# Profitable break crops for management of root lesion nematodes and Rhizoctonia solani AG8

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## Key messages

- Canola is not a break crop in paddocks if root lesion nematodes (RLN) and Rhizoctonia solani are both present
- Weed free legumes, pulses and pastures are the best options where RLN and R. solani co-exist in a paddock
- Cereals substantially increase RLN and *R. solani* levels while canola will also increase RLN

## Aim

To investigate the most effective and profitable break crops in rotation with cereals (wheat) for growers with RLN and R. solani in the same paddock, a two-year (2018 & 2019) break-crop rotation trial was implemented in Dumbleyung and Grass Valley.

## Background to the activity

Root lesion nematodes (RLN) can cause significant yield loss to crop plants; up to 40% in wheat if beginning of season levels are > 25 RLN/g soil for some nematode species (Table 1). However, glasshouse trials in 2017, 2008 and 2007 and field surveys (2013-17) (Loi et al 2005; Vanstone et al. 2008, 2009;Collins et al. 2018) have demonstrated that some pasture and pulse species are resistant to moderately resistant to these plant parasitic nematodes. For instance, lupins have repeatedly demonstrated resistance to the major root lesion nematodes found in Western Australia, Pratylenchus neglectus and P. quasitereoides (Collins et al. 2018) and can offer growers options to manage RLN (Collins et al. 2017). To have full confidence, glasshouse results always need to be confirmed in local conditions the field. In 2017, two WA growers with root lesion nematode infestations offered their paddocks to investigate solutions to these soil disease issues. These paddocks also contained Rhizoctonia solani AG8, a fungal pathogen that causes root rot in a number of agronomically important crops grown in WA, and can cause significant yield losses; up to 50% in wheat if beginning of season levels are > 2.0 log pg DNA/g soil (Table 1). This paper presents the findings from the first year rotation (2018) with broad leaf crops and pastures.

## Methods

Field experiments were implemented at Dumbleyung and Grass Valley in 2018 using break crop options that are potentially suitable in these regions. The trial in Dumbleyung included canola (Bonito), faba beans (Samira), lupins (Jurien) and serradella (Cadiz) in four replicate plots. In Grass Valley, there were six replicate plots for each treatment and the crop types included canola (Stingray), chickpea (Striker), subclover (Dalkeith), field pea (Butler), lupin (Mandelup) and serradella (Eliza) as well as wheat (Calingiri and Mace) and barley (La Trobe). The crops were blocked into broad leaf and cereal crops for appropriate herbicide applications, and a fallow plot (repeated herbicide applications) was included in each block. Nitrogen fertilisers were only applied to the cereals and the canola.

Pre-season sampling included soil nutrient analyses and PREDICTA B testing of each plot (Pi) (Collins et al. 2018) to obtain the baseline soilborne pathogen and nematode pest levels at each site. At mid-season, PREDICTA B soil samples were collected from each plot at Dumbleyung. At the Grass Valley site, ten whole plants were extracted from each plot 10 weeks after sowing and the roots were washed free of soil. Nodulation was rated, where applicable, and plant root health scores taken. Nematodes were then extracted from the roots over a 4 day period using DPIRD's mister system and the nematodes manually counted. The roots of the broad leaf crops were also assessed for the presence of fungal pathogens by DPIRD's Diagnostic Laboratory Services (DDLS) as *R. solani* is not as easy to visually identify in these plant roots as it is in the cereals. Harvest measurements at both sites included biomass yields for the pastures, grain yields for the pulses and cereals, and PREDICTA B soil analyses (Pf). Gross margins were calculated based on trial inputs using 2017 pricing as this is more reflective of the long-term average.

As both of these trials were opportunistic, a one year preparation phase to stabilise the RLN and *R. solani* levels was not possible. Therefore, a regression analysis was performed on the log 10 initial (Pi) versus the log 10 final (Pf) PREDICTA B analyses of RLNs and *R. solani* from the start of the season to the end of the season (Table 4 & 5) in each individual plot (Genstat 18$^{th}$ Edition). This analysis accounts for the variation within each plot at seeding and predicts a result for the end of the season based on a calculated median point. ANOVA was performed on the Pi and Pf PREDICTA B results and nematode counts and multiple comparisons ($P<0.05$) were performed on the log(X+1) or log 10 transformed data using Fisher's LSD.

**Table 1.** Western region yield loss risk categories for root lesion nematodes (RLN); *Pratylenchus neglectus* and *P. quasitereoides*, and *Rhizoctonia solani* AG8 (McKay et al. 2018)

| **Level** | ***P. neglectus*** (RLN/g soil) | ***P. quasitereoides*** (RLN/g soil) | **Potential yield loss in intolerant wheat varieties (%) RLN** | ***R. solani*** **AG8** (log pg DNA/g soil) | **Potential yield loss in wheat (%)** ***R. solani*** |
|---|---|---|---|---|---|
| Below detection | <0.1 | <0.1 | 0 | <0.5 | <5 |
| Low | 0.1-<5 | 0.1-<9 | 0-5 | 0.5-<1.5 | 0-10 |
| Medium | 5-<25 | 9-<35 | 5-20 | 1.5-<2.0 | 5-25 |
| High | >25 | >35 | 20-40 | ≥2.0 | 20-50 |

## Results

At seeding the Dumbleyung site contained medium levels of *P. neglectus* (13 RLN/g soil) and medium levels of *R. solani* (1.9 log pg DNA/g soil) (Table 2), which can cause up to 20 or 25% yield loss, respectively. No *P. quasitereoides* was found at this site. Average pathogen/nematode levels were low across the Grass Valley site for *R. solani*, *P. neglectus*, and *P. quasitereoides*, but reached the medium risk level in a number of individual plots. The potential accumulative increases for yield loss risk where multiple soilborne issues are in the same paddock is not known.

**Table 2.** Pre-season soil sample results and growing season rainfall (April-November) for the Dumbleyung and Grass Valley sites in 2018. Nutrient analysis samples were bulked across the whole site, and the PREDICTA B tests for *Pratylenchus neglectus*, *P. quasitereoides*, and *R. solani* were averaged from each individual plot

| Site | pH | Total N (mg/kg) | P (mg/kg) | K (mg/kg) | Organic C (%) | Growing Season Rainfall (mm) | *P. neglectus* (no./g soil) | *P. quasitereo-ides* (no./g soil) | *R. solani* (log pgDNA/g soil) |
|---|---|---|---|---|---|---|---|---|---|
| Dumble-yung | 5.9 | 23 | 66 | 183 | 2.0 | 230.9 | 13 (19)* | 0 (0) | 1.9 (2.5) |
| Grass Valley | 5.1 | 24 | 39 | 47 | 1.2 | 346.1 | 2 (8) | 7 (21) | 1.2 (2.2) |

* Figures in brackets are highest recorded sample from each site

Due to the late season break in 2018, we were unable to apply a double knockdown herbicide prior to sowing at the Grass Valley site. As a result, the entire site was weedy after crop emergence and the post-emergent herbicide, Select® (broadleaf crops), had about 50% weed control and Decision® (cereal crops) had little effect. The site was hand weeded three times during the season commencing six weeks after crop emergence. Additionally, chickpea failed at this site and was removed from the final analyses.

### Mid-season results

Faba bean (6 RLN/g soil), lupin, (10 RLN/g soil) and serradella (10 RLN/g soil) already reduced numbers of the RLN *P. neglectus* by mid-season at Dumbleyung (using PREDICTA B). In contrast, canola (15 RLN/g soil) increased the RLN numbers.

At the Grass Valley site, instead of PREDICTA B, counts of root lesion nematodes from the roots of plants removed at 10 weeks after sowing were conducted. This allowed root health and rhizoctonia symptom assessments as well as RLN population counts. RLN visual counts include both adult stage, which can be differentiated into species, and juvenile stages (76% of total nematode count) where the species cannot be differentiated. Calingiri, a wheat variety susceptible to both RLN's present at the site, was included as a positive cereal control. In the adult counts, the lowest number of *P. neglectus* /g root was in lupins (51) and field peas (63) and the highest number was found in subclover (2196) (Table 3). For *P. quasitereoides*, the highest number / g root was found in canola (3483) and wheat (Calingri; 1425). The highest total RLN / g root was also found in these crops. *R. solani* was visually detected in all the cereals and detected by plating on agar in field peas and serradella. *Pythium* sp. (another fungal root pathogen) was also detected in lupin and subclover.

**Table 3.** Adult *Pratylenchus neglectus* and *P. quasitereoides,* RLN juveniles and total root lesion nematode (RLN) per gram of dry root from treatment crops collected 10 weeks after sowing at the Grass Valley trial in 2018. Different letters in each column indicates a significant difference at the $p<0.05$ level on log10 transformed data

| Crop/pasture | *P. neglectus* (g/root) | | *P. quasitereoides* (g/root) | | RLN Juveniles (g/root) | | Total RLN (g/root) | |
|---|---|---|---|---|---|---|---|---|
| Canola | 811 | bc | 3438 | b | 6720 | b | 10972 | bc |
| Field peas | 63 | a | 286 | a | 1275 | a | 1632 | a |
| Lupins | 51 | ab | 599 | a | 1128 | a | 1778 | a |
| Serradella | 735 | abc | 169 | a | 4800 | ab | 5704 | ab |
| Subclover | 2196 | d | 198 | a | 5714 | ab | 8111 | b |
| Wheat (Calingiri) | 486 | bc | 1425 | b | 14603 | c | 16521 | c |

## RLN and *R. solani* levels at the end of the season

### *Pratylenchus quasitereoides*

*P. quasitereoides* was only present at the Grass Valley site. Multiplication refers to the amount of population increase for the nematode pest or soilborne disease over the season. At this site, canola and all the cereals were susceptible to this nematode and the highest predicted multiplication of *P. quasitereoides* was found in barley and wheat (Calingiri) (Table 4). At the end of the season, the number of *P. quasitereoides* in the soil after barley (23 RLN/g soil), Calingiri wheat (21 RLN/g soil), canola (18 RLN/g soil) and Mace wheat (13 RLN/g soil) had increased the PREDICTA B risk for yield loss in a wheat crop from low at the beginning of season to a medium risk category by the end of season. All the legumes and the fallows reduced numbers of this plant parasitic nematode, and this reduction was significantly different from the cereals.

### *Pratylenchus neglectus*

Growing legumes reduced *P. neglectus* numbers at both sites, except for subclover at the Grass Valley site, where this RLN population was slightly increased (Table 4). The highest predicted multiplication at Grass Valley was under Calingiri wheat which increased the nematode numbers at harvest (Pf) to 8 RLN/g soil, causing a medium risk for yield loss in the following season if a susceptible wheat is grown. Mace wheat and barley also multiplied this RLN at Grass Valley. Canola multiplied *P. neglectus* at both sites and at Dumbleyung, increased levels of *P. neglectus* from medium to the high yield loss risk category.

### *Rhizoctonia solani* AG8

*R. solani* (log pgDNA/g soil) was significantly lower in subclover and lupin at harvest than barley and wheat at Grass Valley (Table 4). There was no significant difference in *R. solani* levels under any of the crops at harvest at Dumbleyung however, regression analysis predicts that the multiplication of *R. solani* will be lowest under serradella (Table 5).

**Table 4.** PREDICTA B results from the beginning of the season (Pi) and at harvest (Pf) for *Pratylenchus neglectus* (RLN/g soil), P. *quasitereoides* (RLN/g soil) and *Rhizoctonia solani* AG8 (log pgDNA/g soil) and the predicted multiplication (log scale) from the regression analysis

of the log 10 transformed PREDICTA B analyses at harvest (Pf) versus the log 10 transformed PREDICTA B at seeding (Pi) for the Grass Valley site. Back transformed means for the log predictions are in the brackets

| **Crop Type** | **Crop** | ***P. quasitereoides*** | | | ***P. neglectus*** | | | ***R. solani*** | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | |
| Pasture | Subclover | 6.3a | 1.0a | -0.06(1) | 2.3a | 3.1bc | 0.28(2) | 1.5a | 1.3ab | 0.81(7) |
| | Serradella | 6.6a | 1.6a | -0.05(1) | 2.2a | 2.1ab | -0.03(1) | 0.4a | 1.4abc | 1.37(23) |
| Pulse | Field peas | 7.5a | 4.4b | 0.54(3) | 1.7a | 0.7a | -0.38(0) | 0.8a | 1.9abc | 1.36(23) |
| | Lupins | 6.6a | 2.6ab | 0.35(2) | 1.8a | 1.3ab | 0.13(1) | 0.8a | 1.5a | 0.95(9) |
| Fallow | Fallow (Broadleaf) | 6.4a | 5.0b | 0.60(4) | 2.0a | 1.5ab | 0.02(1) | 1.2a | 1.8bc | 1.76(58) |
| | Fallow (Cereal) | 6.9a | 4.4b | 0.59(4) | 1.4a | 2.2ab | 0.23(2) | 1.7a | 1.9abc | 1.62(42) |
| Oilseed | Canola | 8.3a | 17.9c | 1.02(10) | 3.0a | 7.9cd | 0.53(3) | 1.4a | 2.0abc | 1.43(27) |
| Cereals | Barley | 5.8a | 22.8c | 1.38(24) | 2.1a | 4.3bcd | 0.42(3) | 1.0a | 2.3c | 2.06(116) |
| | Wheat (Calingiri) | 5.9a | 20.9c | 1.35(22) | 1.6a | 8.3d | 0.87(7) | 1.3a | 2.4c | 1.88(75) |
| | Wheat (Mace) | 7.3a | 13.0c | 1.04(11) | 2.4a | 4.4bcd | 0.35(2) | 0.8a | 2.3c | 1.96(92) |
| **Average 5% LSD** | | | | 0.29 | | | 0.59 | | | 0.90 |
| **% variance accounted** | | | | 83.7 | | | 55.7 | | | 19.8 |

**Table 5.** PREDICTA B results from the beginning of the season (Pi) and at harvest (Pf) for *Pratylenchus neglectus* (RLN/g soil), and *Rhizoctonia solani* AG8 (log pgDNA/g soil) and the predicted multiplication (log scale) from the regression analysis of the log 10 transformed PREDICTA B analyses at harvest (Pf) versus the log 10 transformed PREDICTA B at seeding (Pi) for the Dumbleyung site. Back transformed means for the log predictions are in the brackets

| | ***P. neglectus*** | | | ***R. solani*** | | |
|---|---|---|---|---|---|---|
| | **Pi** | **Pf** | **Log Prediction** | **Pi** | **Pf** | **Log Prediction** |
| Canola | 11.2a | 25.4c | 1.40(25) | 1.9a | 1.9a | 1.73(54) |
| Faba beans | 14.0a | 4.8a | 0.74(5) | 1.7a | 2.0a | 1.48(30) |
| Lupins | 11.2a | 3.8a | 0.72(5) | 1.8a | 1.5a | 1.12(13) |
| Serradella | 14.6a | 7.6b | 1.06(8) | 1.5a | 1.35a | 1.06(11) |
| **Average 5% LSD** | | | 0.13 | | | 1.25 |
| **% variance accounted** | | | 95.0 | | | -- |

## Grain or pasture biomass yields at harvest

**Table 6** Grain yields and gross margins for pulses, cereals and canola and biomass yields for pasture legumes at Dumbleyung and Grass Valley at the end of the season in 2018

| **Location** | **Crop** | **GIWA Crop Yield Estimates (t/ha) 2018** | **Average yield (biomass/grain) t/ha** | **Gross Margin (2017 pricing) ($/ha)[~]** |
|---|---|---|---|---|
| Grass Valley[#] | Barley (feed) | 3.6 | 1.6 (0.2)[@] | -59.98 |
| | Canola | 1.19 | 0.6 (0.06) | -34.71 |
| | Field pea[^] | 0.5 | 1.1 (0.05) | -$38.30 |
| | Lupin | 1.08 | 1.3 (0.2) | 17.53 |
| | Serradella | - | 3.8 (0.5) | - |
| | Subclover | - | 2.7 (0.2) | - |
| | Wheat (Calingiri) | 1.87 | 1.3 (0.1) | -81.40 |

| | | | | |
|---|---|---|---|---|
| | Wheat (Mace) | 1.87 | 0.7 (0.1) | -248.47 |
| Dumbleyung* | Canola | 1.14 | 1.4 (0.08) | 403.89 |
| | Faba Bean^ | 0.33 | 1.4 (0.1) | 64.44 |
| | Lupin | 1.13 | 3.1 (0.7) | 655.65 |
| | Serradella | - | 2.7 (0.2) | - |

# GIWA Crop Yield Estimates are based on Kwinana Port zone * GIWA Crop Yield Estimates are based on Albany Port zone ^ Field Pea and Faba Bean Crop Yield Estimates are compared with estimates for Pulses in each port zone @ Figures in brackets are standard error of the mean ~Pricing for gross margins for cereals and pulses courtesy of Profarmer Australia

Lupin (3.1 t/ha), faba bean (1.4 t/ha) and canola (1.4 t/ha) yielded above average for the Albany Port zone (GIWA 2019) at the Dumbleyung site (Table 6). At 2017 pricing, lupins, faba bean and canola offer substantial return on investment. Serradella yielded slightly less than expected at this site (2.7 t/ha), possibly due to herbicide drift during post-emergence spraying.

Yields of wheat and barley at the Grass Valley site were lower than average for the Kwinana Port zone (GIWA 2019), but Calingiri (1.3 t/ha) out yielded Mace (0.7 t/ha). Although field pea (1.1 t/ha) and lupin yields (1.3 t/ha) were above average, only lupin offered a return on investment at this site at 2017 pricing. Serradella biomass was higher at this site than at Dumbleyung, reaching an average of 3.8 t/ha. Subclover biomass yields were 2.7 t/ha.

## Conclusions

This investigation, which aims to find effective and profitable break crops in a rotation with cereals (wheat) to manage root lesion nematodes (RLN) and *Rhizoctonia solani* (AG8) in the same paddock, offers clear direction that where both RLN and *R. solani* are present in a paddock, grass-free legumes will offer the most beneficial break.

Serradella, lupin, subclover, field pea and the fallows (cereal and broadleaf) all reduced the total root lesion nematode (RLN) numbers at the Grass Valley site in 2018. Although subclover reduced *P. quasitereoides* numbers by over 80%, it multiplied *P. neglectus*, and this variety (Dalkeith) has been shown to be susceptible to moderately susceptible to *P. neglectus* in previous studies (Collins et al. 2014, 2017, 2018). At Dumbleyung, all the legumes reduced *P. neglectus*; faba bean and lupin reduced RLN numbers by up to 75%. Therefore, in a paddock with high numbers of *P. neglectus*, there are several legume break crop options available but some subclover varieties should be avoided. Serradella, field pea and lupin may be the best option where both RLN species occur together in a paddock.

In the current study, both varieties of canola (Stingray, Bonito) were very susceptible to the root lesion nematodes present in the paddocks and increased the RLN yield loss risk levels. In previous studies (Collins et al. 2017), canola has experienced yield losses (average 16%) in the presence of *P. neglectus* or *P. quasitereoides*, particularly when initial levels are medium to high. Canola yields at the Grass Valley site was lower than regional average and a number of issues including RLN and the late sowing date may have contributed to this.

The cereals at Grass Valley behaved as expected (Collins et al. 2017, 2018), increasing the RLN populations more than the legumes. The cereal yields were also lower than the regional average and may be the result of the disease, weed and pest pressures.  The impact of the weeds on RLN multiplication at Grass Valley is unknown, however, Vanstone and Russ (2001), were able to demonstrate that the two main weed species present in the paddock, *Lolium rigidum* (rye grass) and *Bromus diandrus* (brome grass) are poor hosts for *P. neglectus*. In 2019, the site will be oversown with wheat and yield losses due to high or low nematode presence will be further investigated. At the same time, economic analyses of a break crop- cereal rotation under disease pressure will be determined.

*Rhizoctonia solani* levels were lowest at the end of the growing season in most of the pastures and legume species grown in this experiment. This is in contrast to cereals where *R. solani* was increased to over 2.0 log pgDNA (high risk category) at harvest at Grass Valley. Although this fungus may impact lupin and subclover crops (Harries et al. 2015; Hüberli et al. 2016) this experiment suggests that the carryover of inoculum in the soil may not be as high as the highly susceptible cereal crops such as barley, which is known to be very susceptible to *R. solani*. Therefore, the results in the present study suggest that there may be some advantage to growing lupins, subclover or serradella in paddocks infested with *R. solani*. Future research could include several break crop options with multiple times of sowing under irrigation to tease out the most suitable environment for the pathogens and the crop species.

To reduce rhizoctonia disease effectively, the non-cereal crop rotation needs to be grass-free (Huberli et al. 2016). For example, canola has been shown to be a good break crop for *R. solani* in other studies (Hüberli et al. 2013 and 2016; Harries et al. 2015), however, at Grass Valley the levels were increased under canola. At Grass Valley early weed control was poor which may have caused the increases in the canola plots as well as the fallows and other non-cereal crops.

While the legumes, particularly lupins, serradella and to some extent field peas, and subclover can offer an effective break for RLN and *R. solani* occurring in the same paddock, the most profitable in the first year of this rotation trial was lupin.  However, there are no gross margins available for pastures at this stage, and the results for the second year with the wheat rotation may provide additional profitable solutions.

## References and useful links

Collins S, Wilkinson C, Huberli D (2018) Oats, pastures and other legumes – Are rotations offering breaks for root lesion nematodes or crown rot? GRDC Grains Research Updates, Perth, Western Australia, February 26-27.

Collins S, Wilkinson C, Kelly S, Hunter H, DeBrincat L (2014) Root lesion nematode has a picnic in 2013. The 2014 Agribusiness Crop Updates, Perth, Western Australia, February 24-25.

Collins S, Wilkinson C, Kelly S, Hunter H, DeBrincat L, Reeves K, and Chen K (2017) The invisible threat: Canola yield losses caused by root lesion nematodes in WA. 2017 Grains Research Updates, Perth, Western Australia, February 27-28.

GIWA (2019) GIWA Crop Report - February 2019. Grains Industry Association of Western Australia. http://www.giwa.org.au/2018

Harries M, Anderson G and Hüberli D (2015) Crop sequences in Western Australia: what are they and are they sustainable? Findings of a four-year survey. Crop and Pasture Science. 66: 634:647.

Hüberli D, Connor M, Miyan S, MacLeod W, Desbiolles J, Bogacki P, McKay A (2013) Integrated disease management options to control rhizoctonia bare-patch in cereals. The 2013 Agribusiness Crop Updates, Perth, Western Australia, February 25-26.

Hüberli D, Gupta V, McKay A, Ophel-Keller K, Willhelm N, Desbiolles J, Kirkegaard J, Heuston P (2016) Rhizoctonia Western Region Tips and Tactics. Grains Research and Development Corporation, Canberra, ACT.

Loi A, Howieson JG, Nutt BJ, Carr SJ (2005) A second generation of annual pasture legumes and their potential for inclusion in Mediterranean-type farming systems. Australian Journal of Experimental Agriculture 45: 289-299.

McKay A, Mayfield A, Davies L, Rowe S (2018) Broadacre Soilborne Disease Manual Version 10.1. The South Australian Research and Development Institute (SARDI) http://www.rootdisease.aweb.net.au

Vanstone V, Russ M (2001) Ability of weeds to host the root lesion nematodes *Pratylenchus neglectus* and *P. thornei* I. Grass weeds. Australiasian Plant Pathology. 30:245-250.

Vanstone V, Bhatti A, You M (2008) The role of pastures in hosting root lesion nematode (RLN, *Pratylenchus neglectus*). GRDC Agribusiness Crop Updates. Perth, Western Australia, Department of Agriculture and Food, Western Australia.

Vanstone V, Hunter H, Kelly S (2009) Screening cereal, canola and pasture cultivars for root lesion nematode (*Pratylenchus neglectus*). GRDC Agribusiness Crop Updates. Perth, Western Australia. p. 119-122.

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