PT412
Potato early dying in Australia

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Agriculture Victoria

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INDUSTRY SUMMARY

The purpose of this study was: (1) to determine the incidence of *Verticillium* and *Pratylenchus* in potato crops in Australia, (2) to quantify the levels of the pathogens in potato soil and plants, (3) to develop rapid techniques to predict the occurrence of disease and (4) to evaluate methods of control.

Soil and plant samples were collected from 77 potato fields from South Australia, Victoria, New South Wales, Queensland and Tasmania. Both *Verticillium* and *Pratylenchus* were widespread, with the pathogens found in 90% of the fields sampled. Soil populations of *Verticillium* at levels that caused crop loss overseas were found in 40% of the sites and *Pratylenchus* were found in 75% of the sites sampled. In the USA, *P. penetrans* is the main nematode species associated with premature senescence of potatoes, but in our studies this species was detected at only 1 site in the South East of South Australia. The main species detected in our studies were *P. crenatus* and *P. neglectus*. Both these species of *Pratylenchus* may also be inhibiting potato growth, but further work in this area is needed.

Seed potato tubers were also sampled and 75% of the batches sampled were infected internally with *Verticillium* with up to 22% of the tubers infected in some samples. This suggests that the disease is widespread as a result of the use of infected seed.

Soil sampling was successful in detecting and measuring levels of both *Verticillium* and *Pratylenchus*, but further studies will be required to determine if the sampling can be used to predict the occurrence of disease.

Fumigation with metham sodium applied at 400L/Ha before planting controlled potato early dying in field experiments and in one experiment resulted in a 26% yield increase. Metham sodium significantly reduced soil levels of both *Verticillium* and *Pratylenchus* but did not eradicate them completely.

Indian mustard, Ebony mustard and Rangi forage rape were incorporated into the soil before planting to evaluate their efficacy as green manure crops to reduce levels of *Verticillium* and *Pratylenchus*. The results from these experiments showed a trend to suppress nematode numbers but not *Verticillium*, indicating that further work needs to be done in this area.

Overall this project has shown that potato early dying is an unrecognised disease widespread in Australia and that the problem could be significantly depressing yields by up to 30% in some areas. Further studies are required to develop resistant cultivars and to evaluate chemical, biological and cultural methods for control.

TECHNICAL SUMMARY

Soil samples from 77 potato fields in South Australia, Victoria, New South Wales, Queensland and Tasmania showed that *Verticillium dahliae* and several species of *Pratylenchus* were widespread throughout Australia.

Levels of *Verticillium* ranged from 0 –18 colony forming units (cfu's)/g of soil whereas *Pratylenchus* levels ranged from 0 – 19 vermiforms/g.

Verticillium levels of 4 cfu/g and above are considered to cause significant yield losses without the presence of nematodes whereas levels of 2 cfu/g and above cause yield losses in the presence of *Pratylenchus*. Levels of 4 cfu/g or higher with *Pratylenchus* were found on 40% of the sites sampled.

V. dahliae was the main *Verticillium* species isolated from soil and plants, although other species (yet to be identified) were found in less than 1% of the soil samples. *P. penetrans* is the main nematode associated with senescence of potatoes overseas, but in our studies this species was found at only one site. The main nematode species detected in our samples were *P. neglectus* and *P. crenatus* and at one site in NSW, *P. coffeae*. Nematodes were detected in 75% of the sites sampled with *P. crenatus* and *P. neglectus* in 71% and 24% of the samples respectively.

Certified seed tubers were sampled to determine the level of infection in potato seed. *Verticillium* was detected in 15 out of the 20 batches sampled and in some batches up to 22% of the tubers were infected.

The soil fumigant metham sodium and nematicides Temik and Nemacur were evaluated as a means of delaying senescence in several field and glasshouse experiments. Metham applied at 400L/Ha significantly reduced the levels of *Verticillium* and *Pratylenchus* in soil and in one field site increased yield by 26%.

Green manure crops of Indian mustard, Ebony mustard and Rangi forage rope were incorporated into the soil before planting to evaluate their efficacy in reducing levels of *Verticillium* and *Pratylenchus*. The results were not conclusive and indicated that considerable fine tuning will need to be undertaken before the technique can be recommended and provide reliable results.

TECHNICAL REPORT

INTRODUCTION

Premature senescence and reduced yields have been long recognised as a problem, particularly of Russet Burbank potatoes. In America the condition is known as potato early dying (PED) and is found in both irrigated and non-irrigated areas. Yield reduction of 30% to 50% have been reported from many potato growing areas in America (Powelson and Rowe, 1993). The PED disorder is caused primarily by the verticillium wilt fungus *Verticillium dahliae* in association with the nematode *Pratylenchus penetrans* (Powelson and Rowe, 1993 and Kotcon *et al*, 1985).

Distinct pathotypes of *Verticillium* have been reported on potato (Powelson and Rowe, 1993) with specific interaction with *Pratylenchus*, namely *P. penetrans* (Bowers *et al*, 1996). It is assumed that nematodes feeding on potato roots either provide entry points for the *Verticillium* fungus or they reduce the natural host defence mechanism of the potato, enabling the fungus to attack the roots and grow though the plant. *Verticillium* alone can attack potatoes, but it seems that the most severe reaction is in combination with the nematode.

Premature senescence of Russett Burbank potatoes has been reported in Australia (M. Heap – personal communication) which suggested that the PED complex may also be present. Verticillium wilt has been recorded on potatoes in Australia (Sampson, 1980) but few studies have been done on the levels of *Pratylenchus* and their possible interaction with the fungus in this country.

The aim of this project was to (1) determine the incidence of *Verticillium* and *Pratylenchus* in potato crops in Australia (2) quantify the levels of these pathogens in potato soils and plants (3) develop a rapid technique to predict the occurrence of the disease and (4) evaluate methods of control.

GENERAL METHODS

Soil and plant sampling and assessment methods

Soil and plant samples were collected from potato fields using a diamond shaped sampling pattern with a minimum of 25 equally spaced assessment sites per field. The size of each sample area varied due to the different size of each potato field. Soil samples were collected twice in the growing season, the first before planting and the second 80 - 100 days after emergence. Plants were collected with the second soil sample. Soil was sampled with a 2.5cm diameter corer used to remove at least a 100 g soil sample from within the root zone. Samples from each site were bulked and mixed by rotating the soil in the plastic bag in which the samples were collected. A bulk sample of 1Kg was stored at 4°C until analysed for *Verticillium* and *Pratylenchus*.

Plant senescence was rated visually on a total of 100 plants per paddock (4 per site) using the same diamond sampling method as above. The development of senescence was rated using a 0-5 scale, based on the percentage of foliage with wilting, chlorosis, necrosis, or stunting typical of verticillium wilt as shown in Fig 1. For analysis the rating was adjusted to 0-100 scale (Wicks *et al*, 1994).

Extracting nematodes from soil and plant tissue

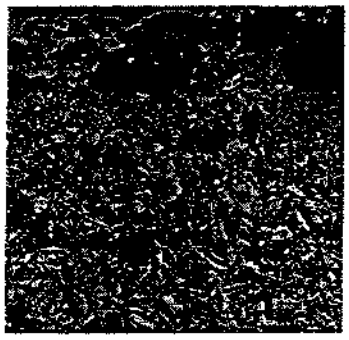
Two to five 200g sub samples of soil from each site were sieved to remove root fragments and then placed individually on modified Baermann trays described by Hooper and Evans (1993). A 50gm sample of soil was kept aside and air dried to determine dry soil weight. The soil was spread uniformly over paper tissue placed on a plastic screen in a tray and water added to just cover the soil surface. The samples were incubated at 21-24°C and after 3 days nematodes were collected from the trays by decanting the water through a 25 um screen. Dirty water was cleaned by pouring it through a 500um-mesh sieve first.

Roots were collected 100 days after plant emergence to compare numbers extracted from roots with those from the soil. Roots were washed free of soil, chopped into 5mm segments and then placed in Baermann funnels in a heated intermittent mist. Extracted nematodes were collected every 48hrs for a period of 5 days. Roots were removed from the mist chamber and air dried to determine the dry weight.

The extracted nematodes were stored up to 2 months at 4°C until counts of the species were completed. Several batches of the extracted nematodes were killed by immersing them in a water bath at 60°C and then fixing them with 2% formalin. This was abandoned in later samples, as nematode identification became difficult once they were fixed.

Figure 1. Rating system for Verticillium wilt based on a 0 – 5 scale where 1 = 1-12%, 2 = 13-25%, 3 = 26-50%, 4 = 51-75%, and 5 = 76-100% of foliage wilted.

1



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Nematode identification

A random sample of at least 50 *Pratylenchus* spp per soil or plant tissue were identified by Francis Raye (nematode taxonomist).

Determination of Verticillium levels in soil and plant tissue

A 200 - 500g soil sample was air dried at room temperature (15 to 25°C) for 3-4 weeks to eliminate short-lived propagules such as conidia and mycelial fragments. After drying the soil was homogenised and sieved through 850, 500, 250 and 45 micron sieves. The resulting soil sample was analysed for the presence of *V. dahliae* by plating out five sub samples of 0.01mg onto separate plates of selective media NP10 (Sorenson *et al*, 1991) using an Anderson Sampler in the method described by Butterfield and DeVay (1977). Soil was stored at 4°C until analysed.

Plates were incubated at 22-25°C for 2 weeks and then examined under a bifocal microscope for microsclerotia typical of *V. dahliae*. The number of colony forming units (cfu's) per gram of unsieved soil was calculated using the formula (X/0.01) * Y/200, where:

Total weight of unsieved soil = 200gms
Total weight of soil plated out = 0.01gms
Total weight of sieved soil = Y
Total Number of cfu's on plate = X

To verify the identity of the *V. dahliae* colonies, 10 random isolates were selected and subcultured onto PDA for 2 weeks to compare sporulation and cultural characteristics typical of the species.

Two methods were used to determine the presence and level of *Verticillium* in plant tissue,. The first used a modified method of Hoyos *et al* (1991), where at 100 days after planting, potato stem sections approximately 15cm long were cut with a sterile knife from the base (soil line), put into plastic bags, placed in an ice chest for transportation to the laboratory and processed within 1-4 days. Stem sections were washed in running tap water, immersed in a 1% NaOCl solution for 30 sec and rinsed in sterile water. Sap was extracted from the basal portion of each stem section using sterile pliers, or by placing them in a sealable polyethylene bag and crushing the tissue with a rubber hammer. A 0.1ml aliquot of the plant sap was pipetted into a 100mm petri dish containing

20 ml of the selective NP10 media. The second method involved plating thin 2mm stem sections from the basal portion of stems onto NP10 selective media. Plates with sap or stem segments were incubated at 22-25°C for up to 2 weeks.

Preparation of Verticillium inoculum and soil infestation

Soil was prepared by mixing recycled soil and peat moss at 4:1 v/v then adding 200gm of potassium sulphate, 100gm of super phosphate and 200 gm of agricultural lime (calcium carbonate) per 500 L of soil. Sub samples of this mixed were then prepared by moistening 1L with 100ml of sterile water, autoclaving at 121°C for 1hr on each of two successive days, air drying for 7 days at room temperature and sifting through three nested sieves of 1-mm, 850-um and 425-um mesh. The finest soil was then used to make a concentrated soil inoculum source of *V. dahliae*.

V. dahliae was grown on PDA plates for two weeks to allow for microsclerotia formation, after which the agar was blended for 30sec in distilled tap water and adjusted to produce a suspension of 50,000 microsclerotia per ml (Botsea *et al,* 1994). Approximately 300ml of the spore suspension were poured onto 3L of the fine soil and mixed by hand. This concentrated infested soil was stored in plastic bags at 5°C and if the storage period was longer than 4 weeks, inoculum density was reassayed before use.

Infested soil required for experiments was prepared by adding appropriate amounts of the concentrated microsclerotia inoculum to obtain the desired inoculum density and mixing with the soil in a hand operated cement mixer for 3 min in batches of 20 or 30 L. Ten 200g sub samples were collected from each treatment and plating onto NP10 selective media using the Anderson Sampler to determine the final inoculum density.

Sieves, mixers and all other devices used in the above procedures were rinsed with water and then disinfested with 0.4% NaOHCl prior to and after use to prevent cross contamination.

Preparation of nematode inoculum and soil infestation

Nematode inoculum was prepared by multiplying the nematodes on carrot callus. Carrots were peeled, soaked in alcohol, flamed and repeeled before incubation for at least 2 weeks at 22°C to

form callus. Nematodes were then sterilised by soaking for 3 hours in a 1% antibiotic solution of streptomycin and penicillium, rinsed 3 times in sterile distilled water and placed on the callus. After 3 months the nematodes were extracted by placing the carrot callus in Baermann funnels under a heated mist chamber. Nematodes were collected every 48hrs over a 5 day period and stored at 4°C until required. Soil and plants were artificially infested by pipetting aliquot's of nematode suspensions with the desired number of *P. crenatus* into four holes (4.6cm deep and 5cm from the plant stems) two weeks after emergence.

Source of seed tubers used in glasshouse experiments

Mini tubers of the cultivars Russet Burbank and Sebago, both highly susceptible to *V. dahliae*, were obtained from Toolangi, Victoria. The tubers were stored in moist vermiculite for approximately 3 months at 22°C until dormancy was broken and 0.5-1cm long sprouts had developed. Sprouted tubers were cool stored at 5°C until 2 – 5 days before planting when they were returned to 22°C.

LEVELS OF *VERTICILLIUM* AND *PRATYLENCHUS SPP* IN POTATO SOILS AND PLANTS

Soil and plant samples were collected from 77 potato fields throughout Australia between 1995 and 1997 to measure the levels of *Pratylenchus* and *Verticillium* in potato soils.

Methods:

Soil was collected before planting and 80 - 100 days after planting, air dried for 4 weeks then plated onto selective media using an Anderson sampler. Four plants from each of the 25 assessment sites were collected between 80 - 100 days after planting and stem sections assayed for the prescence of *Verticillium* as previously described.

Results and Discussion:

The results summarised in Table 1 show that *Verticillium* is present in most potato soils in Australia, with soil levels as high as 18cfu/g recorded. In some samples, 100% of the plants were infected with *Verticillium*. Similarly *Pratylenchus* was widespread with levels up to 19 vermiforms/g soil in some areas. *P. crenatus* was the main species detected, which differs to America where *P. penetrans* is the main nematode species involved with PED. Other species found in Australia were *P. coffea* and *P. neglectus*, but no testing was undertaken to clarify their role in the PED complex.

Table 1. Levels of *Verticillium* in soil/plant stems and *Pratylenchus spp* in soil

State	No. *Verticillium* cfu/gm soil mean (range)	No. *Pratylenchus.spp* /gm soil mean (range)	No. sites sampled (soil)	No. stems infected by *Verticillium* (%) mean (range)	No. sites sampled (stems)
SA	3 (1 - 10)	1 (0 – 6)	35	39 (0 - 100)	15
QLD	6 (0 - 19)	0.2 (0 – 1)	30	33 (0 – 94)	13
NSW	3 (0 - 9)	0.5 (0 – 1.3)	3	-	-
VIC	6 (0 - 14)	9.2 (3 – 19)	12	51 (10 – 100)	5
TAS	5 (0 - 10)	0.9 (0 – 14)	4	37 (6 – 68)	2

Powelson and Rowe (1993) suggest that the threshold levels for the development of PED are 4 cfu/g of *Verticillium* in the absence of *Pratylenchus* and at least 2 cfu/g in the prescence of *Pratylenchus* at 0.1 vermiforms/g soil . Using these values, the incidence of paddocks in each state that were at or

above the threshold level justifying soil treatment with fumigants were, SA 38%, Vic 75%, NSW 33%, Qld 31% and Tas 25%.

One of the aims of this project was to develop a rapid technique to predict the occurrence of PED. Soil sampling and plating methods successfully detected the pathogens, however results were not obvious until at least 2 weeks after sampling. While this could not be considered rapid, the soil sampling could be carried out prior to soil preparation for sowing, therefore results could be obtained in time to recommend fumigation if growers sampled early enough. This method is offered as a commercial service in both the U.K. and U.S.A.

Although molecular tests for the detection of *Verticillium* in plants and soil have been developed overseas (Robb and Nazar, 1996) it is uncertain whether these will be any more efficient or faster. In any case these new techniques will require considerable fine tuning and development before they can be used commercially. Another complicating factor is the occurrence of distinct pathogens of *Verticillium* (Powelson and Rowe, 1993, Botseas and Rowe, 1994). Isolates of *V. dahliae* are known to vary in the level of virulence on potato and further tests are required to identify the pathotypes in Australia.

These results are likely to be conservative, however they do show that in most states at least one third of the potato fields have levels of *Verticillium* and *Pratylenchus* that could significantly reduce yields. In these situations, soil fumigation with metham sodium is warranted and could potentially result in yield increases of up to 30%.

In summary this survey has shown that PED is an unrecognised and widespread disease in the potato industry in Australia and is likely to be significantly reducing potential yields in most potato growing areas.

INCIDENCE OF *VERTICILLIUM* IN POTATO SEED TUBERS

This work was undertaken to determine the incidence of *V. dahliae* and other tuber borne pathogens on certified and uncertified seed tubers used in South Australia.

Methods:

Fifteen growers throughout the state were sampled, with 100 seed tubers collected at random from up to 16 half tonne bins from each grower. The tubers were carefully washed in running water to remove most of the soil adhering to the surface, and examined for the presence of the diseases Black scurf (*Rhizoctonia solani*), Black dot (*Colletotrichum coccodes*), Silver scurf (*Helminthosporium solani*), Powdery scab (*Spongospora subterranae),* Common scab (*Streptomyces scabies*) and *Fusarium* rot.

The stem end of all tubers was sliced and where staining was obvious in the vascular tissue, 2 pieces of approx 1mm^2 were removed with a scalpel and plated on to selective media. After 14 days incubation at 22 to 25°C, plates were examined microscopically and scored for presence or absence of *Verticillium, Colletotrichum* and *Fusarium.*

Results and Discussions:

The results in Table 2 show that none of the certified potato tubers from either New South Wales or Victoria were disease free. The incidence of disease in different samples was extremely variable, for example of the 4 Russet Burbank samples from Victoria, the incidence of Black dot was 0, 0, 3 and 79% and *Rhizoctonia* 0, 0, 1 and 10%. There were also variations in the incidence of Black dot between the internal and surface examinations. For example no internal infection was found where 79% external infection was detected and conversely 16% internal infection was detected on tubers with no external symptoms. Common scab was not detected in any sample and the incidence of Powdery scab on all samples was less than 7%. The high level of disease on potato seed tubers sampled in this survey confirmed previous studies in Australia (de Boer and Wicks, 1994, Wicks *et al,* 1996) and indicates that some batches of certified seed tubers may need to be treated with fungicides.

The isolation of *Verticillium* and *Colletotrichum* from vascular tissue is of concern as these fungi

are unlikely to be affected by fungicide treatments applied to tubers for the control of other diseases.

Table 2a. Incidence of external diseases on certified potato seed tubers.

Cultivar & *source*	No. of Certified Growers	Mean Disease Severity Rating (%) (and range)		
		Black Dot	Rhizoctonia	Silver Scurf
NSW				
Coliban	4	15(10-20)	21((10-35)	51(40-56)
Vic				
Atlantic	5	4(0-12)	17(7-25)	51(44-80)
R/Burbank	4	21(0-79)	3(0-10)	61(35-97)
Bison	1	1	13	30
Crystal	1	1	51	4

Table 2b. Incidence of internal diseases on certified potato seed tubers.

Cultivar & *source*	No. of Certified Growers	Mean Disease Incidence (%) (and range)		
		Black Dot	Verticillium	Fusarium
NSW				
Coliban	4	4 (0-15)	3 (1-6)	11 (5-21)
Vic				
Atlantic	5	8 (2-18)	5 (0-10)	22 (17-33)
R/Burbank	4	6 (0-7)	12 (2-22)	9 (5-11)
Bison	1	0	0	18
Crystal	1	15	3	9

Whilst this survey was not extensive it has shown that (a) much of seed potato tubers planted in South Australia are diseased (b) the health status of seed lots is extremely variable and (c) certified seed tubers are not a guarantee of disease freedom or a low incidence of disease.

These studies have shown that drastic changes in the potato certification schemes need to be undertaken to improve the health status of potato seed tubers used in Australia.

GLASSHOUSE AND FIELD EXPERIMENTS

Experiments 1 and 2

Two experiments were undertaken to determine the effect of Metham, Temik and Benlate on senescence in Russet Burbank potatoes and on soil levels of the root lesion nematode *Pratylenchus* and the fungus *V. dahliae*.

Methods:

Soil naturally infested with both *Pratylenchus* and *Verticillium* was collected from two commercial potato sites in the Adelaide Hills. Soil from each site was collected randomly across the paddock and mixed thoroughly. The clay loam soil from site one (Experiment 1) was placed into foam trays each holding 18L of soil, whereas the sandy loam from site two (Experiment 2) was placed into 25cm diameter plastic pots each holding 15L of soil. Replicates of eight trays and twelve pots were treated with chemicals as outlined in Table 1. Five weeks after Metham was applied, tubers were planted to a depth of 5cm with 10 mini tubers per tray and 4 mini tubers per pot. Plants were watered by hand using care to avoid splashing and possible contamination between treatments.

Preplant population densities of *V. dahliae* and *Pratylenchus* were 14 and 7 cfu/g soil and 8.5 and 5.5 vermiforms/g soil from Experiment 1 and 2 respectively. Nematodes were identified as *P. crenatus*.

In both experiments plant senescence was rated and soil assessed for *Verticillium* and *Pratylenchus* at 89, 96, 103 and 110 days after emergence for Experiment 1 and 67, 81, 93 and 112 days after emergence for Experiment 2. Stems and roots were assayed for *V. dahliae* and *Pratylenchus* as previously described at 103 and 110 days after planting by choosing five plants per tray or two plants per pot.

Table 3: Treatments and method of application of soil naturally infested with *Verticillium* and *Pratylenchus*.

TREATMENT & RATE	APPLICATION METHOD
1. Metham 400L/ha (423g ai/L Metham Sodium)	Applied as a drench to soil surface, then watered to field capacity.
2. Temik 3.5Kg/ha (150g ai/kg aldicarb)	Applied as granules to the soil, then watered to field capacity.
3. Benlate 100gms/100L (500g ai/Kg benomyl)	Applied as a drench to soil surface at planting and 3 weeks after tuber emergence
4. Temik + Benlate (as above methods)	As above for single Temik and Benlate treatments.
5. Control	Untreated, watered to field capacity

Results:

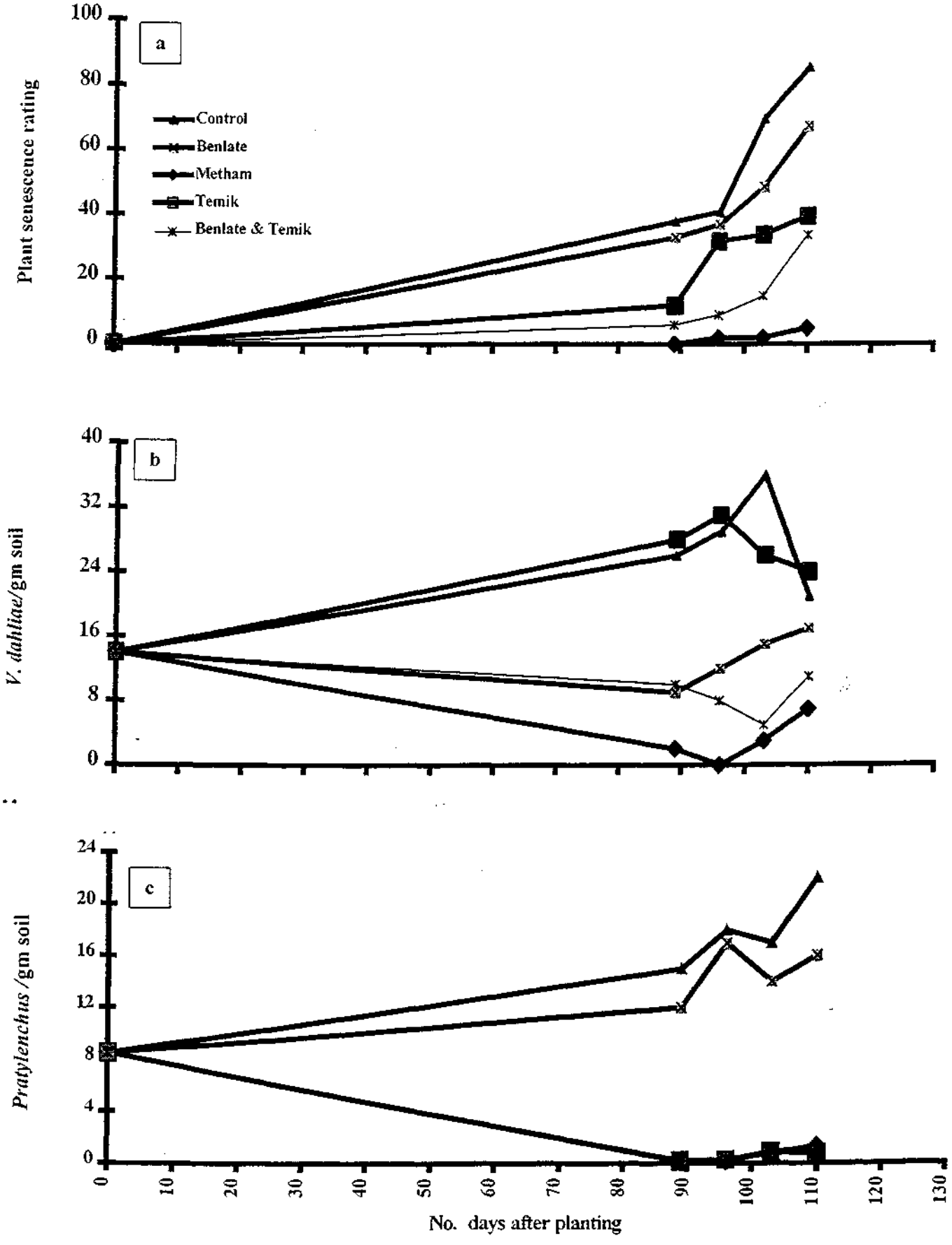
Experiment 1

Senescence was first observed 13 weeks after planting. Initial symptoms were characteristic of PED, with unilateral and/or interveinal chlorosis followed by progressive leaf necrosis. This developed to whole leaves becoming chlorotic and/or necrotic with 50% of plants dying 4 weeks after the symptoms were first noticed.

Symptoms were most severe in plants grown in untreated soil and those with Benlate alone (Fig 2a) compared to those in the other treated soils. Senescence development was delayed in Temik, Benlate + Temik and Metham treatments. All treatments except Metham and Benlate + Temik had plants that were infected with *V. dahliae*.

In the soil, *Verticillium* cfu/g increased by approximately 50% in the untreated and Temik treatments, but reduced by approximately 50% when treated with Metham or Benlate + Temik (Fig 2b). The level of *Verticillium* in the soil treated with Benlate remained fairly constant. The number of *Pratylenchus* vermiforms/g soil increased up to 150% in the untreated and Benlate treatments whereas the remaining treatments were reduced by nearly 100% (Fig 2c).

F igure 2 (a – c). The development of senescence (a) levels of *V. dahliae* (b) and *Pratylenchus* spp (c) in soil following treatment with Metham, Temik, Benlate and Benlate + Temik.

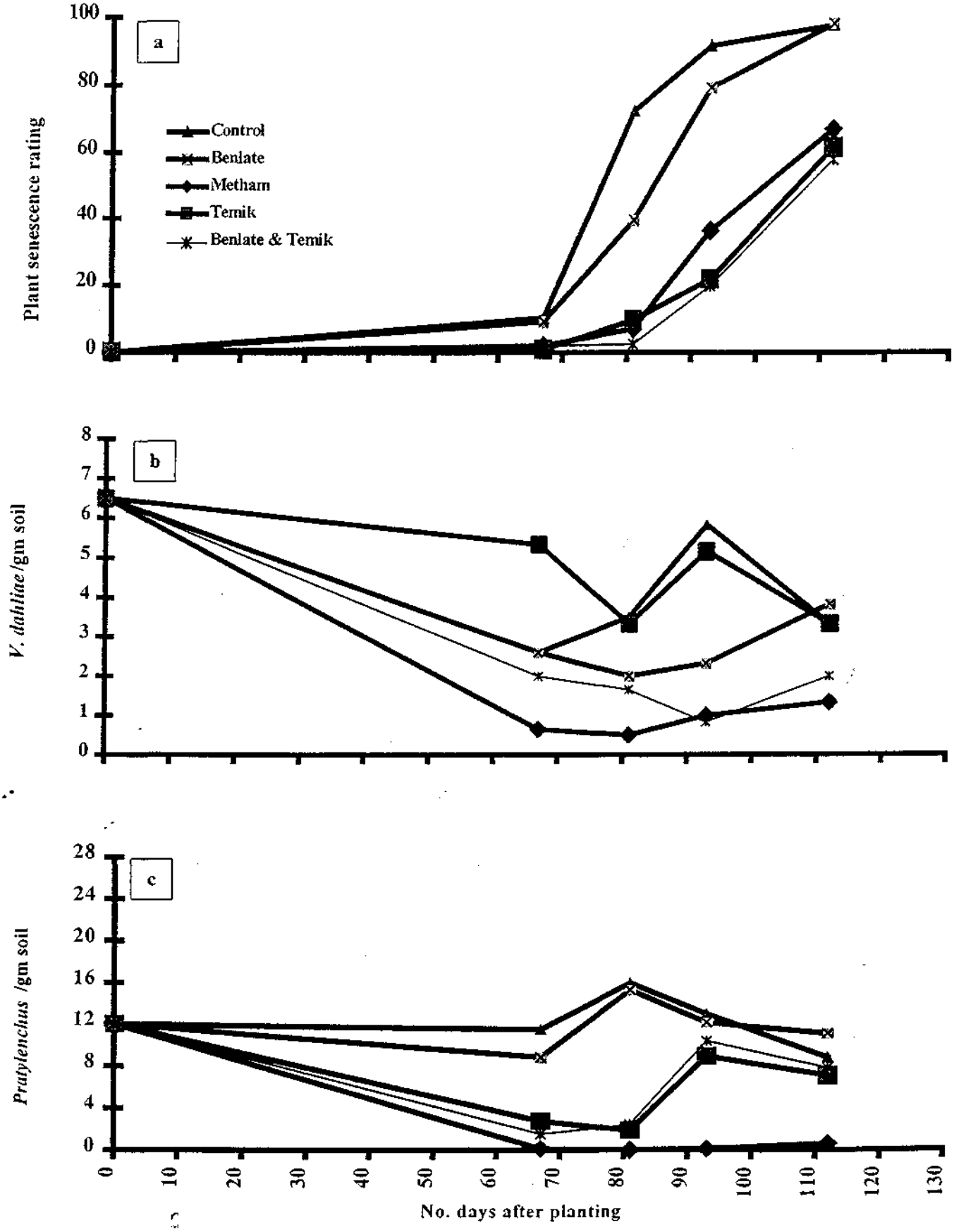


Experiment 2

Senescence was first observed 9 weeks after planting with 50% of plants dying by 3 weeks after the symptoms were first observed. Symptoms were again most severe in plants grown in untreated soil and those with Benlate alone (Fig 3a) compared to those in the other treated soils. All treatments except Metham had plants that were infected with *V. dahliae*, and senescence development was delayed in Temik, Temik + Benlate and Metham treatments. *Verticillium* cfu/g soil were reduced in all treatments, with Metham and Benlate +Temik reducing populations significantly (Fig 3b).

Ninety days after planting, *Pratylenchus* vermiforms/g soil increased gradually in the control and Benlate treatments whereas in the Metham, Temik and Benlate + Temik treatments, numbers were significantly reduced (Fig 3c).

Figure 3 (a – c) The development of senescence (a) levels of *V. dahliae* (b) and *Pratylenchus* spp (c) in soil following treatment with Metham, Temik, Benlate and Benlate + Temik.



At 103 days after planting *Verticillium* was detected in 45, 35 and 45% of plants in the control, Benlate and Temik treatments respectively, whereas none was detected in the Benlate + Temik and Metham treatments. A noticeable increase in infected plants was detected in all treatments except Metham by 112 days after planting (Fig 4). *P. crenatus* was recovered from roots of all treatments with the lowest level found in those treated with Metham (Fig 5).

Figure 4: Percentage of plants yielding *V.dahliae* at 103 and 112 days from planting following soil treatments with Metham, Temik, Benlate and Benlate + Temik.

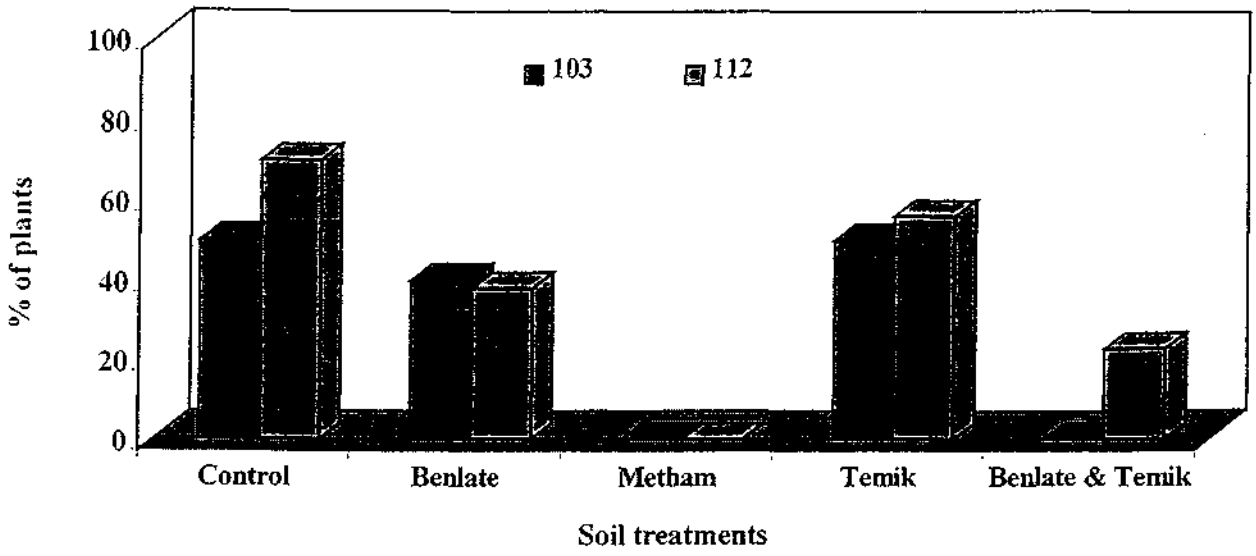
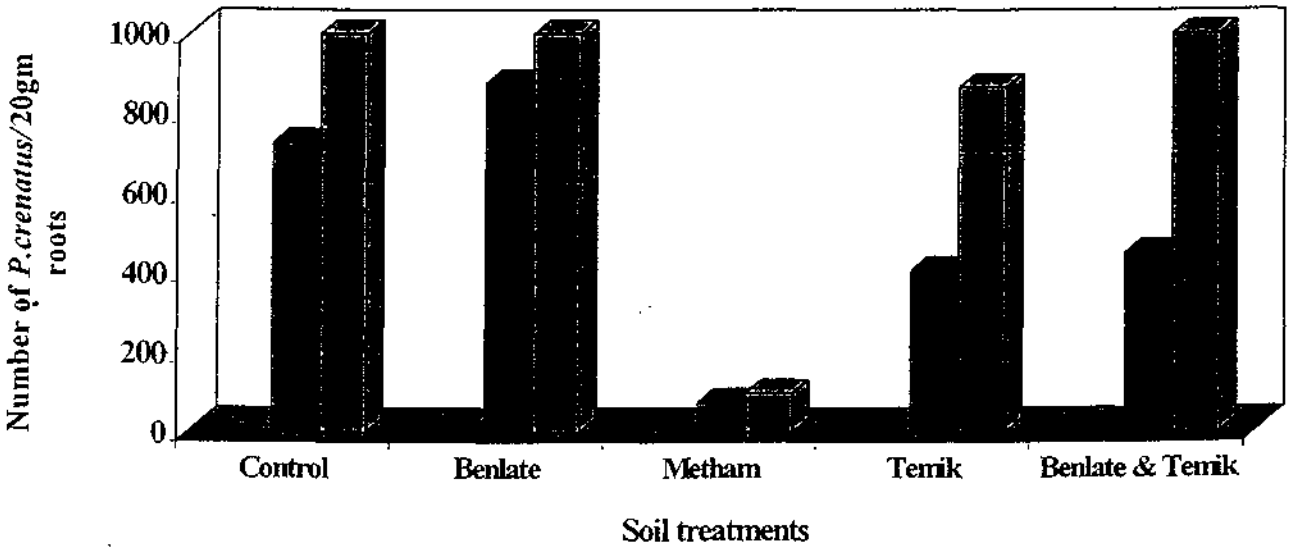


Figure 5: Population of *P.crenatus* in 20 gm of roots extracted at 103 and 112 days after planting following soil treatments with Metham, Temik, Benlate and Benlate + Temik.



Discussion:

These results show that Metham applied to the soil surface and leached through the soil significantly reduced the level of both *Verticillium* and *Pratylenchus* in the soil but did not completely eradicate these organisms. In both experiments there were indications that near the completion of the experiment the levels of *Verticillium* and *Pratylenchus* in the soil had begun to increase from the initial low level. While Temik was highly effective in reducing levels of *Pratylenchus* in the soil it had little effect on *Verticillium*. Nevertheless in both experiments senescence in plants grown in soil treated with Temik was delayed compared to those untreated soil showing that nematode control is important in delaying senescence.

Although Benlate reduced levels of *V. dahliae*, it had little effect on *Pratylenchus* and had no significant effect on the development of senescence in either experiment.

Overall these results show that metham sodium is the preferred treatment to delay senescence caused by infection by *V. dahliae* and *P. crenatus*.

Experiment 3 & 4

Two experiments were set up to evaluate the effects on disease development in Russet Burbank potatoes the effects of interaction of *P. penetrans and P. crenatus* with *V. dahliae*, by combining *Pratylenchus* and *V. dahliae* at high and low population densities.

Methods:

Foam trays containing 18L of pasteurised "Mt Compass sandy loam" were used in experiment 3 and plastic pots containing 15L of the same soil were used in experiment 4. Both experiments were set up as a randomised complete block design to compensate for slight temperature gradient across the greenhouse. The treatments compared various levels of *V. dahliae* and *Pratylenchus* nematodes alone or combined. Soil was artificially infested at either low (1cfu/gm of soil) or high (10cfu/gm soil) levels prior to planting by adding concentrated inoculum made with a mixture of *V. dahliae* microsclerotia from four different isolates as described previously. Two weeks after emergence, nematodes were applied to the soil as previously described using *P. crenatus* and *P. penetrans* at low (0.1 vermiforms/gm soil) or high (1 vermiforms/gm soil) levels alone or combined with the *Verticillium* infested soil. The various combinations are outlined in Table 4 and were compared with a non infested soil and replicated ten times.

In Experiment 3, six mini tubers of the potato cultivar Russet Burbank were planted 5cm deep into each tray and in Experiment 4, one commercial seed tuber of the same cultivar was planted per pot. Tubers from commercial sources were dipped in 5% formalin for 20 minutes to control surface pathogens such as *Rhizoctonia*. Plants were fertilised fortnightly with a solution of 20-20-20 (N-P-K) prepared at a concentration of 2 g/L, insecticides applied as needed to control whitefly and thrips and plants were watered by hand using great care to avoid splashing and contamination between treatments. Plants were grown for 3 months in Experiment 3 and for 2 months in Experiment 4 in a greenhouse with the average maximum temperature from 22°C to 25°C.

Table 4: - Levels of *Verticillium* and *Pratylenchus* inoculum added to soil before planting potato tubers

Sterilised Soil
Verticillium (low rate)*
Verticillium (high rate)†
Verticillium (low rate) + *Pratylenchus crenatus* (low rate)*
Verticillium (low rate) + *Pratylenchus crenatus* (high rate)**
Verticillium (high rate)† *Pratylenchus crenatus* (low rate)*
Verticillium (high rate) † *Pratylenchus crenatus* (high rate)**
Pratylenchus crenatus (low rate)*
Pratylenchus crenatus (high rate)**
Pratylenchus penetrans (low rate)*
Verticillium (low rate) + *Pratylenchus penetrans* (low rate)*
Verticillium (low rate) + *Pratylenchus penetrans* (high rate)**

Where			
	Verticillium:	low = 1 cfu/gm soil +	high = 10 cfu/gm soil†
	Pratylenchus:	low = 0.1 veriform/gm soil*	high = 1 veriform/gm soil**

Three core samples each of 250cc were taken from each tray at 30, 40, 71, 79 and 88 days after planting and mixed uniformly before extracting nematodes from a 200gm sub sample from each replicate.

Levels of *V. dahliae* in soil were also measured in another 200gm subsample per replicate taken from the above soil sample by plating onto selective media using the Anderson Sampler as previously described.

Senescence was assessed and potato stems and roots were assayed for *Verticillium* and *Pratylenchus* as previously described.

Results and Discussion:

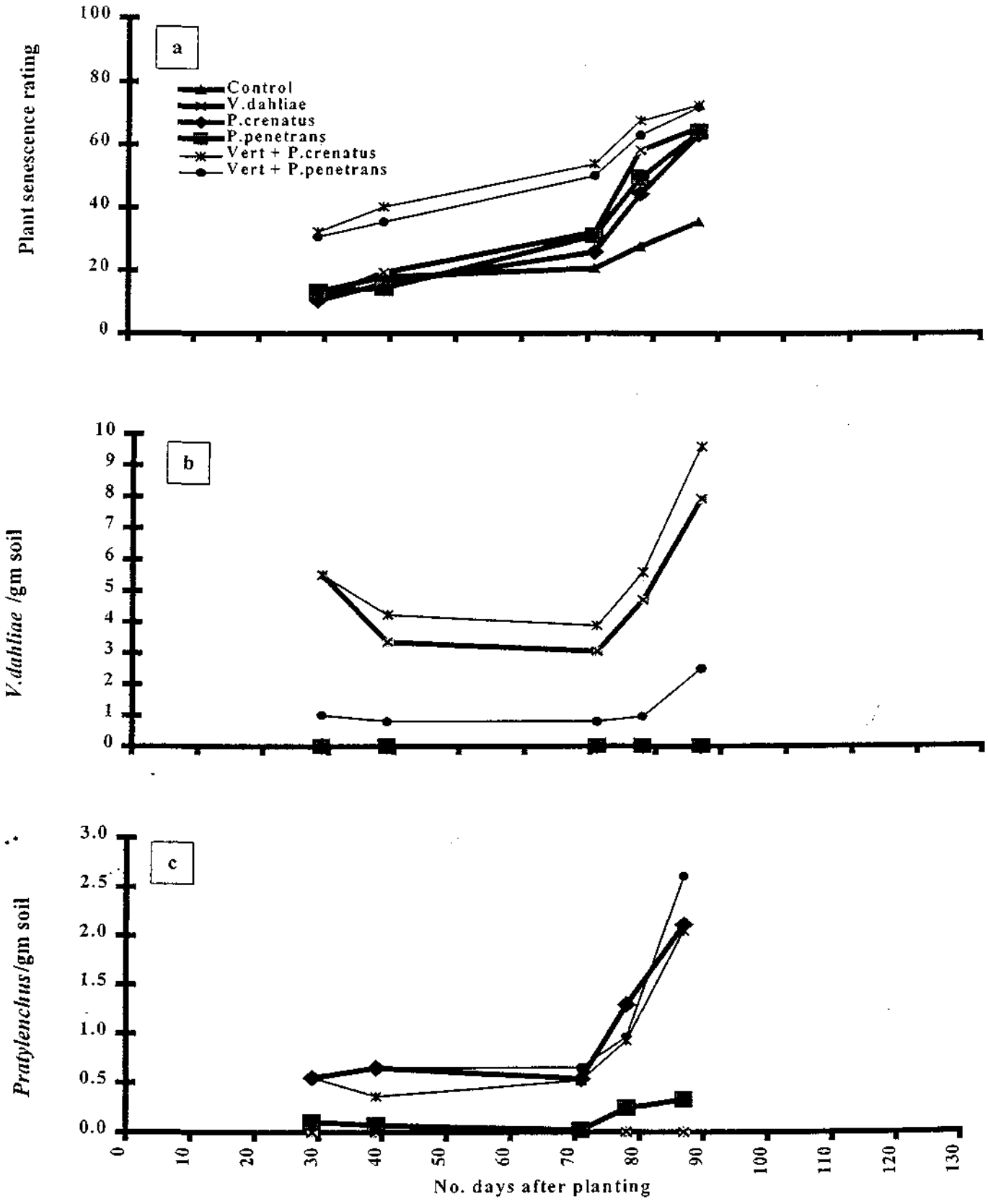
Results from Experiment 4 are not presented, as the potato plants became infected with an unknown virus which resulted in most plants dying within 56 days after planting.

Since trends for both high and low levels of *Verticillium* and *Pratylenchus* inoculation were similar results of senescence ratings nematode and *Verticillium* levels have been combined. The results from Experiment 3 (Fig 6a) show that senescence through most of the growing period was most severe when plants were inoculated with both nematodes and *Verticillium*.

Nematode levels increased rapidly in some treatments after 70 days and by the end of the 12 week growing period numbers had increased from 0.5 to 2.0 vermiforms/g soil in the *P. crenatus*, *P. crenatus* plus *Verticillium* and *P. penetrans* plus *Verticillium* treatments (Fig 6c). Similar increases occurred with *Verticillium* levels (Fig 6b), particularly where high levels of inoculum were applied For example *Verticillium* increased from 5.5 to 8 cfu/g when applied alone, whereas in the presence of *P. crenatus* numbers increased from 5.5 to 9.6 cfu/g.

These results confirm overseas reports on the interaction of *Verticillium* and *Pratylenchus* that results in increased severity of the potato early dying complex. However, the American studies indicate that the interaction is specific to *P. penetrans* (Riedel and Rowe, 1985, Bowers *et al*, 1996) whereas our studies indicate that *P. crenatus* may also be involved in the interaction. This is not surprising considering that *P. crenatus* was the most widespread species of *Pratylenchus* found throughout potato paddocks in Australia and that *P. penetrans* was detected at only 1 site.

Figure 6 (a – c) The development of senescence (a) levels of *V.dahliae* (b) and *Pratylenchus sp* (c) in artificaly inoculated soil.



Experiment 5

This experiment was conducted to compare senescence in Russet Burbank potatoes in soil naturally infected by *V. dahliae* and in the presence of low and high levels of *Pratylenchus*.

Methods:

A sandy loam soil naturally infested with 3.2 vermiforms/g *P. crenatus* and 3cfu/g *V. dahliae* collected from a commercial potato field in the Mallee region of SA was thoroughly mixed and placed into foam trays each containing 18L of soil. Trays were treated with Temik at the equivalent rate of 3.5 Kg/ha by applying as granules to soil surface 2 weeks prior to planting and then watering the soil to field capacity. Seven replicates of Temik and untreated soils were set up as a linear block design and two weeks after treatment 10 mini tubers were planted in each tray.

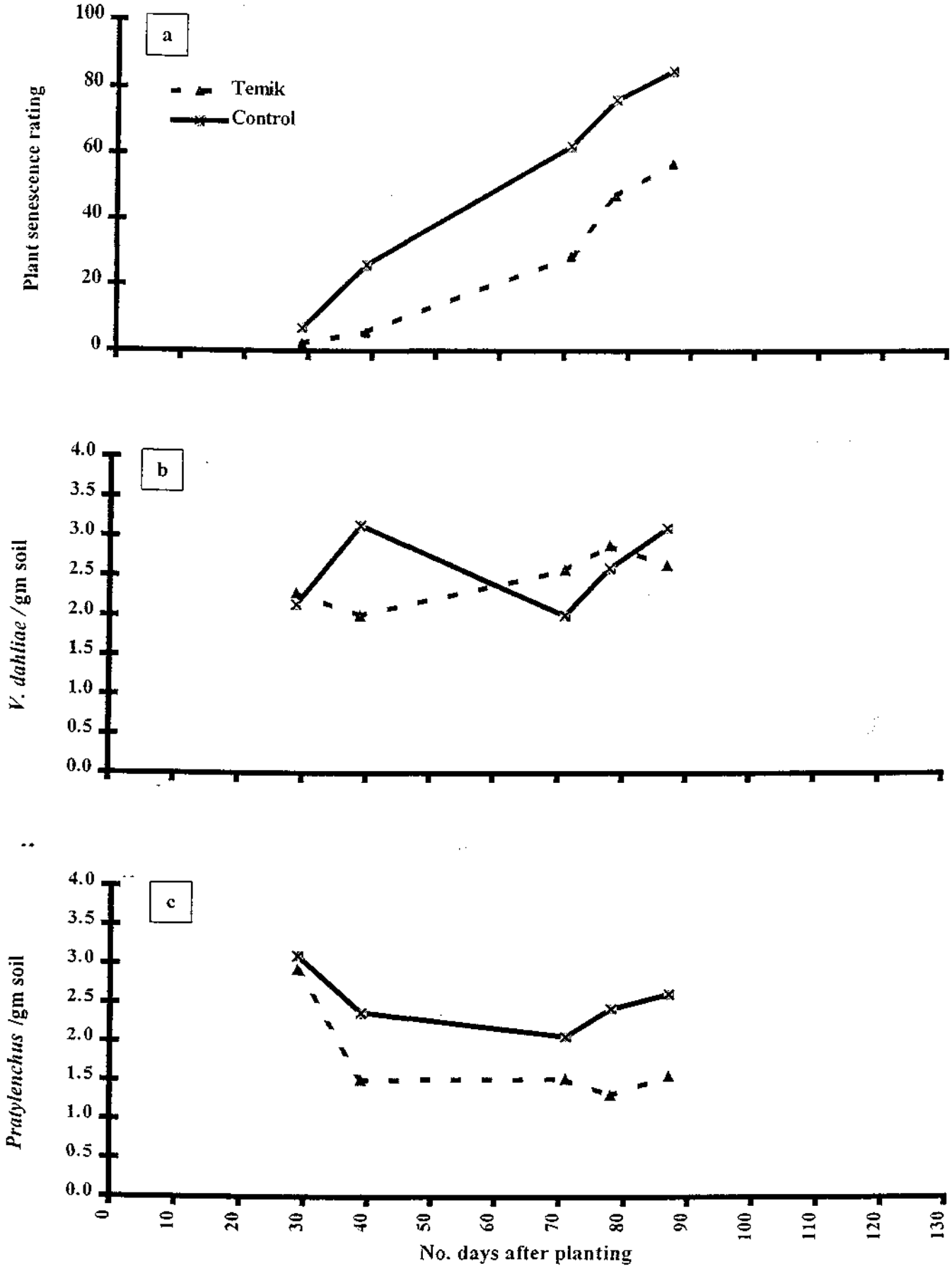
Plant senescence and the *Verticillium* and *Pratylenchus* levels in soil and plants were recorded at various times as previously described.

Results and Discussion:

Senescence was delayed in plants grown in Temik treated pots compared to those in the non treated pots. (Fig 7a). Nematode numbers were significantly reduced by the application of Temik and remained lower than those of the untreated at all sampling times (Fig 7c). *Verticillium* levels were not markedly affected by Temik (Fig 7b).

Whilst plant senescence was delayed and nematodes levels reduced by 51% in the Temik treated pots, the population of nematodes was not reduced to the levels suggested by Powelson and Rowe (1993) as threshold levels for an interaction with *V. dahliae*. Temik reduced the levels of *Pratylenchus* in the soil and delayed the development of senescence even in the presence of high levels of *V. dahliae*, confirming the importance of nematode control .

Figure 7 (a – c). The development of senescence (a) levels of *V. dahliae* (b) *and P.crenatus* (c) following the application of Temik.



Experiment 6

In this experiment the effect of metham sodium fumigation on levels of *V. dahliae* and *P. crenatus* and the development of senescence was measured on a commercial properties.

Methods:

On 19th November 1995, 3Ha of clay loam soil on a commercial potato property in the Adelaide hills was treated with 400L/Ha metham sodium using a 5 bladed injection unit, set at a depth of 25cm. A further 3Ha in the same paddock was left untreated. The soil was cultivated to a fine tilth just before fumigation. Both areas were planted with cv. Atlantic on 4th November and harvested 14th April. Plants were watered and fertilised as other commercial plantings within the same area. The development of senescence was rated using a 0-5 scale and soil levels of *V. dahliae* and *P. crenatus* measured at 30, 60, 95 and 120 days after planting as previously described. Yield was measured by hand digging 3m of row from each of 5 randomised areas in each treatment. Soil levels of *Verticillium* were also measured at harvest.

Results and Discussion:

Senescence was delayed and less severe within the fumigated area compared to the unfumigated area (Fig 8a). Nematode numbers were significantly reduced after the application of metham sodium and remained less than 0.05 vermiforms/g soil through to harvest. In contrast nematode levels remained between 0.5 and 0.85 vermiforms/g soil in the untreated area. Fumigation had a similar effect on the levels of *Verticillium* as around 5 cfu/g were detected in untreated soil compared to levels of 0.7 cfu/g in the fumigated soil (Fig 8b).

At harvest the levels of *Verticillium* were 6 and 1.3 cfu/g and *Pratylenchus* levels 8 and 0.07 vermiforms/g soil in the unfumigated and fumigated areas respectively. Total yield in the fumigated area was 38 ton/Ha compared to 28 ton/Ha in the untreated area.

Fumigation with metham sodium reduced the rate at which senescence developed, increased yield by 26% and significantly reduced the levels of *Verticillium* and *Pratylenchus* in the soil (Fig 9). For example plant senescence was reduced by 27% and nematodes and *Verticillium* numbers by 91% and 87% respectively in the fumigated areas.

Figure 8. The development of senescence (a) levels of *V. dahliae* (b) and *Pratylenchus* spp (c) following the application of Metham sodium.

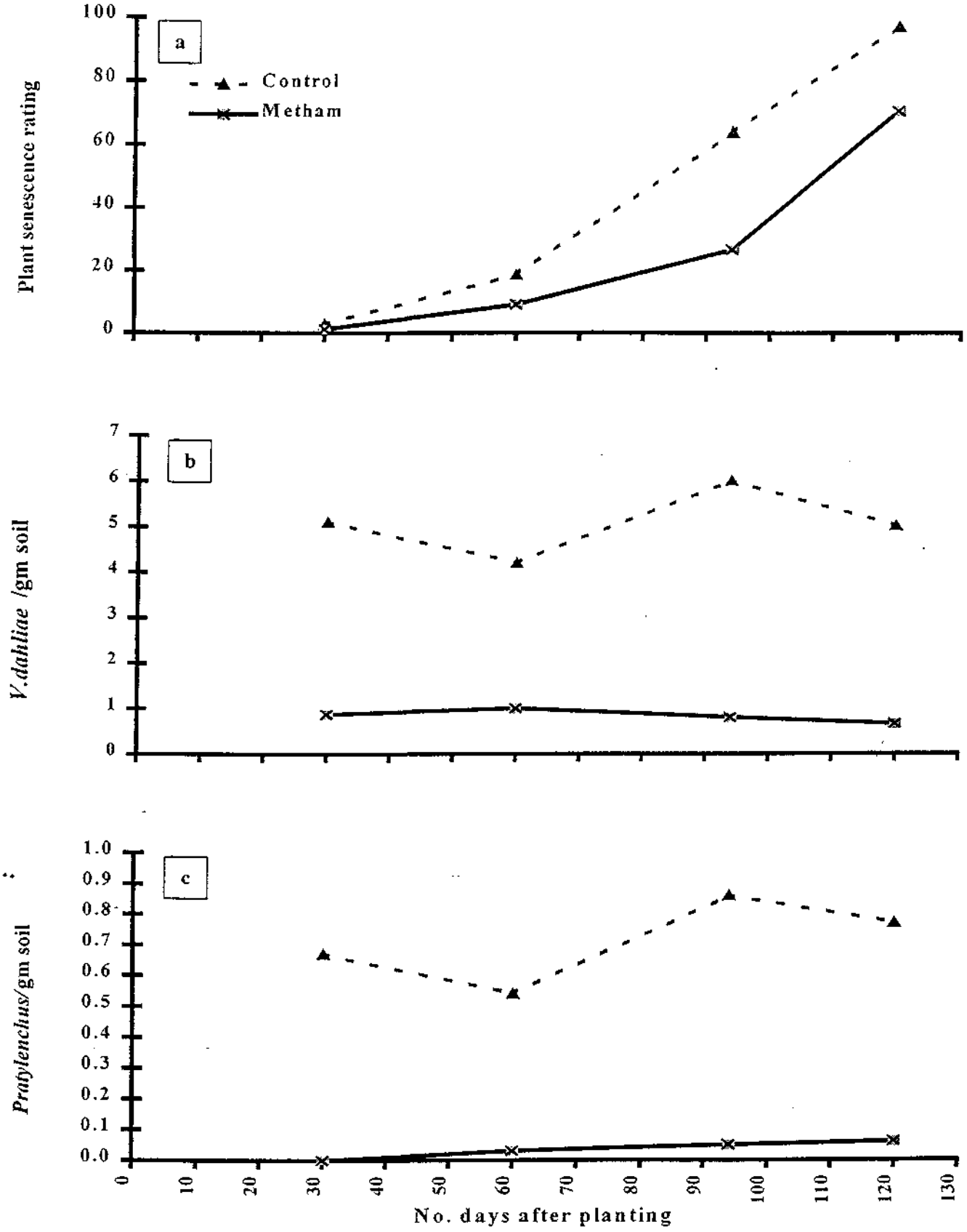
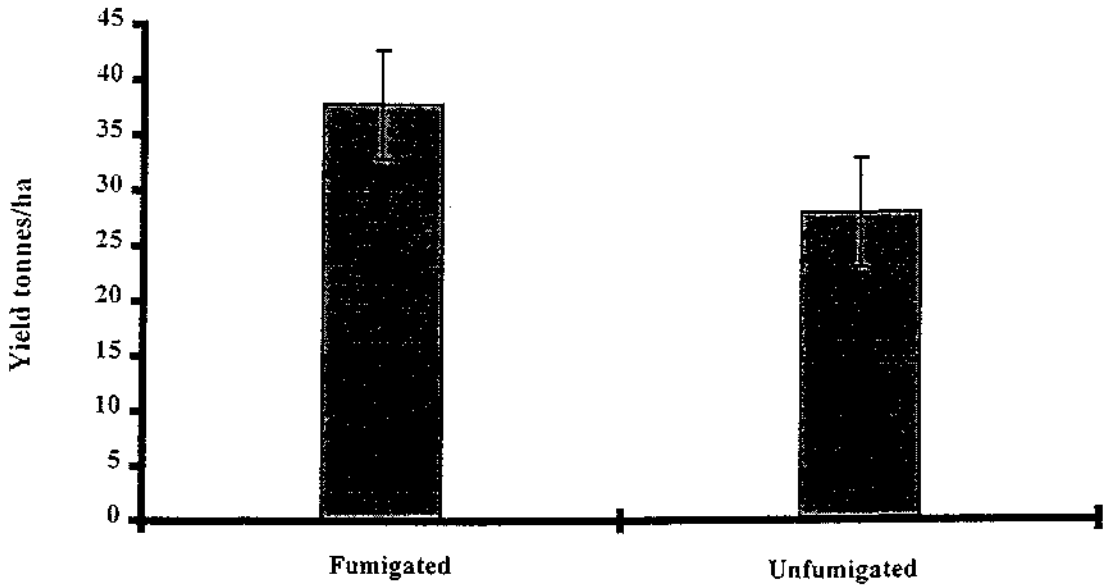


Figure 9. Effect of Metham fumigation on potato yield.



Fumigation reduced populations of both organisms to where no interaction was likely to occur (Powelson and Rowe, 1993), and provided a significant yield increase of 26%, similar to that achieved in the USA. Fumigation increased marketable yield and premium tubers by 17% equated to 13.4 ton/Ha or $2,728/Ha, and as fumigation costs were around $450 /Ha, a profit of $2,278 per Ha would be achieved.

Experiments 7, 8, 9 & 10

Four field experiments were undertaken to determine the effect of fumigation with sodium metham and nematicide treatments on the levels of *Pratylenchus* and *V. dahliae* and the development of senescence.

Methods:

These experiments were conducted on four commercial properties, two in the Adelaide Hills on a heavy clay loam and two in the South East of South Australia on light sandy loam. All properties were naturally infested with both *Pratylenchus* and *Verticillium*. Metham at 400 L/ha and Nemacur at 100 kg/Ha was applied at all sites to plots 5.4 m x 5.4 m and replicated 4 times in the South East sites and 8 times in the Adelaide Hills sites. Metham was applied to the soil surface and watered in at the South East sites, whereas at the Adelaide Hills sites Metham was injected. Nemacur was applied as granules to the soil surface and rotary hoed at all sites. Treatments were applied 11 November 1996 in the Adelaide Hills and 29 November 1996 in the South East sites.

Potatoes were planted four to six weeks after the Metham treatment and thereafter the trial sites were managed as per commercial practices. Yield was determined by hand digging 4 x 2 m strips in each treatment.

Senescence was assessed at approximately 50 and 80 days after emergence and every 10 days thereafter using a subjective rating scale as described previously.

Verticillium and nematode levels in the soil were assessed just prior to fumigation at planting and at 50, 80, 90, 100 and 110 days after emergence using methods previously described.

The incidence of *Verticillium* in tubers was assessed by collecting at random 100 tubers from each treatment at harvest. The stem end of all tubers was sliced and where staining was obvious in the vascular tissue, 2 pieces of approx $1mm^2$ were removed with a scalpel and plated on selective media . After 14 days incubation at 22 to 24°C, plates were examined microscopically and scored for presence or absence of *Verticillium, Colletotrichum* or *Fusarium.*

Results and Discussion:

As results from the two sites at each locality were similar, data from only one experiment from each of the Adelaide Hills and the South East sites is given.

In the Adelaide Hills sites the first appearance of senescence was delayed in both Metham and Nemacur treatments (Fig. 10a) but once started the senescence developed at the same rate as in the untreated plots. Nematode numbers remained low throughout the growing season (Fig. 10c) with around 4 vermiforms/g in the control plots. Both the Metham and Nemacur treatments reduced the level to less than 1 vermiforms/g. *Verticillium* levels remained around 4 cfu/g in both the control and Nemacur treatments throughout the season but were significantly reduced to less than 1 cfu/g by the application of Metham (Fig 10b).

In the South East sites, senescence was less severe than in the Adelaide Hills and both Metham and Nemacur reduced the rate of senescence compared to the control (Fig 11a). Within 80 days after planting nematode numbers declined in all treatments, but were lowest in the Metham and Nemacur treatments (Fig 11c). *Verticillium* levels remained around 1 to 1.5 cfu/g at all sampling times for the control and Nemacur treatments, but were reduced to below 0.5 cfu/g at most times in the Metham plots (Fig 11b).

The incidence of *Verticillium* in tubers collected from the Adelaide Hills was 18%, 17% and 12% for the Metham, Nemacur and control treatments respectively with no significant difference detected between treatments. In the South East site less than 1% of tubers in Metham treatment were infected with *Verticillium* and none were detected in tubers from the other treatments.

Yield at the Adelaide site was 33 ton/Ha in the control plots and was not significantly different to yield in the Metham and Nemacur plots, 31.5 and 32.5 ton/Ha. At the South East site, the yield of 25 ton/Ha in the control plot was significantly less than the 31 ton/Ha and 34.5 ton/Ha obtained from the Metham and Nemacur treated areas respectively. Yields of the Metham and Nemacur

treatments were not significantly different.

Overall these results show that Nemacur reduced *Pratylenchus* numbers, Metham reduced both *Verticillium* and *Pratylenchus* numbers and both slightly delayed the rate of senescence.

The significant increase in yield at the South East site following Metham and Nemacur treatments was unexpected considering the low numbers of nematode and *Verticillium* compared to other sites. However the soil at the SE site was mostly sand and in this soil the threshold levels where *Verticillium* and *Pratylenchus* damage occurs may be lower than in heavier soils. The effect of soil type on these threshold levels needs to be evaluated further.

Figure 10 (a – c) The development of senescence (a) levels of *V. dahliae* (b) and *Pratylenchus* spp (c) in soil following treatment with Metham and Nemacur in the Adelaide hills.

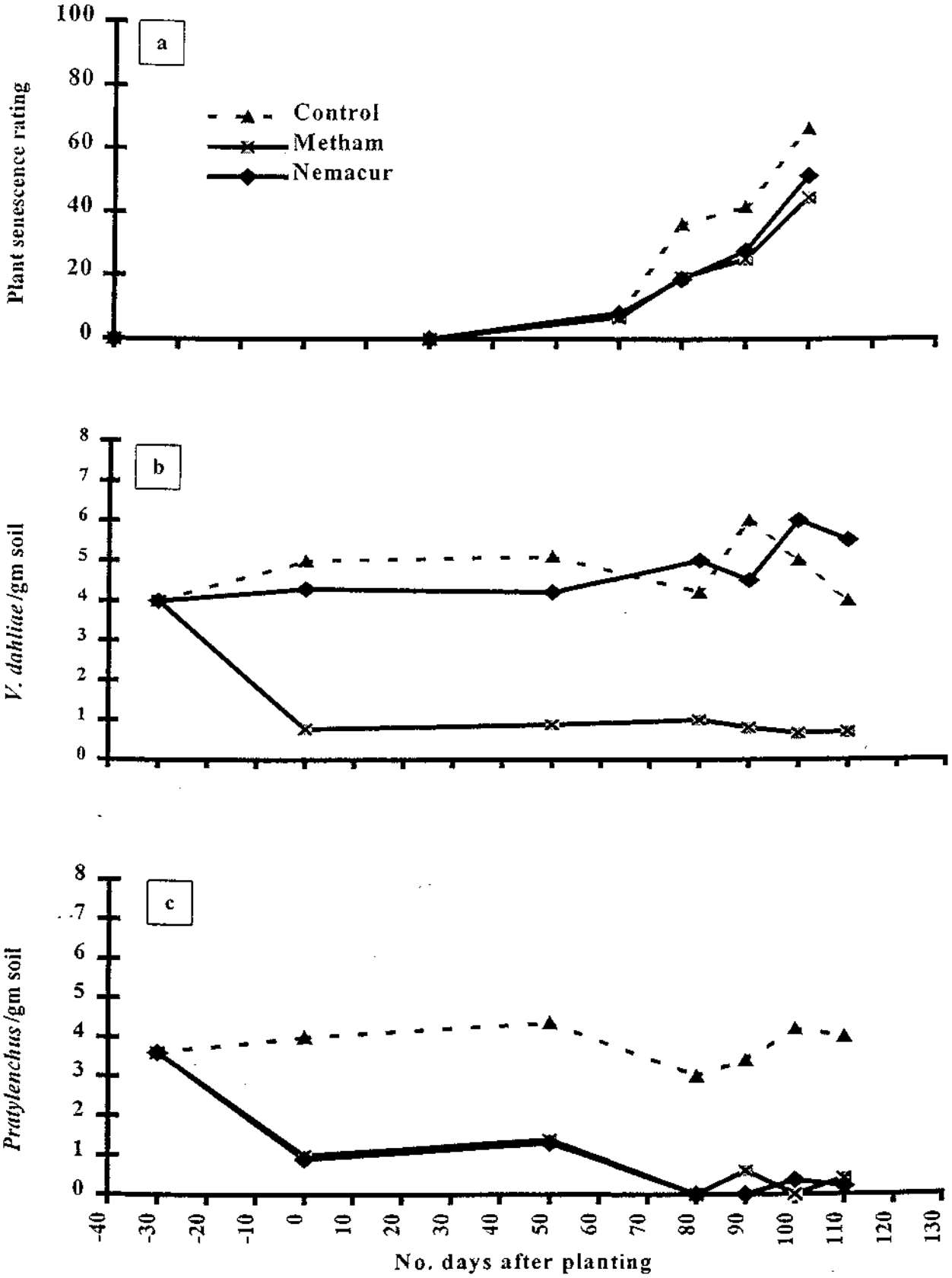
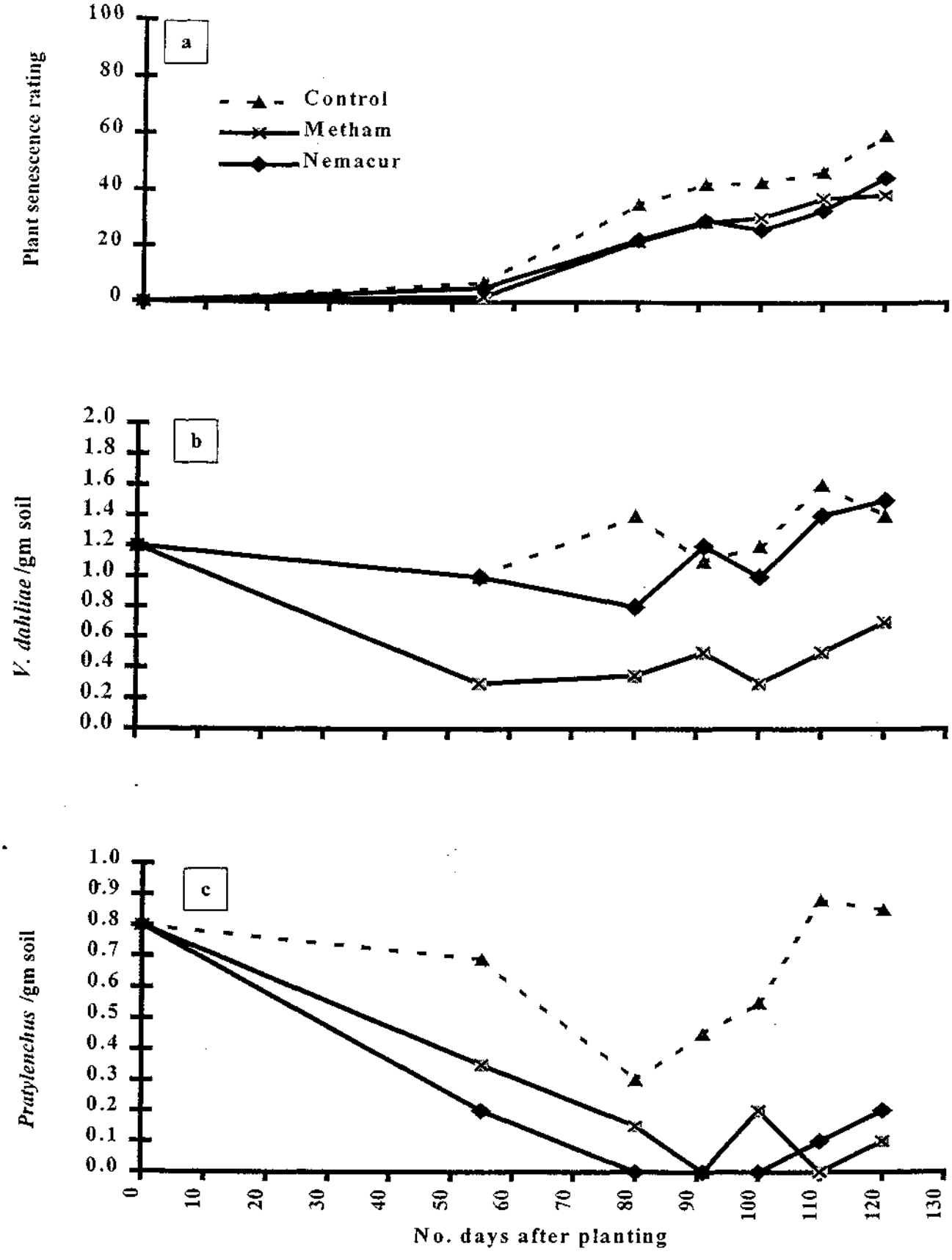


Figure 11 (a –c) . The development of senescence (a) levels of *V. dahliae* (b) and *Pratylenchus* spp (c) in soil following treatment with Metham and Nemacur in the South East.



Experiment 11 & 12

These experiments were undertaken to compare the effects of green manure crops on soil levels of *V. dahliae* and *Pratylenchus.* sp and the development of senescence. In one experiment potato yield and quality were measured.

Experiment 11

Methods:

Green manure crops of oats cv. Coolabah and forage rape cv. Rangi were planted using a sod seeder at 187kg/Ha & 57Kg/Ha of seed respectively in the Adelaide Hills on the 10th May 1995. After six months growth the manure crops were rotary hoed into the soil and after a further 6 weeks potatoes cv. Atlantic were planted. Plots were approximately 5m x 5m each replicated 6 times with a 1m buffer between each treatment.

Plant senescence was assessed approximately every 10 days on 12 occasions from emergence using a subjective rating scale as described previously.

Verticillium and nematode levels in soil were also sampled at similar times using the methods previously described.

Results and Discussion:

The development of senescence as well as the level of *Verticillium* was similar in both the oat and rape plots. For example the levels of *V. dahliae* prior to planting varied from 10 - 20 cfu/g soil and these levels fluctuated as the season progressed but at all times were similar in soils where either the oats or rape had been incorporated (Fig 12a). Initial *Verticillium* levels of around 15cfu/g fell to around 5 cfu/g 10 days after incorporation, but after 30 days approached original pre incorporation levels, rape 14.1cfu/g soil and oats 12.7cfu/g soil (fig 12b).

The levels of *P. crenatus* prior to planting varied from 1.6 to 5.2 vermiforms/g soil between replicates. Just prior to incorporation the *P. crenatus* level for rape was 7.2 vermiforms/g soil and

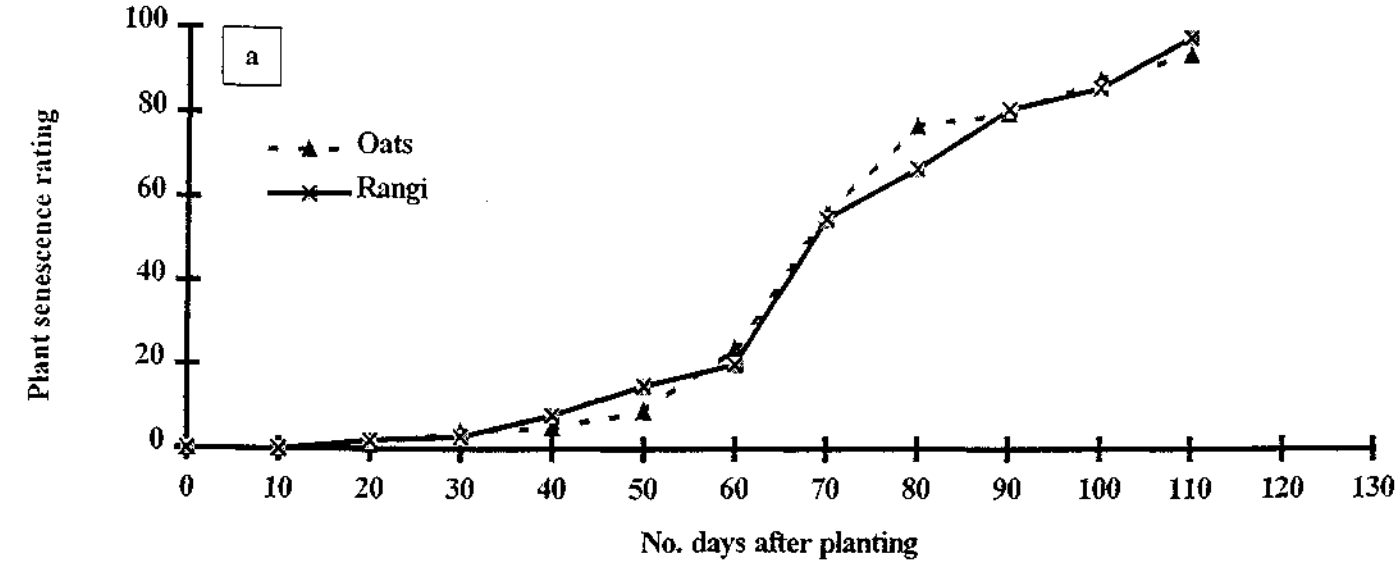
oats 2.9 vermiforms/g soil (Fig 12c). Ten days later levels in the rape dropped to 3.5/g soil but increased slightly in oats to 3.2/g soil.

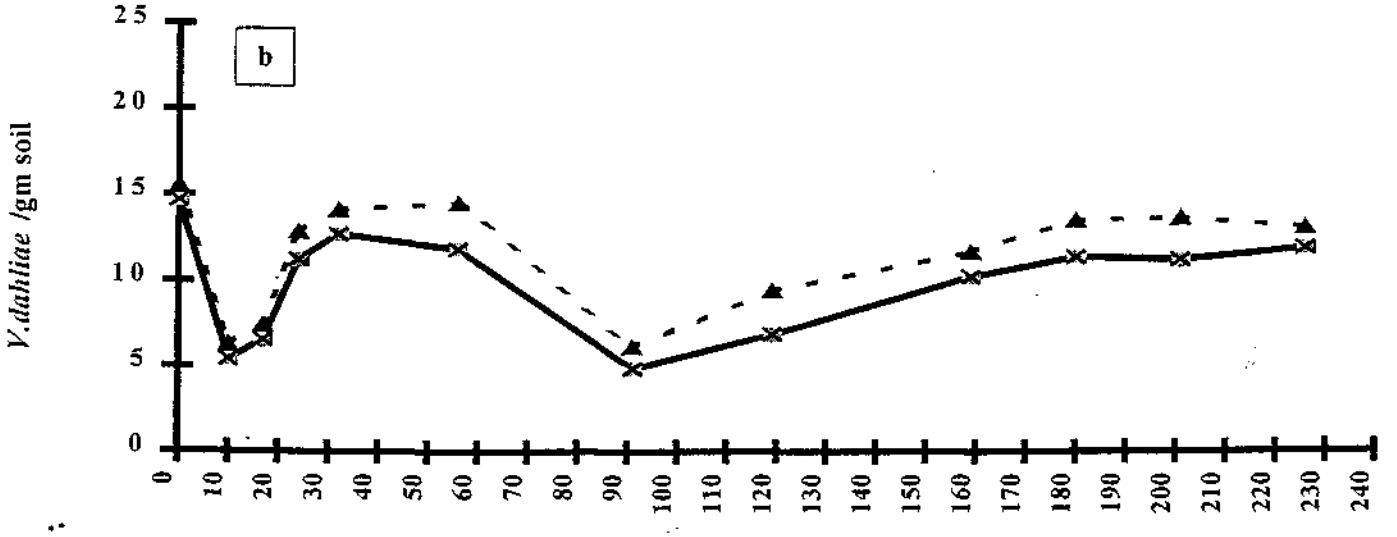
Thirty days after incorporation, nematode levels were the lowest at 2.0 vermiforms/g soil in oats and 1.5 vermiforms/g soil in rape. The levels in both the oats and rape rose steadily to around 3.5 vermiforms/g soil until potatoes were planted. They continued to rise slightly in the oat treatments but levelled within the rape treatments. Fifty days after planting the potatoes, levels diverged with those in the oats increasing to 5 vermiforms/g and those in the rape decreasing to 2.5 vermiforms /g.

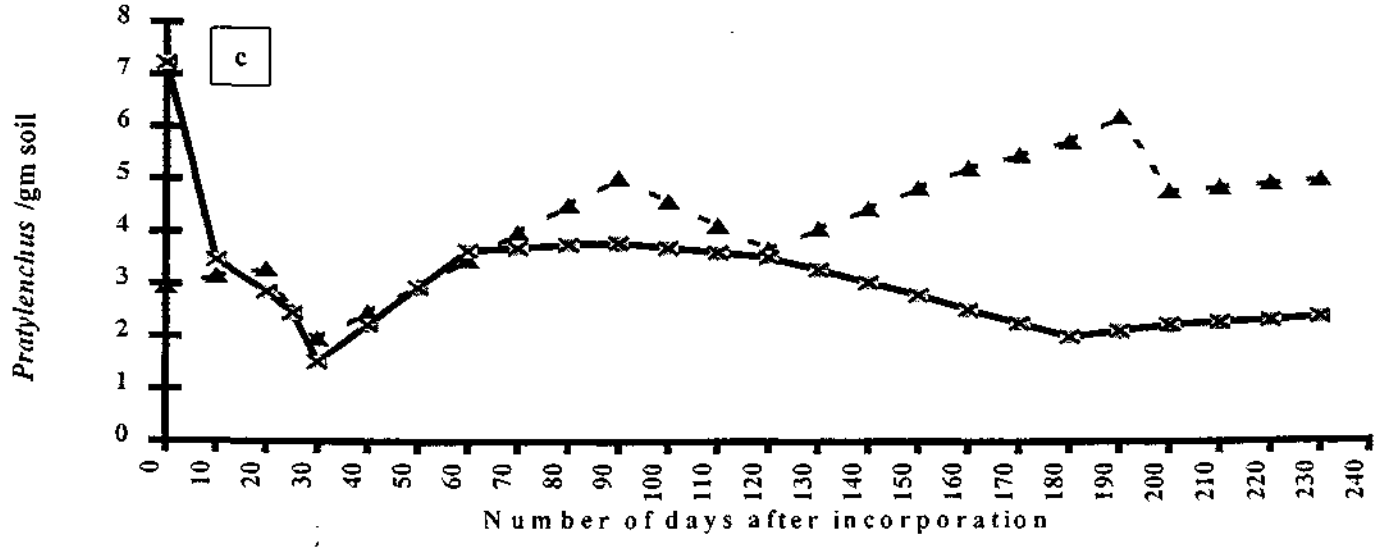
The yield of potatoes was 22% higher in the rape plots compared to that in the oat plots. These results show that Rangi rape is an ideal host for *P. crenatus* as nematode levels in soil from around these plants were at least twice that in soil from around oats. Nevertheless the biofumigation effect of the Rangi rape reduced nematode levels to that similar to those in the oat treatments, showing that there was no overall reduction in nematode numbers from the original planting level.

These results have not shown any significant reduction in *Verticillium* or *Pratylenchus* levels in the soil following incorporation of Rangi rape, indicating that further work on the use of Brassica green manure crops is required.

Figure 12 (a – c) The development of senescence (a) levels of *V. dahliae* (b) and *Pratylenchus* spp (c) following the incorporation of different green manure crops.







Experiment 12

Methods:

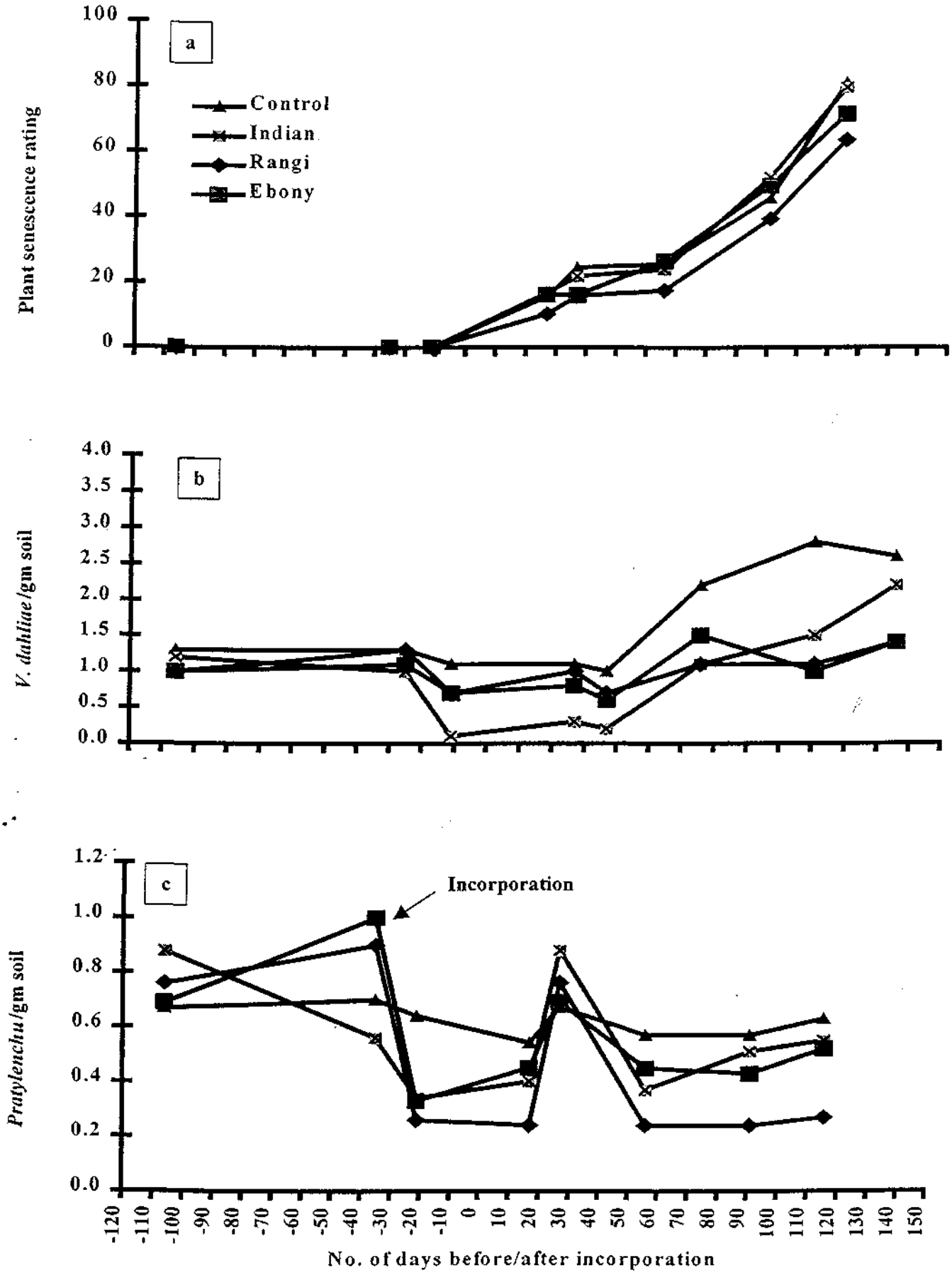
Brassica green manure crops of Indian Mustard, Ebony Mustard and forage rape cv. Rangi were planted in the Lower Murray region 4th October 1995 at 9kg/Ha on a sandy loam soil. The area had previously been planted to barley and onions. Each treatment was replicated 10 times using plots 50m long and 3.75m wide and arranged in a randomised complete block. The insecticide Baldock 25 EC (a.i. 25g/l Beta-Cyflthrin) was applied at 700ml/ha on 5th December to control cabbage moth. Brassicas were incorporated into the soil on 8th December, 65days after planting and potatoes cv. Ottway were planted 1st January and cv. Coliban 1st February 1996. Both were harvested on the 18th June.

Using the methods previously described, senescence was assessed on five occasions from emergence and *Verticillium* and nematode levels in soil were measured in samples collected approximately every 14 days.

Results and Discussion:

Senescence developed at the same time and at similar rates in all treatments (Fig 12a). Previous studies by Wicks and Harding (unpublished) showed that volatiles from Indian Mustard killed *Rhizoctonia, V. dahliae* and *Colletotrichum* mycellium in closed petri dishes, demonstrating the potential of this plant material to control these fungi when incorporated into the soil as a green manure crop. However this was not achieved in field experiments where green manure crops of both Indian Mustard and Ebony Mustard failed to significantly reduce soil levels of *Rhizoctonia*. The reasons for this were unclear although it may be associated with the biomass of green manure incorporated into the soil, as the mustard plants developed poorly in this experiment and suffered nutrient deficiencies and insect attack. Although attempts were made to rectify these problems, they may have influenced the production of glucosinolates. There was no significant trend in any of the Brassica incorporation treatments, although the Rangi rape had the lowest levels of *Pratylenchus* at most times during the potato growing season (Fig 13c). Similarly with *Verticillium* there was no trend to significantly reduce levels, although levels in all the Brassica treatments were less than those in the control (Fig 13b). Davis *et al* (1996) also showed that two cultivars of rape did not significantly reduce soil levels of *Verticillium* when they were used as a green manure crop.

Figure 13 (a – c). The development of senescence (a) levels of *V. dahliae* (b) and *Pratylenchus* spp (c) following the incorporation of different green manure crops.



FIELD EXPERIMENTS – NEW SOUTH WALES

Experiment 14: Len Tesoriero & Stephen Wade, NSW Agriculture NSW 1995/96

Aim:

I. To identify the cause of PED in the NSW Riverina potato crops.

II. To compare the effectiveness of cultural (fallow, crop rotation & *biofumigation*) and chemical (nematicide & fumigation) in managing early dying disease of potatoes.

Methods

Preliminary observations

Autumn crops were surveyed in 1994 and samples of plants and soil were collected and assessed in a pathology laboratory (BCRI, Rydalmere) for potential causal organisms. An unreplicated field trial was conducted in a potato crop (cv. Coliban) on 'Homelea', Finley. Nemacur ® was sprayed (24 l/ha) in a band along the furrow just after plant emergence. An untreated area (approximately 1/3 of the field) was left as a control area. Observations were made as plants started to senesce and two-metre lengths were dug after which tubers were graded and weighed. Four lengths were dug from each treatment.

Field Experiment

A trial was established at 'Claredale', Berrigan on a Sandmont sand and within the outer two spans of a centre pivot irrigator. A replicated complete block design was used with six replicates of five treatments (fallow, crop rotation, *biofumigation*, nematicide, & fumigation). Treatment plots were six rows wide (4.9m)and ten metres long. Rows were spaced 81 cm apart. Potatoes (cv. *Sebago*) were sown at 10 inch intervals giving 40 plants per row. The crop was sown on 8th February 1996 and hilled twice. The trial was harvested on July 10th and tubers were counted, weighed and graded into chats (0-80g), smalls (80-200g), medium (200-350g), large (350-450g) and oversize (>450g).

Soil was sampled in late August 1995 to give a pre-treatment estimate of the nematode population and distribution . Ten composite samples were collected with a soil corer (10 mm dia.) within each

plot. Nematodes were extracted and counted from 500 g sub-samples.

Ten random plants were sampled from each plot in mid-February 1996, just prior to flowering. Roots were cut into 5cm pieces and 300g samples were placed in plastic Baerman funnels from which nematodes were extracted over 4 days in a misting unit. Nematodes were collected on sieves, eluted with 30 ml of water into a beaker and counted under a microscope. This process was repeated in June as plants had started to senesce with PED symptoms. Stem pieces approximately 5 cm long were cut from plants adjacent to ground level. They were dipped in paraquat herbicide and incubated (6 stems per treatment x 6 replicates corresponding to field plots) in humid chambers for 15 days at 25°C. They were scored for the presence of microsclerotia of *Verticillium* under a dissecting microscope.

Results & Discussion:

Preliminary Observations

The fungus *Verticillium dahliae* was consistently isolated from roots and vascular tissue from lower stems of affected plant. The root lesion nematode, *Pratylenchus coffeae*, was identified by Rod McLeod, Nematologist at BCRI, Rydalmere, from affected roots. This species of nematode has been previously found on roots from wheat and grapes in the Riverina and appears to be the first record on potatoes in Australia (McLeod, Reay and Smyth, 1994), although there are reports of *P. coffeae* on potatoes from India, Japan and South America (Prasad *et al*, 1984; Inagaki, H, 1984; Curi *et al*, 1990). Typical brown necrotic roots that were consistent with those described for root lesion nematode damage of potatoes were observed under a dissecting microscope (photo). Root lesion nematodes were also found associated with lesions on tubers (photo). Plants remained greener in the nematicide treated areas of the observation trial while untreated areas showed typical early dying symptoms. There was an average 43% increase in yield of No.1 Grade potatoes (80-350g) in the nematicide treated area of the observation trial (Table 5). This result suggested that root lesion nematodes were associated with early dying disease in the NSW Riverina.

Table 5. Mean yields (t/ha) of potatoes (cv. Coliban) from observation trial, Finley 1994

	Nemacur®	Untreated	% Difference
Smalls (0-80g) t/ha	6.58	6.7	-2%
No. 1 Grade (80-359g)	20.82	14.57	+43%

Field Experiment

Pratylenchus counts in soil collected from the trial site prior to treatments ranged from 3-11 per 100g. Their distribution appeared to be spatially variable but uniform between treatment plots. *Pratylenchus* counts from root samples taken just prior to flowering showed that the metham sodium treatment to be superior to all other treatments while the nematicide and biofumigation treatments were significantly more effective than the control. Results are shown in Table 6.

Table 6. *Pratylenchus* counts from potato (cv. Coliban) roots (300g) sampled just prior to flowering

Treatment	***Pratylenchus* per 300 g roots**	±s.e.
Control	150 a*	47.5
Oats rotation	98 a b	31.1
Biofumigation	52 b	16.4
Nematicide	45 b	14.3
Metham sodium	15 c	4.8

* values followed by a different letter are significant at p=0.05

Nematode counts from root samples of the senescing crop were highly variable between treatments and replicates (data not presented). There was no significant trend in this data suggesting that treatment effects had not persisted. Similarly, *Verticillium dahliae* was detected on most (84-90%) stem pieces with no significant difference between treatments. Total and

marketable (80-450 g) yields were not significantly different between treatments. These data were consistent with field observations that there was a random distribution of early dying symptoms within the trial area. This contrasted sharply with observations and yield estimates outside the trial area where metham sodium treated areas showed no visible signs of early dying and marketable yields were increased by 57%. One major reason for the non-persistence of treatments within the trial area is that hilling operations would have moved soil and would most likely to have spread pathogens between treatments. This would have occurred at sowing and then with the two subsequent hilling operations. Future trials would require hilling operations to be done by hand.

Warm soil temperatures (25-28^{o}C) experienced in Riverina autumn crops have been shown in overseas studies to be optimal for invasion of potatoes by *P. coffeae*.

Further research is required to determine the benefit/cost and environmental sustainability of chemical controls as well as the evaluation of other *Brassica* cultivars for optimum *biofumigation* effects. Legislation may prevent the future application of metham sodium through overhead irrigation systems due to occupational health and safety concerns. Contamination of groundwater has led to the banning of this method of application in some production areas in the USA (W. Stevenson pers. Comm.). Alternative soil injection methods need to be evaluated in these cropping systems for optimum efficacy.

TECHNOLOGY TRANSFER

Oral presentations:-

(1) Riverina Potato Growers:-

Len Tesoriero, Stephen Wade & Russell Fox:

'Potato Early Dying - Research Progress',

October, 1996 at Finley RSL Club.

(2) South East Potato Growers - Connawarra – July 1997

R. Harding

(3) Murraylands Potato Grower - Murray Bridge – August 1997

R Harding and T. Wicks

(4) IAMA Field Day – Peebinga – August

R. Harding

(5) Virginia Potato Growers – Virginia – January 1998

R. Harding and T. Wicks

Extension articles

The Grower, October 1995. Premature deaths mean lower yields. T. Wicks and R. Harding

Spud Speak, May 1996. Potato Early Dying. T. Wicks and R. Harding

Spud Speak, Autumn 1997. Potato seed tubers as a source of disease causing fungi.

R. Harding and T. Wicks

The Growers, July 1997. Keeping an eye on potato seed quality. R. Harding and T. Wicks

Potato Australia, 1998. Potato Early Dying. Harding and Wicks (in preparation)

Research articles:

Wicks, T.J., Harding, R.B., Hitch, C. and Hall, B. (1997). Incidence of diseases on potato seed tubers. Australasian Plant Pathology Society 11th Biennial Conference.

Wicks, T.J., Harding, R.B. and Hitch, C. (1997). Verticillium wilt of potatoes. Australian Plant Pathology Society 11th Biennial Conference, 188.

Wicks, T.J. and Harding R.B. (1988) Potato Early Dying in Australia (in preparation). Australian Journal of Experimental Agriculture.

RECOMMENDATIONS

Extension/Adoption

In potato fields where premature senescence has been observed, soil and plant samples should be tested for the present of *Verticillium dahliae* and *Pratylenchus* spp. Where levels of 2 cfu/g soil or higher of *V. dahliae* are detected in the presence of *Pratylenchus*, fumigation with metham sodium is recommended before the next crop of potatoes is planted. Nematicides alone may be required in situations where *Pratylenchus* nematode was detected without *Verticillium*.

Potato seed tubers should also be tested for internal infection by *V. dahliae* and infected batches discarded, particularly where new areas are to be planted or when paddocks have been fumigated to control soil borne diseases.

Brassica green manure crops rotary hoed into the soil before planting may be of benefit in some situations in reducing pathogens, however further work is required to determine the most appropriate Brassica cultivar, seeding rate, time of planting and turning of the green manure crop.

Directions for Future Research

These studies have shown that *Verticillium* is widespread in the potato growing areas of Australia and is likely to be reducing yields significantly in some areas. Studies should be undertaken to compare the resistance or susceptibility of the main potato cultivars grown in Australia as well as new lines so that resistant cultivars can be suggested for disease prone areas. Other factors such as removal of infected plant debris after harvest to reduce carry over of the fungus should be evaluated as well as the further evaluation on use of green manure crops such as brassicas to reduce soil borne inoculum of the pathogens. The high incidence of *Verticillium* in seed potatoes indicates that this is the main means of spreading the disease and that further work needs to be done to ensure the production of healthy seed.

Other factors such as early season irrigation management have a significant impact on disease development overseas (Cappaert *et al*, 1994) and this aspect needs investigation in Australia.

Several species of *Pratylenchus* nematodes were found in potato plants and soils throughout Australia. The role of these nematodes in potato production, particularly their effect on yield in

different soil types needs further investigation.

Further studies on chemical control with soil fumigants and nematicides needs to be undertaken to evaluate if these treatments are warranted economically. Long term studies looking at the feasibility of biological control, and the integration of chemical and biological (antagonists or crop rotations) also need to be undertaken.

In summary the areas for future research are:-

- Evaluation of resistant cultivars
- Production of healthy seed tubers
- Evaluation of green manure crops in rotation
- Removal of infected debris after harvest
- Influence of *Pratylenchus* nematodes in potato yields
- Impact of irrigation on disease management
- Chemical and biological control of PED

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APPENDIX

POTATO EARLY DYING - NORTH QUEENSLAND

Introduction

Potato early dying (PED) is a disease caused by a complex of organisms. The major organisms associated with PED are root lesion nematode (*Pratylenchus* spp.) and the soil borne fungus *Verticillium dahliae.* A range of other organisms have also been implicated with PED, including the bacterium *Erwinia carotovora* var *carotovora* and the fungi *Collectotrichum coccodes* and *Fusarium* spp (Otazu *et al.*, 1978).

The syndrome is a factor limiting production in many areas, including USA, and Israel (Rowe, 1983; Krikun & Orion, 1977). In long established areas in the United States, the early dying syndrome has developed slowly over many years. In some cases, growers do not realise that their yield expectations are low and have come to consider early maturity the normal situation for their land (Rowe *et al.*, 1985).

The symptoms of PED are difficult to distinguish from normal senescence and may initially involve only reduced growth. Confusion may occur in the diagnosis of PED because the symptoms are highly variable and also may be associated with other diseases or physiological problems.

PED is perceived by potato growers on the Atherton Tableland as a major problem. There was evidence that Sebago crops were very short lived in the north Queensland environment and that the life span of crop had declined over the years of cropping. In north Queensland, the average life of the Sebago potato is 14-16 weeks. In other districts of Australia it is 18-20 weeks. Overseas this cultivar is regarded as very late maturing.

Some issues, such as seed source had been identified as possible causes for decline in Sebago but were later discounted. *V. dahliae* was known to be present in some soils as it causes minor disease losses in peanut crops, and forage sorghum was being used as a green manure crop prior to planting peanuts, which may enhance the build up of root lesion nematodes.

The purpose of this study was to determine which organisms are present in potato tissue that may lead to PED in north Queensland.

In addition to the procedures used by Harding (this report) which were able to detect and quantify *Verticillium dahliae* in soil and plant tissue, and root lesion nematode, a method based on the use of paraquat to aid detection of fungi in plant tissue (Cerkauskas and Sinclair, 1980) was used. In soybean, the use of paraquat induced the formation of fungal lesions and fruiting bodies of four pathogens about 2 wk before symptoms appeared on non treated tissues. Detection of fungi by dipping surface disinfested tissue in paraquat and then incubating them on moist filter paper was found to be easier and more accurate than plating tissues on an agar medium (Cerkauskas and Sinclair, 1980).

Material and Methods

Sampling Procedure

Ten samples were received from Lockyer Valley (South Queensland) in 1995, and samples were taken from paddocks in north Queensland in 1995 and 1996. Seven potato farms were sampled from north Queensland in 1995, and a further three were selected for evaluation of latent infection fungi. In 1996 seven farms were selected for evaluation of latent infection fungi.

Healthy, green potato stems were sampled at approximately 100 days (some crops were sampled earlier as senescence set in) after planting according to the methods outlined in the PED sampling protocol described by Harding (this project).

Detection of latent infection

Stem pieces were cut into 4cm sections and treated with paraquat according to the method of Cerkauskas and Sinclair (1980). Stem pieces were washed in tap water to remove soil, immersed in 95% ethanol for 3-4 sec, immersed in 0.05% NaOC1 for 4 mins and rinsed in sterile water. Some stem pieces were treated with 0.3% paraquat (1,1 - dimethyl -4, 4' - bipyridinium dichloride) prepared with sterile water and filter sterilized. Some stem pieces were not treated with paraquat and remained as a control. The treated and non treated stem pieces were placed separately on moist filter paper in 9-cm diameter deep dish petri dishes and incubated for 4 days at room temperature with natural light.

Tissue pieces were examined for fungal fruiting bodies in situ at 50 x magnification under a stereo microscope. Slides were made of fungi that could not be identified at this level, and were examined under a compound microscope. Fungi that had not produced fruiting bodies or were difficult to identify were pure cultured onto potato dextrose agar and carnation leaf agar. Fusarium like colonies were generally difficult to identify and this procedure was used for those colonies.

Incidence of *V. dahliae* and *C. coccodes* in soil and on seed.

Certified seed of cv. Sebago was obtained, and planted into steam sterilized UC mix or unsterilized field soil. Potato stems were removed from the plants, divided into below ground level and above ground portions and treated with paraquat. The presence of *V. dahliae* and *C. coccodes* was observed.

Pathogenecity testing of latent infection fungi.

Mini tubers of cv. Sebago were planted into steam sterilized UC mix, in order to obtain sufficient quantity of seed for testing. Sprouts from seed planted in UC mix revealed that the plants were already infected by latent infection fungi (principally *Fusarium* spp.) and pathogenicity tests were not conducted.

Results And Discussion

Detection of Latent Infection Fungi

Lockyer Valley

A range of fungi developed after four days incubation on potato stems treated with paraquat. Untreated stems remained green and healthy. Some paraquat treated stems rotted rapidly due to bacteria.

V.dahliae was recovered from six locations and *C.coccodes* from five, indicating that the fungi associated with early dying are widespread in the region. Other fungi recovered included *Alternaria sp.*, *Fusarium sp.*, *Colletotrichum sp.*, and some colonies which resembled *V.dahliae* but failed to produce microsclerotia in the potato tissue.

Atherton Tablelands

1995: A wide range of fungi developed on stems treated with paraquat. Untreated stems remained healthy. Fungi recovered from the tissue included *Colletotrichum coccodes*, *Verticillium dahliae*, *Fusarium moniliforme*, *F.oxysporum*, other unidentified *Fusarium sp.*, *Phoma sp.*, *Alternaria alternata*, *Alternaria solani*, *Cylindrocarpon sp.*, *Glomerella cingulata* and *Cercospora sp.*. The most commonly occurring fungi were *Fusarium sp.* *Verticillium dahliae* was recorded at a very low frequency.

The low frequency of *V.dahliae* was in contrast to the findings of Harding (Table , this report) who found that five of the seven sampled farms had a heavy infection level of verticillium, and two samples with light infection levels. Paraquat treated stems may allow for rapid growth of fungal endophytes and latent infection fungi which compete successfully with *V.dahliae*. However, *V.dahliae* was recovered readily from samples received from Lockyer Valley.

1996: A similar array of fungi was recovered from samples in 1996. *V.dahliae* was recovered at a very low frequency, again in contrast to the findings of Harding, where *V.dahliae* was recovered in high numbers from six of the seven paddocks sampled. Macrophomina sp. (*phaseolina*?) was recovered from treated stems, which had not been observed in previous tests.

The occurrence of a variety of potato pathogens (*V.dahliae*, *A.solani*, *Macrophomina* sp., *Fusarium* sp.) indicates the success of this method in detecting latent infection fungi. As in soybean (Sinclair 1991) the role of these latent infection fungi in the epidemiology of these pathogens requires further investigation. Early infection that does not result in conspicuous signs or symptoms may weaken the plant, predisposing it to other stresses or diseases.

The incidence of root lesion nematode was low in north Queensland sites, indicating that nematodes may not be involved in the PED syndrome as it occurs in this region. Although *V.dahliae* levels were generally high in the preplant and post plant (100 days after planting) sample. This was not confirmed by the paraquat test. *V.dahliae* was only occasionally recorded by the paraquat method, indicating that this method may not be entirely satisfactory for detection of this fungus.

PED in north Queensland may be caused by an interaction of fungi. The prevalence of *Fusarium spp.*, *Colletotrichum coccodes* and *Macrophomina sp.*, in paraquat treated

samples warrants further attention. These fungi are all capable of causing disease to potato (Hooker, 1981) and their combined effect may lead to a syndrome similar to PED.

Incidence of *V.dahliae* and *C.coccodes* in soil and on seed.

There appeared to be no difference in the incidence of *V.dahliae* and *C.coccodes* from potato stems sampled from below ground level or above ground. *V.dahliae* was recovered from stems grown in unsterilized soil, whereas *C.coccodes* was recovered from stems grown in both steam sterilized and unsterilized soil. *C.coccodes* was most likely present on the seed used, unlike *V.dahliae* where the primary source of inoculum was the soil.

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