | 1.46 |
| LE 081 (Yates) | 1.80 |
| 8016 (NW) | 1.93 |
| LE 064 (Yates) | 2.00 |
| 8019 (NW) | 2.07 |
| 8015 (NW) | 2.47 |
| 8017 (NW) | 2.53 |
| 8009 (NW) | 2.53 |
| LE 064 (4/8) (Yates) | 2.60 |
| 8018 (NW) | 2.73 |
| 8010 (NW) | 3.13 |
| 8012 (NW) | 3.33 |
| 8014 (NW) | 3.40 |
| 8011 (NW) | 3.60 |
| 8013 (NW) | 3.73 |
| Target (Yates) | 4.53 |
| Fraser (NW) | 5.13 |
| LSD P = 0.05 | 0.95 |
| LSD P = 0.01 | 1.25 |

Experiment 3. For all three cultivars, direct seeding led to significantly higher disease ratings than either of the transplanting treatments (Table 25). The experiment confirmed previous evaluations of the susceptibility of the cultivars to corky root. When direct seeded, Fraser had significantly higher disease ratings than 8015 or 8005 while 8015 was significantly more susceptible than 8005. Differences between cultivars were not as large when they were transplanted. The disease ratings for Fraser were significantly higher than those for 8005 but not 8015. There was not a significant increase in disease severity due to the pre-planting inoculation of seedlings.

**TABLE 25**

**Severity of corky root in three lettuce cultivars when established by direct seeding or transplanting using clean or inoculated seedlings**

| | Disease severity (0-6) | | |
|---|---|---|---|
| **Cultivar** | **Direct seeded** | **Transplanted (clean)** | **Transplanted (inoculated)** |
| cv. Fraser | 4.95 a* | 1.70 c | 1.55 c |
| cv. 8015 | 2.60b | 1.10 cde | 1.40 cd |
| cv. 8005 | 1.47 cd | 0.60 e | 0.75 de |

* Values followed by the same letter are not significantly different (P = 0.05)

**DISCUSSION**

Experiment 1 was conducted during warm weather and disease symptoms were more severe than in the other trials conducted during winter. Under these conditions, SPS761 showed very high resistance although evaluation was limited to two plants. No other varieties, except Misty Day, showed promise.

The breeding lines from Yates and New World, which were included in experiment 2, showed that resistant varieties will be an effective method to control corky root. In some lines, segregation appeared to be continuing since individuals within a line showed variable

resistance to the disease. Lines such as NW8006, NW8007, NW8008 and LE081 were practically free of symptoms and had vigorous, white, disease- free root systems. Perhaps the most important finding from the cultivar evaluation is that the varieties bred for resistance to American strains of *R. suberifaciens* are also resistant to local strains.

In experiment 3, transplanting reduced the severity of corky root in the three cultivars tested. For the susceptible cultivar Fraser, direct seeded plants were slower growing than transplants and roots were severely affected. Transplants of Fraser showed only light-moderate disease symptoms. It is probable that in fields of low or moderate disease risk, transplanting a susceptible line such as Fraser would result in a commercially acceptable crop while direct seeding would not.

The benefits from transplanting were not so great with resistant cultivars. Even direct seeding cvs. 8015 and 8005 produced light-moderate symptoms. Commercially these varieties could probably be direct seeded into infested soil and produce an acceptable crop.

The reason why transplanted crops are less affected than direct seeded is not completely understood. The root systems produced by the two establishment systems are vastly different. Plants resulting from direct seeding have long tap roots while those from transplanting have short stubby tap roots with numerous laterals. Factors involved in the lower disease ratings of transplants could include length of time root is associated with causal bacterium; young seedling tissue more susceptible than 4-5 week old transplant; conditions for infection may be more favourable at the greater soil depths which tap roots explore. Van Bruggen and Rubatzky (1992) suggest that there is a form of mature plant resistance which comes into effect when seedlings are about four weeks of age.

## PROJECT CONCLUSIONS

- Both corky root and black root rot have the potential to be very damaging to lettuce production.

- Identification of the particular disease is the first step to initiating effective control measures. As a general guide, corky root mainly affects the tap root while black root rot destroys the feeder root system. Sometimes both diseases occur on the same plant.

- Corky root inoculum is soil-borne and can be maintained on weed hosts such as milk thistle in the absence of lettuce.

- Corky root control is based on avoiding heavily infested fields (determined by a soil prediction test or observation of previous lettuce crops); using resistant varieties, e.g. Misty Day, SPS761; and using transplants instead of direct sowing.

- Black root rot inoculum (*Chalara elegans*) is probably first introduced with infested peat for seedling mixes, then infected seedlings introduce the organism to the field. Unless effective decontamination of seedling trays is undertaken, successive sowings will be infected. The disease can also occur in hydroponically grown lettuce if peat has been used for seedling production.

- *Chalara elegans* exists as many strains with different adaptations to hosts. The lettuce isolates are also highly pathogenic to some ornamentals, e.g. *Viola* spp. They are different from strains affecting cotton at Narrabri. They are similar to isolates obtained from peat.

- Control of black root rot in nurseries can be achieved by washing seedling trays in benomyl solution. Chlorine was not effective at the rates tested.

- Resistant varieties (large range available) and long rotations (>18 months with no alternative weed or crop hosts) are effective field control methods for black root rot.

- Several fungicides used as drenches will control black root rot but are not necessary provided nursery hygiene and resistant varieties are used as management practices.

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## APPENDICES

Scientific and advisory articles relating to this project.

### 1. Scientific

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