Pea root rot survey

2008 – 09 season

Ian Harvey and Penny Turpin
PLANTwise Services Ltd, Lincoln

Julie Sime and Nick Pyke
Foundation for Arable Research, Lincoln

Summary:

- Forty pea crops were sampled for root rot during December 2008 from throughout Canterbury. Approximately 30 plants were collected in a W pattern across each crop.

- Roots and lower stems were returned to the laboratory, washed free of soil and scored for root and tap-root rotting on a 0 – 5 scale. From these scores, a disease index (DI) was calculated on a 0 -100 scale.

- Tap-roots scoring 2 and above out of 5 were surface sterilized and plated out onto malt extract agar (MEA). Isolated fungi were identified after 4 days at 25^{o}C in the dark and 3 days under nUV light sporulation stimulation.

- *Fusarium solani* was the most abundant fungus isolated, being recovered from the roots of 82% of the crops sampled. Within these crops there was an average of 28.6% of plants infected. *F. oxysporum* was isolated from 75% of the crops but was not considered the wilt strain. Several other *Fusarium* spp. were also isolated during the study and may have contributed to the root rot complex.

- *Phoma medicaginis* var. *pinodella,* the cause of collar and root rot of peas was isolated from 53% of the crops surveyed. Within these crops, there was an average of 60% of plants infected.

- Other root rot pathogens, namely *Aphanomyces euteiches* and *Thielaviopsis basicola* were seldom isolated but were detected by direct microscopic observation in 22.5% and 7.5% of crops sampled respectively.

- The mean DI calculated for the crops sampled was around 50 and ranged from 20 to 85 (out of 100).

- There was a negative correlation (r^2 = -0.33) between *F. solani* isolations and years since a legume crop (eg. peas and / or clover). There was also a negative relationship between the general DI of a crop and the years since a legume, but this was a less clear relationship with r^2 = -0.23.

- There was only a weak relationship between the general root rot DI and crop yield (r^2 = -0.17).

Objective:

Reports of root and collar rots of peas in Canterbury being caused by pathogens other than Aphanomyces and the "Ascochyta / Phoma" complex prompted a survey of pea crops throughout the region. Although it has been acknowledged that root and collar rots are present in pea crops in New Zealand (Greenwood, *et. al;* 2008), the extent of the damage or incidence of the causal fungi has not been determined. Thus, the main thrust of the study was to determine the incidence, severity and role of *Fusarium* spp. and other fungi in the rotting of pea roots and collars in the region.

Methods:

Forty paddocks were selected by seed merchants and pea processors from throughout Canterbury and sampled almost exclusively throughout December 2008. Information on location, paddock size, cultivar, irrigation availability, sowing time, growth stage at sampling and previous cropping history were included in the information collected with the samples (see form used in Appendix I). Appendix II tabulates this data.

Samples were taken by visiting the paddocks and digging approx. 30 plants in a W pattern from the site. The plants were wrapped in damp kitchen paper towels and placed in un-sealed plastic bags. Samples were then delivered to the laboratory and cool stored at 4^{o}C until processed.

As soon as practical after delivery to the laboratory, the plant had the tops removed and the soil thoroughly washed off the roots. Roots were then placed in 5 groups according to the 0-5 disease scale, with 0 being healthy and 5 being the worst, as illustrated in Appendix III. Once assessed, a disease index (DI of 0 – 100) was calculated as per the following formulae:

$$DI = \frac{\text{Sum of (Disease score x no. plants with that score) x 100}}{\text{No. of plants in sample x 5}}$$

Groups 0 & 1 were discarded as they were considered to have levels of infection too low for meaningful isolation results. Groups 2, 3, 4 & 5 had 1mm sections cut out between the stem base and roots. These pieces were placed into labeled vials. The remaining pea roots were put into labeled bags and placed back into the cooler. The 1mm sections were sterilized with 1% sodium hypochlorite for 40 sec, then washed twice with sterile water and dried between paper towels. These sections were then put onto MEA (malt extract agar) with 3-4 pieces per plate. Plates were sealed in cling wrap and put into the incubator at 25ºC for 2-3 days to stimulate fungal growth

After 2-3 days the plates were removed from the incubator, unwrapped and placed under near UV light to promote sporulation. Fungal colony identification was carried out under dissecting and compound microscopes.

Results and discussion:

Disease index (DI) range:

Of the 40 crops surveyed, 50% were assessed as having a DI of 50 (out of 100) or more (Fig. 1). The range was from around 20 up to 85. Most of the crops surveyed had a DI between 40 and 70 (Fig. 2).

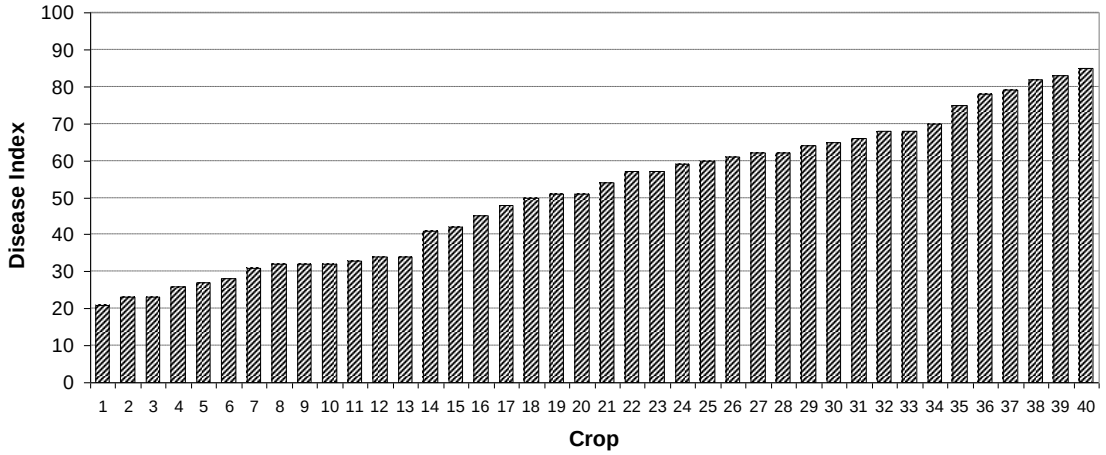


Figure 1: Spread of disease index ratings over the 40 paddocks sampled in Canterbury 2008 - 09

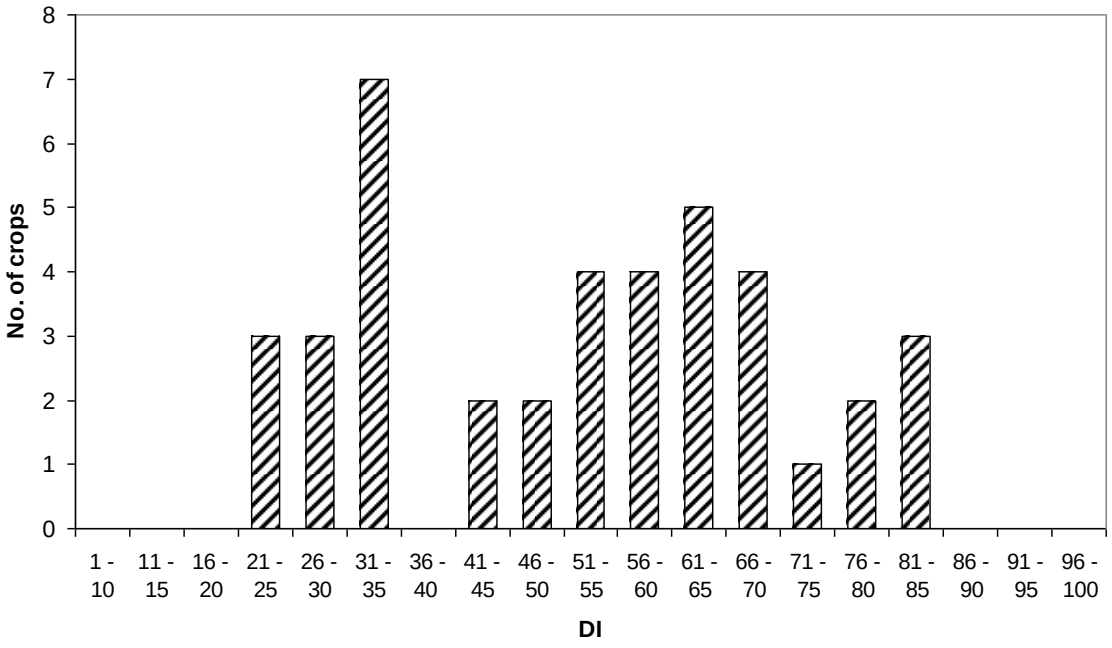


Figure 2: Distribution of disease index (DI) ratings from 0 -100 over the 40 paddocks sampled in Canterbury 2008 - 09

This level of root rotting is considered high. Coupled with the incidence of roots that yielded *Fusarium* spp. (especially *F. solani*) these results strongly suggest that this group of fungi are a major cause of root and foot rotting in Canterbury pea paddocks.

Detected fungi in the samples:

The following fungal pathogens were either isolated from or detected on the pea stem bases and root tissue in this survey:

1. *Fusarium solani* - the cause of pea foot and root rots
2. *F. oxysporum* - the cause of pea root rot
3. Other *Fusarium* spp. - the cause of pea root rot
4. *Aphanomyces euteiches* - the cause of pea common root rot
5. *Thielaviopsis basicola* - the cause of black root rot
6. *Phoma medicaginis* var. *pinodella* - the cause of collar and root rots

Visual examination of peas that exhibited general root, collar and foot rotting (Fig. 3A) could not distinguish between the diseases caused by *Fusarium* spp. and *P. medicaginis* var. pinodella.

Pathogens *A. euteiches* and *T. basicola* are not readily isolated into culture in the presence of other pathogens and / or secondary tissue invaders. Thus, they were rarely encountered in the isolations to MEA culture medium. However, they were often recognised during the index scoring (Figs. 3B and 3C) and the causal agents detected using compound microscope examinations. These samples are noted in Appendix IV.

Figure 3:
A: Foot, collar and root rot complex of peas
B: Root rot caused by *Aphanomyces euteiches*
C: Black root rot caused by *Thielaviopsis basicola*

Incidence of isolated fungi:

Fusarium spp. were the most predominant fungi detected as the cause of root rotting in the peas sampled from the 40 paddocks (Table 1).

F. solani was the most predominant fungus isolated overall within the study (Fig. 4), being isolated from 84% of the 40 crops samples and with a mean incidence of approx. 29% of plants infected within the infected crops.

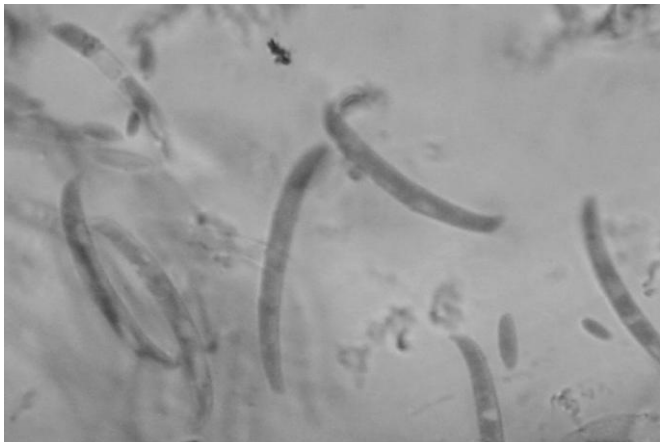


Fig. 4: Macro and micro conidia of *Fusarium solani* isolated from diseased pea root tissue.

Table 1: Percentage of crops infected, mean incidence within infected crops and range of incidence of the main fungi isolated from pea tap roots exhibiting rot symptoms

	%		
Fungus:	crops infected	Mean incidence	Range
Fusarium solani	84	28.6	3 - 91
F. oxysporum	75	12.2	3 - 33
Chaetomium globosum	65	21.4	3 - 91
Other *Fusarium* spp.	63	20.6	3 - 58
Phoma medicaginis var. *pinodella*	53	11.4	3 - 47

(note: almost all samples had multiple fungal isolates – see Appendix IV)

Even though 75% of the paddocks yielded *F. oxysporum* (Table 1), none of the peas sampled examined and scored appeared to have typical symptoms of Fusarium wilt (viz. lower leaf down-curling, wilting and dying of plants and vascular reddening). It is suspected that all the isolates were either the root rotting strains or secondary saprophytic strains of this species.

The remaining *Fusarium* spp. were located in 63% of the surveyed crops but these were not identified to species level, as there was a wide range which were suspected as weak or secondary root invading fungi.

Over half the paddocks (53%) with root rotting yielded the foot rot fungal pathogen - *Phoma medicaginis* var. *pinodella*. There was a mean incidence of 11.4% plants infected within these infected crops. No other fungi in the "Ascochyta" disease complex were isolated or detected in this study.

C. globosum is an aggressive soil-borne secondary fungal invader of compromised root tissue located on 63% of the paddocks surveyed. Many of the samples from which this fungus was isolated exhibited honey-coloured root rotting typical of that of common root rot caused by *A. euteiches*. Moreover, when these tissues were examined under the compound microscope, typical oospores of the pathogen (Fig. 5) were often detected, with 22.5% of crops sampled found to have such infection (Appendix IV). It is surmised that the majority of times when this fungus was isolated in samples with high DI ratings,

that common root rot was the most likely cause of the rotting in the absence of any of the above pathogens.

The black root rot pathogen, *T. basicola* was seldom isolated into culture. However, on many of the samples with a high DI and showing typical blackened root symptoms of this disease, characteristic black arthrospores of the pathogen (Fig. 6) were readily detected with both the dissecting and compound microscopes; with 7.5% of crops sampled observed to have such infection (Appendix IV).

Fig. 5: Oogonium and oospore of *Aphanomyces euteiches* in diseased pea root tissue.

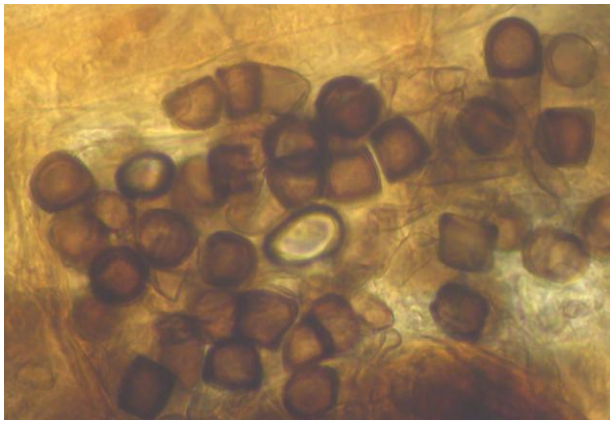


Fig. 6: Arthrospores of *Thielaviopsis basicola* in diseased pea root tissue.

Relationship between root rot fungi and previous crops

In the survey, where possible, information of previous crops for the last 5 years was obtained from each grower (data not presented, but see Appendix I form). It was surmised that legume crops / stands may be a major source of inoculum for *Fusarium* spp., especially *F. solani* (see Leslie and Summerall, 2006). Thus, when years since a legume (especially peas and clover / mixed pasture) was provided and correlated against the incidence of *F. solani* (no. out of approx. 40 plants sampled yielding this pathogen) than there is a negative relationship (r^2 = -0.33 – Fig. 6). When the general disease index (DI) is correlated with years since a legume, there is a less strong negative relationship (r^2 = -0.23 – Fig. 8).

From these relationships, it indicates that there is a weak, but definitely negative relationship between how long since legumes were the predominant plants in the paddock and the incidence of *F. solani*. In other words, the closer a pea crop being grown to another pea crop in a rotation sequence or since there was a legume in the paddock, then the higher chance there is of having a high incidence of *Fusarium* spp. causing root damage. However, the incidence and survival of this pathogen after a legume crop or stand depends on many factors including disease incidence in the previous plants, soil type and moisture content, types of break crops and the nature of the cultivation, especially as it relates to debris breakdown and pathogen carryover in the form of chlamydospores either in this debris or free in the soil.

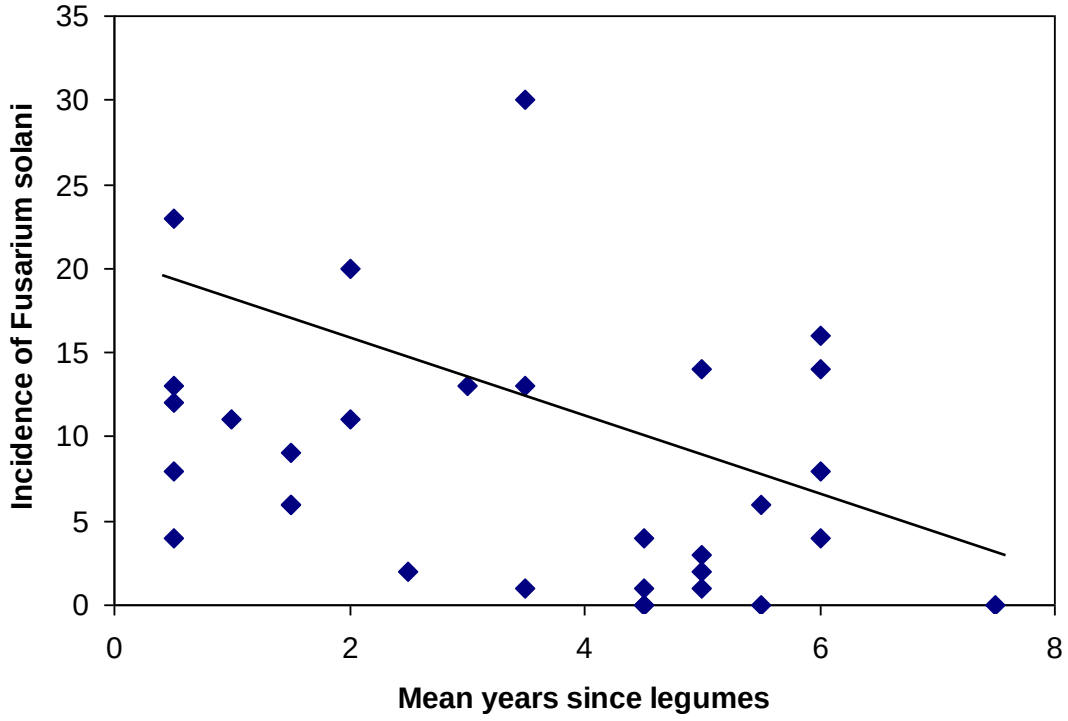


Figure 7: Relationship between the incidence of *F. solani* in paddocks and years since a legume crop / stand. R2 = - 0.33.

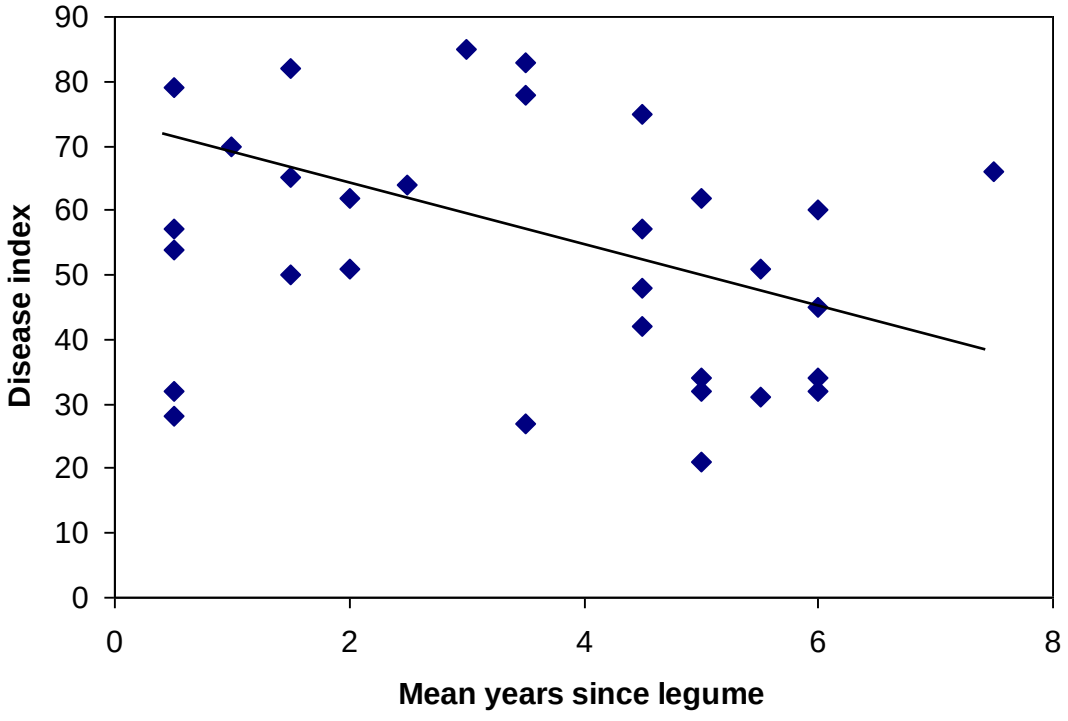


Figure 8: Relationship between pea root rot disease index and years since a legume crop / stand. R2 = - 0.23.

Relationship between root rot DI and yield

Some of the crops that were surveyed were either not harvested because of hail damage (2) or damage severely by this event or subjected to flooding. These factors also had a major impact on final disease. The mean yield of those crops for which this data was available was 3.9 t/ha.

When DI was correlated to final yield, there was no relationship detectible, with the correlation coefficient (r^2) being -0.17. Thus, even though there was a high level of disease in the crops surveyed other factors such as weather and fertility problems probably contributed to the low and varying yields experienced in these crops.

General conclusions:

- The root rot disease index (DI) ranged from 21 to 85, with a median of 51 out of 100. The use of this index as a measure of pea crop health may have application in crop sequence planning and in evaluating the efficacy of any control measures that may be applied to a crop or pea trial.

- *Fusarium solani* was the most abundant fungus isolated from the diseased tap roots, with 85% crops yielding this pathogen. The overall mean incidence was 23.6%, but the incidence within the infected crops being 28.6% (range 3 – 91% plants infected).

- The collar and root rot fungus – *Phoma medicaginis* var. *pinodella* was isolated from 53% of the crops surveys. The overall mean incidence was 6%, but the incidence within the infected crops was 21.4% (range 3 – 46.7% plants infected).

- The common root rot pathogen, *Aphanomyces euteiches* was not isolated from pea roots in this study with the laboratory methods utilised. However, it was detected, through the presence of oospores, in 22.5% (T = 9) of the crops surveyed. The growth of the common root colonising fungus *Chaetomium globosum* was considered a possible indicator of the presence of this pathogen using the isolation techniques utilised in this study.

- The black root rot pathogen *Thielaviopsis basicola* was seldom isolated into culture, but was detected in roots exhibiting typical symptoms in 7.5% (T = 3) of the crops surveyed. This disease is becoming a concern in pea and bean crops for processing (Heinz Watties / PLANTwise diagnostic records – *pers. comm*.). Further work on the damage, aetiology and control of this disease may be warranted.

- There was a negative correlation (r^2 = -0.33) between *F. solani* isolations and years since a legume crop (eg. peas and / or clover). There was also a negative relationship between the general DI of a crop and the years since a legume, but this was a less clear relationship with r^2 = -0.23. Further work could be carried out on the length of time between legumes in a paddock is optimum to minimise the damage from this disease complex involving *Fusarium* spp. that attack pea roots. Further, ways of controlling this disease complex, especially through the use of seed treatments may also be warranted.

- There was only a weak relationship between the general root rot DI and crop yield; r^2 = -0.17.

April 2009

References:

Greenwood, R., C. Aves and D. Catherwood 2008 (eds): Making Peas Pay. Pea Industry Development Group, Foundation for Arable Research. Lincoln, Canterbury: 68pp.

Leslie JF and BA Summerall 2006: The *Fusarium* Laboratory Manual. Blackwell Publishing Asia; 388pp.

APPENDIX I - Field sampling form

Root rot survey

Field sheet 2008 -

Agent	**CODE**
Grower	**Date**
Location	

Paddock size

Cultivar / type

Irrigated ☐ **Dryland** ☐

Sowing date

Total nodes (mean)
Nodes in pod
Nodes in flower

Soil moisture – dry to_____cm

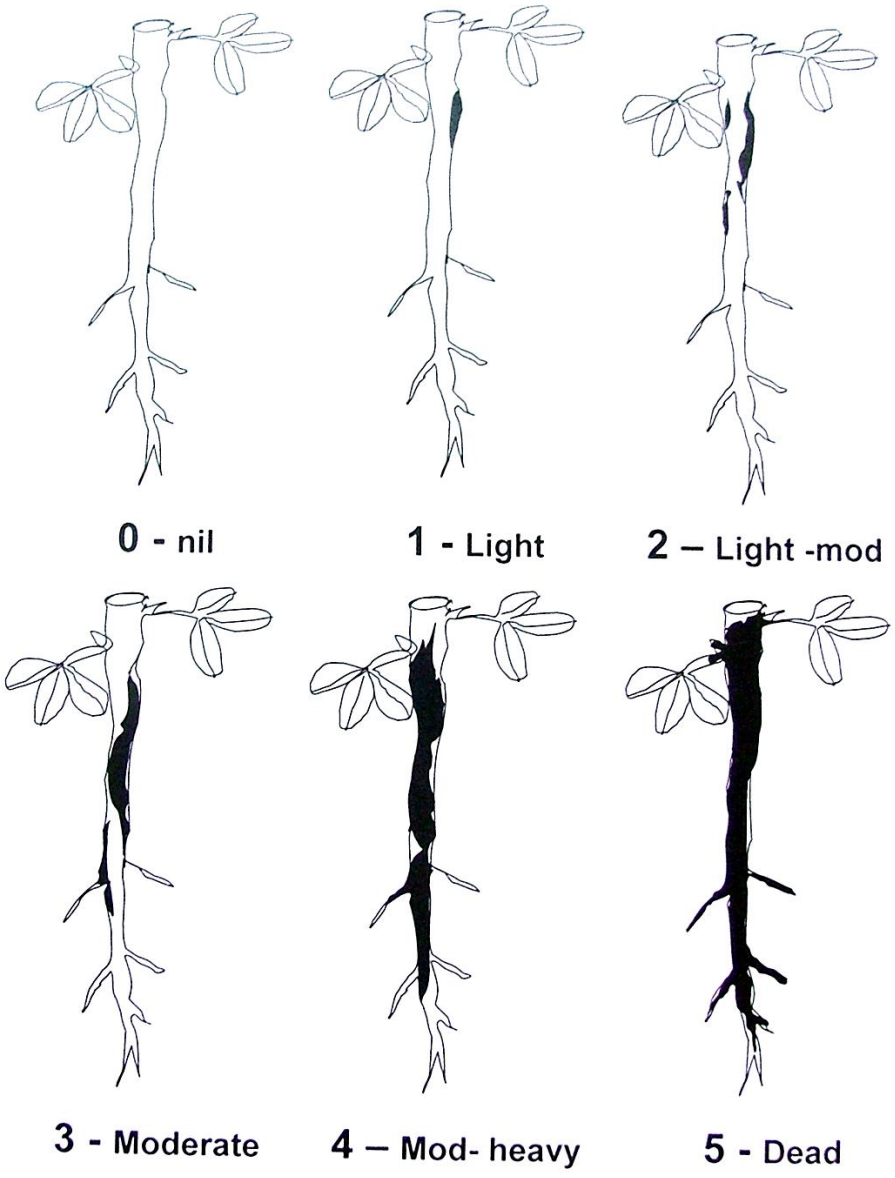
Previous cropping history
2002 – 3
2003 – 4
2004 – 5
2005 – 6
2007 – 8

Notes

APPENDIX II - Grower list information and sampling schedule (NA = not available).

Grower	Code	Location	Sowing date	Cv.	Sampled	Irr.	Nodes		
							Total	In pod	In flower
Butcher	P1	Lincoln	NA	NA	28-Nov	Y	9	0	0
John Morrish	P2	Lincoln	31-Oct	Ashton	8-Dec	Y	9	0	1
J. McCarttney	P3	Tai Tapu	11-Oct	Bingo	8-Dec	Y	13	0	2
John Court	P4	Lincoln	28-Oct	Ashton	8-Dec	y	8	0	1
R. Orchard	P5	Lincoln	31-Oct	Sonata	8-Dec	Y	8	0	0
A. Redmond	P6	Kirwee	13-Oct	Ashton	8-Dec	y	12	0	2
P. Cockburn	P7	Darfield	28-Oct	Ashton	8-Dec	y	8	0	1
N. Watson	P8	Lincoln	28-Oct	Ashton	8-Dec	Y	7	0	1
G. Wilson	P9	Kirwee	16-Oct	Ashton	8-Dec	Y	12	0	2
A.Ward-Smith	P10	Lincoln	28-Oct	Ultimo	8-Dec	Y	10	0	1
A &W Seaton	P11	Kirwee	3-Oct	Ashton	12-Dec	Y	12	1	0
A. Gillanders	P12	Darfiled	10-Sep	Onwards	12-Dec	N	16	3	0
A. Fisher	P13A	Ashburton	25-Sep	Marrowfat	15-Dec	N	20	5	1
A. Fisher	P13B	Ashburton	25-Sep	Marrowfat	15-Dec	N	17	4	1
D. Bennett	P14	Ashburton	NA	Cant. 100	15-Dec	Y	16	2	4
L. Sheed	P15	Geraldine	3-Oct	Novella	17-Dec	NA	18	4	1
Peter Maw	P16	Methven	21-Sep	Aragorn	17-Dec	N	NA		
Tony Corbett	P17	Ashburton	30-Oct	Marrowfat	17-Dec	Y	NA		
G. Foster	P18	Methven	26-Sep	Marrowfat	16-Dec	N	16	3	1
B. Perry	P19	Barhill	7-Nov	Ashton	16-Dec	Y	NA		
A. Molloy	P20	Pendarvis	26-Sep	Marrowfat	18-Dec	N	16	3	1
M. Sampson	P21	Darfield	30-Sep	Prelado	21-Dec	Y	15	3	0
G Lovett	P22	Ashburton	24-Sep	Aragorn	18-Dec	Y	17	4	3
Brosnahan	P23	Temuka	9-Oct	Biktop	21-Dec	Y	9	2	2
S. Bierema	P24	Methven	2-Oct	Marrowfat	21-Dec	N	15	3	1
A. West	P25	Ashburton	20-Oct	Marrowfat	18-Dec	Y	16	0	3
P. Wilkinson	P26	Somerton	7-Oct	Marrowfat	18-Dec	N	15	4	1
Arable Site	P27	Chertsey	NA	NA	23-Dec	Y	10	0	0
S. Skurr	P28	Darfield	20-Sep	Miami	22-Dec	N	16	3	1
A. Maw	P29	Methven	6-Nov	Nachos	21-Dec	N	13	0	0
R. Williams	P30	Waimate	16-Oct	Zhondas	23-Dec	N	15	0	1
G. Simmons	P31	St Andrews			23-Dec	NA	15	0	2
R. McIlraith	P32	St Andrews	25-Sep	Marrowfat	23-Dec	Y	20	5	2
J. Linton	P33	St Andrews	16-Oct	Dakota	23-Dec	N	12	3	0
J. Wright	P34	Methven	NA	NA	22-Dec	NA	8	0	0
D. Wright	P35	Methven	26 Sept	Marrowfat	22-Dec	N	8	0	0
H. Porter	P36	St Andews	NA	NA	23-Dec	NA	16	0	3
M. Porter	P37	St Andrews	14-Oct	Marvella 2	23-Dec	N	15	1	1
J. Hughes	P38	St Andrews	4-Oct	Ashton	23-Dec	N	15	0	0
J. Evans	P39	Dorie	NA	NA	16-Jan	Y	NA		

APPENDIX III – Root rot scoring diagram



Pea stem disease rating scale (0 – 5)

APPENDIX IV – Paddock disease assessment and isolation incidence and observations of disease/pathogens and yields

			% incidence					Observed -		
Grower	Code	DI	*F. sol*	*F. oxy*	*F.* spp.	*Phoma*	*Cheato*	*Aphano.*	*T. bas.*	Yield
Butcher	P1	23	7.3	4.9	0.0	14.6	0.0			1.7
John Morrish	P2	32	26.7	0.0	0.0	0.0	6.7		*	7.4
J. McCarttney	P3	78	90.9	15.2	0.0	12.1	0.0		*	NA
John Court	P4	27	2.9	14.7	0.0	20.6	0.0		*	5.0
R. Orchard	P5	57	71.9	3.1	0.0	0.0	6.3			7.1
A. Redmond	P6	34	43.8	3.1	0.0	0.0	0.0			9.6
P. Cockburn	P7	28	12.9	3.2	0.0	0.0	0.0			8.3
N. Watson	P8	32	25.0	9.4	0.0	9.4	6.3			6.6
G. Wilson	P9	60	45.2	12.9	0.0	0.0	0.0			4.0
A.Ward-Smith	P10	51	66.7	16.7	6.7	0.0	0.0			7.0
A &W Seaton	P11	54	40.0	10.0	23.3	46.7	0.0			3.6
A. Gillanders	P12	70	33.3	15.2	51.5	24.2	0.0			3.0
A. Fisher	P13A	65	17.6	11.8	32.4	0.0	17.6			3.8
A. Fisher	P13B	82	27.3	18.2	18.2	0.0	39.4			4.3
D. Bennett	P14	64	5.9	2.9	0.0	0.0	91.2			2.7
L. Sheed	P15	34	13.8	10.3	3.4	3.4	3.4			2.9
Peter Maw	P16	50	20.7	17.2	10.3	0.0	6.9			2.7
Tony Corbett	P17	32	6.5	6.5	3.2	0.0	16.1	*		3.0
G. Foster	P18	75	10.5	10.5	18.4	5.3	15.8	*		3.0
B. Perry	P19	42	2.9	8.8	5.9	2.9	20.6			7.4
A. Molloy	P20	79	43.3	10.0	23.3	20.0	0.0			3.8
M. Sampson	P21	62	32.4	26.5	14.7	17.6	0.0			NA
G Lovett	P22	57	0.0	0.0	0.0	0.0	54.1	*		2.5
Brosnahan	P23	62	3.0	21.2	0.0	3.0	57.6	*		3.3
S. Bierema	P24	59	6.1	12.1	57.6	0.0	12.1			1.5
A. West	P25	45	51.6	25.8	19.4	3.2	9.7	*		NA
P. Wilkinson	P26	83	43.3	33.3	36.7	10.0	0.0			3.5
Arable Site	P27	51	20.0	3.3	26.7	13.3	10.0			NA
S. Skurr	P28	85	43.3	0.0	10.0	3.3	3.3	*		NA
A. Maw	P29	48	0.0	10.0	0.0	0.0	43.3	*		NA
R. Williams	P30	61	35.7	3.6	7.1	7.1	42.9	*		2.4
G. Simmons	P31	21	10.3	0.0	0.0	0.0	3.4			0.4
R. McIlraith	P32	66	0.0	0.0	32.1	10.7	0.0	*		3.7
J. Linton	P33	68	43.8	0.0	21.9	0.0	6.3			1.8
J. Wright	P34	23	0.0	0.0	6.9	3.4	3.4			NA
D. Wright	P35	41	0.0	0.0	10.0	0.0	50.0			NA
H. Porter	P36	33	6.5	12.9	35.5	0.0	0.0			1.8
M. Porter	P37	68	31.3	12.5	28.1	3.1	3.1			1.1
J. Hughes	P38	31	0.0	0.0	13.3	0.0	25.0			0.7
J. Evans	P39	26	0.0	0.0	0.0	7.1	3.6			3.9
	Means	51.5	23.6	9.1	12.9	6.0	13.9			3.9