Multi Species Demonstration Trials – Invertebrate Analysis

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The invertebrate component of the mixed species cover crop project was extremely successful with key learnings extended to growers through 9 talks, 5 publications, 1 podcast and 17 social media posts.

Proposed aims were modified in the initial stages of the project to account for grower and scientific requirements. The revised contract aimed to deliver 2 outcomes:

> **Outcome 1:** Provide scientific evidence of improved soil biodiversity and tools to growers to quantify this benefit.
>
> **Outcome 2:** Limit pesticide usage that reduces invertebrate biodiversity and ecosystem service benefits provided by cover cropping.

To achieve these the invertebrate component was divided into two activities:

1/ Soil macroinvertebrate monitoring and interpretation at a minimum 15 demonstration sites in the final cash crop.

2/ Integrated Pest Management support with five (5) key growers spread across five (5) grower group participants.

The second activity expanded beyond the demonstration sites to include larger fields. A follow on from the second activity was 3/extension and Integrated Pest Management support to all participants and a specific focus on 4/ IPM support for Ag KI.

Soil macroinvertebrate monitoring and interpretation at 17 demonstration site

Seventeen (17) demonstration sites were monitored for soil invertebrates in the final cash crop (2021), with some success in extracting soil macro fauna using 2ullgren funnels. Interpretation of results is awaiting data from the associated soil component as initially set out in the project. Sandy soils and dry spring conditions hindered to collection of intact soil cores, hence limited the number of macro-invertebrates extracted. Taking deeper cores, i.e. 10 – 20 cm did to some extent overcome this problem, however only 4 sites had data that enabled further analysis, hence interpretation. Overall, there the abundance varied greatly with high numbers of invertebrates due mainly to the presence of Hypogastrura. The diversity of springtails at all sites was low and infested with exotic species. The demonstration sites sampled were:

AgKI_Pontifex	SANTFA_Johnson
AgKI_Stanton	SANTFA_March
LEADA_Morgan	SANTFA_Patterson
LEADA_Phillis	SANTFA_Robinson
MFMG_Jaeschke	SANTFA_Scholz
MFMG_Langley	UNFS_Nottle
SAMDB_Candy	SFS_Rokewood (Carr)
SANTFA_Clarke	SFS_Bairnsdale (GAG)
SANTFA_Dolphin	

Pest and beneficial monitoring at 5 demonstration sites

Twenty (20) trials were carried out with 33 data sets generated. Some details and data from trials not presented here can be found in previous project reports or has been published as part of extension activities. Data was collected from the following demonstration sites:

Grey field slugs – *Deroceras reticulatum*

A demonstration site located at Rokewood, south west Victoria, was setup after a canola crop in 2019 using a fully replicated randomised block design with 12 m wide treatments. Tillage radish sown Jan 2020 as the single species and the mixed species summer cover crop composed of tillage radish, sorghum, millet, forage rape (SummerMax from AGF seeds Smeaton VIC). Faba beans (Zara) was the winter cash crop in 2020. Dec 2020 summer cover crop treatments were: Fallow – faba bean stubble Single – soybeans (*Glycine max*), Multi – soybeans, sunflowers, sorghum (*Sorghum bicolor*), forage rape (*Brassica*), leafy turnip (*Brassica rapa* var. *rapa*), tillage radish (*Raphanus sativus*), millet (*Panicum miliaceum*). Wheat (Revenue [*Triticum*]) was the cash crop sown in 2021. The rainfall was above average over the two summers cover crops were grown.

The number of slugs was quantified using surface refuge traps that consisted of 500 by 500 mm carpet mats in tillering wheat following a summer cover crop to test the grower's assumption more slugs would be carried over in the mixed species cover crop over summer. This was not the case with no differences between treatments detected (Fig 1). The wheat established successfully with final yield also showing no response to cover crop treatments: $F_{2,6} = 1.5$; $P = 0.296$.

A North American study evaluating slug damage to soybean crops following winter cover crops found when terminated two weeks before soybean planting, slug damage was greater in the single species rye cover crop compared to the 3-way mix and the no winter fallow. Planting green with termination a week after planting resulted in a significant, though small, reduction in slug damage, but not pest populations: seedling damage was not closely related to slug active density. Multi species cover crops probably do not make slug damage worse, despite increased numbers (https://extension.umd.edu/resource/slug-damage-soybeans-do-cover-crops-help-or-hurt accessed 10/4/2022).

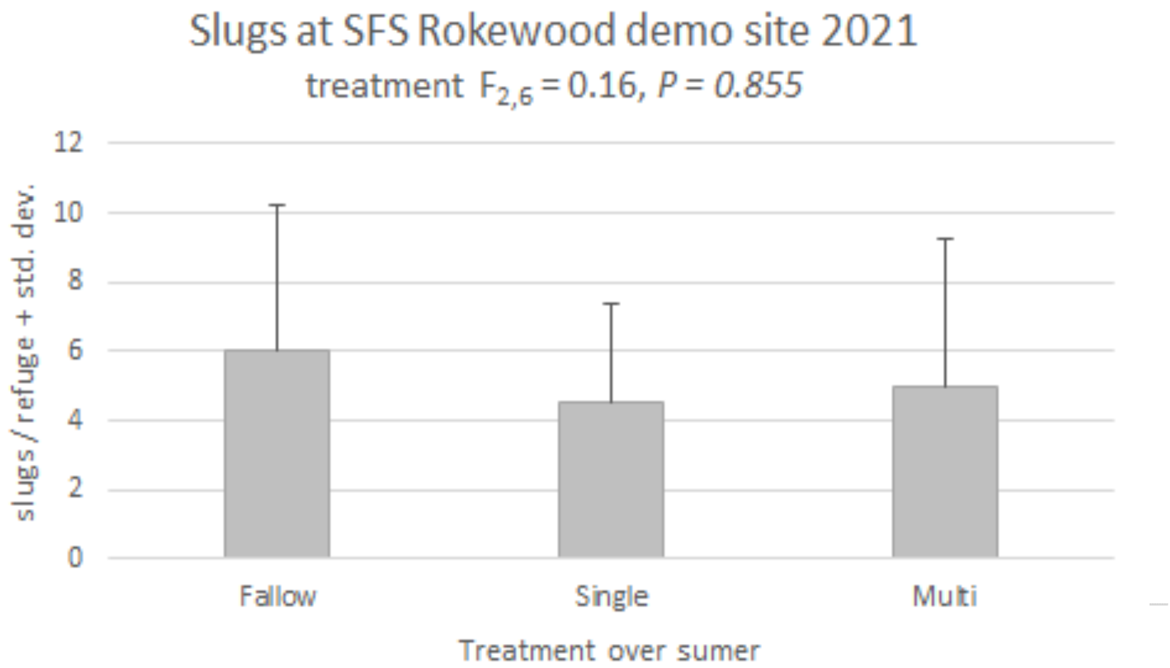

Figure 1. Mean number of grey field slugs under a refuge in wheat south west Victoria June 2021 in each of the three summer treatments: Fallow – faba bean stubble, Single – soybeans, Multi – soybeans, sunflowers, sorghum, forage rape, leafy turnip, tillage radish, millet.

Russian Wheat Aphid (RWA) - *Diuraphis noxia*
A demonstration site located at Ungarra, Eyre peninsula South Australia, was setup in 2019 using a repeated paired design. The 36 m by 300 m multi treatment was sown within a barley (Compass) crop. The 2019 winter cover mix was composed of vetch (*Faba sativa*), barley (*Hordeum vulgare*), winter canola, tillage radish, peas, and lentils (*Lens culinaris*). Treatments sown Jan 2020 were: Fallow – stripper stubble 24 m wide by two strips, single – Millet 12 m wide by two strips, Mutli -millet, tillage radish, winter canola, and sunflowers 36 m wide. Faba beans (Bendoc) was the winter cash crop in 2020, with the middle 26 m Multi treatment sown to faba beans and canola (Stingray). Dec 2020 summer cover crop treatments were: Fallow – bean stubble, Single – millet and sunflowers, Multi – Shirohie millet (*Echinochloa esculenta*), white millet (*E. frumentaceae*) sorghum, sunflowers, buckwheat (*Fagopyrum esculentum*), tillage radish, purple top turnip, mung beans (*Vigna radiata*), and 3 cultivars of clover (*Trifolium*). Wheat (Scepter) was the cash crop sown without insecticide dressings in May 2021.

Figure 2. Mean number of Russian Wheat Aphids (RWA)/ 100 wheat stems scored Oct 2021, Ungarra, South Australia in each of the three summer cover crop treatments: Fallow – faba bean stubble, Single – millet & sunflowers, Multi – millet, forage sorghum, sunflowers, buckwheat, tillage radish, purple top turnip, mung beans, and 3 cultivars of clover.

The number of RWA was quantified by randomly selecting 100 wheat heads/tillers per sampling point, 10 m by 10m, and counting the number of aphids on that “head”. Four sampling points were randomly chosen per treatment. No significant differences were detected between treatments (Fig. 2). These results are concordant with the literature: millet is a poor over summer host for RWA. Favoured summer grass hosts are: *Bromus* spp.; Barley grass, *Hordum* spp.; native grasses, *Enneapogon*, *Rytidosperma*, and *Austrostipa* spp. (13).

Italian round snails – *Theba pisana*

Experiment 1

At the same demonstration site, Ungarra, Eyre peninsula South Australia, round snails were also quantified by counting the number found on 100 randomly selected wheat head/tillers per 10 m by 10 m sampling point. No significant differences were detected between treatments, although numbers were numerically greater in the Multi species treatment (Fig. 3).

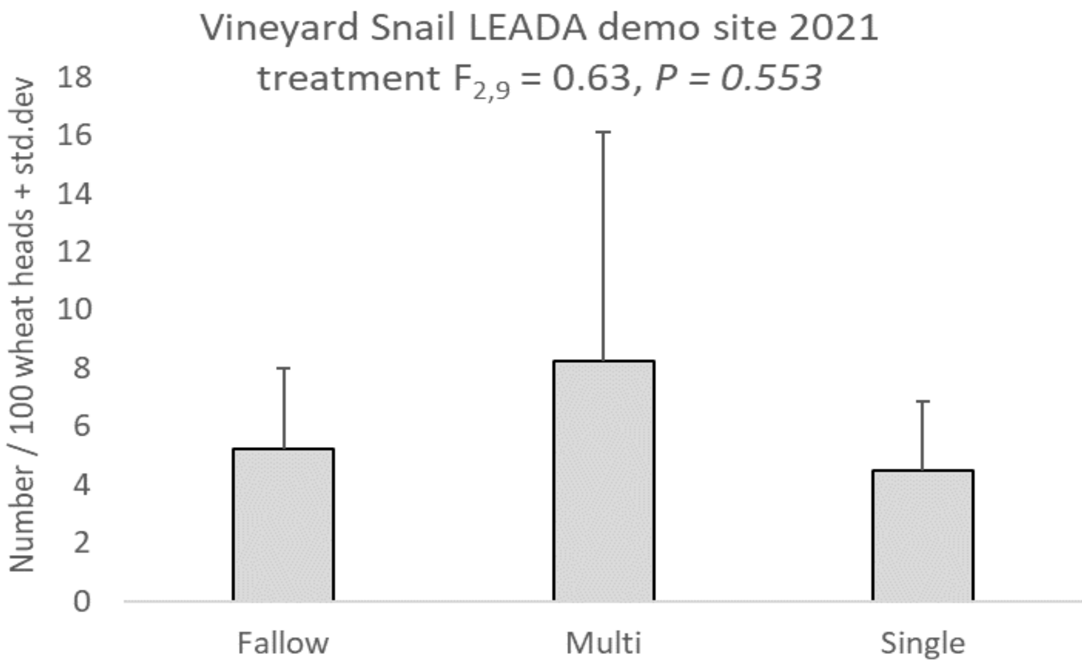

Figure 3. Mean number of Italian snails on 100 wheat stems scored Oct 2021, Ungarra, South Australia in each of the three summer cover crop treatments: Fallow – faba bean stubble, Single – millet & sunflowers, Multi – millet, forage sorghum, sunflowers, buckwheat, tillage radish, purple top turnip, mung beans, and 3 cultivars of clover.

Experiment 2

A replicated (n=6), randomised block experiment was established (June 2021) at Warooka, Yorke Peninsula, South Australia, to test what species (Fig. 4) may cause an increase in snail numbers when sown as part of a mixed cover crop. Species were sown individually and in combination, with a mixed species treatment either including or excluding brassicas. The full mix of species was lentils, phacelia (*Phacelia tanacetifolia*), saia oats (*Avena strigose*), marigold (*Tagetes patula*), linseed (*Linum usitatissimum*), turnip, tillage radish. Combinations of a legume (lentils) or wheat (Scepter) and various individual species were also included in the treatments.

Snail numbers were scored by counting the number per 1.8 by 10m plot. The results indicate what species are included in cover crops can have a significant effect on snail numbers ($F_{19,95} = 6.2$; $P < 0.001$), with the most snails observed where tillage radish was grown, either as a single species or as part of a mix. Snail population increase due to the growing of brassica species was expected because snail population increases are often observed in canola crops. In areas where snails are present, growing a winter cover crop that contains brassicas, in particular tillage radish and/or canola, should be avoided, else additional management of snails will be required.

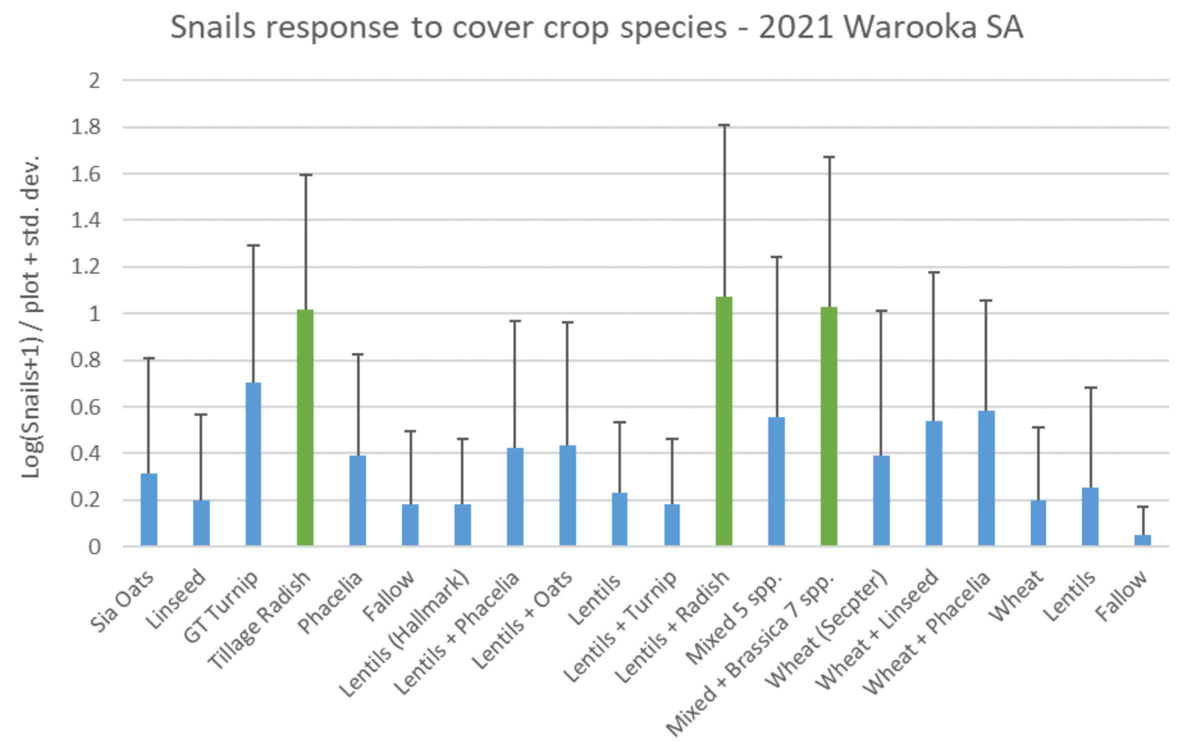

Figure 4. Mean number of Italian snails per plot scored Oct 2021, Warooka, South Australia to test what cover crop species increased populations. Green bars represent treatments where tillage radish was included in the mix. Mixed species treatment either including (Mixed + Brassica 7 spp.) or excluding brassicas (Mixed 5 spp.) and was made up of the following species: lentils, phacelia, sia oats, marigold, linseed, turnip, tillage radish. The marigold failed to establish.

Moths – Various species

Due to scale limitations replicated experiments could not be conducted to test the likelihood of increased moth numbers in mixed species crops. Paired paddock observations from seven sites using pheromone traps found varied response with either no difference in moth numbers, or significantly less in the mixed species crops (Table 1). Some results were based on real time data generated by smart traps (DTN.com) that record images of moths daily, hence were not spatially replicated (n=1, Table 1). These traps often failed to upload data resulting in missing data that could not be analysed. Results did not investigate actual damage caused so should be treated with some caution. The in season monitoring of moth flights was not followed up with monitoring in the following season, nor was the level of natural enemy function assessed. Further research is required to tease apart the often interactions occurring when plant diversity is increased.

Table 1. Experiments testing the influence of crop diversity on moth numbers, assessed using pheromone traps. A positive mean difference indicates more moths were recorded in the multispecies crop, hence a negative value indicates lower numbers. Significance of observed differences (bold) were based on T-tests ($P < 0.05$). The frequency of sampling and number of replicates (n) is indicated in Location column. NA indicates not analysed due to missing data due to technological issues.

Location	**Pest**	**Single**	**Multi**	**Date**	**Sig.**	**Mean/day difference**
Bairnsdale VIC daily n=1	Native Budworm	forage rape	Various plots of mixed species	7 Mar – 8 Apr 2020	NA	-0.4
Yorke Peninsula SA weekly n =3	Native Budworm	faba beans	faba beans, lentils – alleys	Aug - Nov 20	*0.599*	0.27
	Etiella	lentils		Sep – Nov 20	*0.769*	0.11
Mid North SA weekly n =3	Etiella	lentils	lentils, oats, linseed, barley, tillage radish	Sep – Oct 20	*0.307*	-0.53
Kangaroo Is. SA monthly n = 3	Diamond Back Moth	canola	canola, faba beans – mixed rows	9-Sep 20	*0.267*	7.1
				28-Sep 20	*0.183*	2.1
				26-Oct 20	*0.841*	-0.4
				29-Nov 20	*0.205*	3.2
Kangaroo Is. SA daily n=1	Diamond Back Moth	volunteer canola	tillage radish, fodder rape, sorghum, shirohie millet, french white millet, kikuyu & sunflowers	Mar – May 21	<0.001	**-1.2**
Minnipa SA varied n = 3	Native Budworm	peas	peas, canola	27-Aug-21	*0.356*	-1.67
				28-Sep-21	*0.272*	-0.15
				15-Oct-21	*0.037*	**-1.04**
Yorke Peninsula SA monthly n=1	Native Budworm	faba beans	tillage radish, mustard, canola, barley, vetch, medic, phacelia	5-Oct-21	NA	-0.1
				15-Nov-21	NA	0.2

Rokewood VIC – Carr 2021 slugs

Eyre Peninsula SA – Phillis 2021 Russian Wheat Aphid and Snails

This component was expanded to include eight (8) additional paired paddocks (sites) in addition to the demonstration sites and one full replicated randomised block trail conducted to test snails' response to the different species.

Bairnsdale VIC – Gippsland Agricultural Group Native Budworm
Kangaroo Island SA – Pontifex 2020 Diamond Back Moth

Kangaroo Island SA – Jenny Stanton 2021 Diamond Back Moth

Mid North SA - Tom Robinson 2020 Etiella

Curramulka SA – Hickmann 2020 Native budworm

Curramulka SA – Hickmann 2020 Etiella

Curramulka SA – Hickmann 2021 Native budworm

Minnipa SA – Scholtz 2021 Native budworm

Warooka SA – Hayes 2021 replicated trial snails

Mid North SA - Tom Robinson

Monitoring was shifted to larger paired paddock where a winter mixed species cover crop was being grown.

2021: Monitoring of pests in winter mixed species cover crop, which included a cereal species of oats, adjacent to a wheat crop under sown with lentils indicated the presence of armyworm, with significant less common armyworm larvae (2-3 instar) observed: mixed species, 1-2 / 5 by 10 sweeps (50 in total) vs cash crop, 10-15 / 10 sweeps. Further investigation indicated sap brix levels in the cereal grown with a diversity of species with significantly greater than that of adjacent cereal being eaten by armyworm larvae.

A separate report is being prepared for SANTFA and Mid North group on armyworm that will contain information (above) from this project.

2020: Winter and spring surveys (pitfall trapping) found lower relative abundance and species numbers of ground dwelling invertebrates in the cover crop paddock (Table below). However, a greater number and diversity of ants in the cover crop paddock in spring could provide greater soil porosity.

-	Winter	-	Spring	-
-	Cover crop	Lentils	Cover crop	Lentils
abundance	83	161	571	1,947
species	3	6	22	28

2020: A comparison of ground active invertebrate communities between crimped and herbicide (Spray Seed) termination treatments found lower abundance in the area sprayed compared to the crimped plots, but a similar number of species (see Table below). More Portuguese millipedes were observed in the sprayed treatment, concordant with literature suggesting this species increases in abundance where system function is disrupted by pesticide use.

	Crimped	Sprayed
abundance	2,692	1,182
species	15	17

2019: Four (4) surveys (Feb, May, Jun & Jul) using soil cores and/or pitfalls were completed with limited insect activity due to dry conditions. Four "sites" included two from one field: one side with cover crop 2018 vs Canola 2018 all sown to wheat 2019; and two from demonstration field with sparse cover crop summer 2018/2019 sown on canola 2018 with two treatments 2019 being treated wheat vs untreated wheat seed. The aim was to test the effects of seed treatments on crop establishment and is being supported by lab trials investigating the effects of fungicide and insecticide treatments on seed establishments. See "Warm and cool season mixed cover cropping for sustainable farming systems in south eastern Australia – seed treatment information sheet" for details.

East Gippsland – Gippsland Agricultural Group

2021: Pitfall traps set in late Jan did detect differences between treatments with an increased abundance of ants in the least diverse fallow treatment, which is concordant with the literature. One species of springtail (Hypogastruridae) was also observed to be in greater abundance in the fallow treatment.

2020: Cabbage aphid migrating into the demonstration site from adjacent forage rape was quantified using Yellow sticky traps with the least wing aphids found in the mixed species treatment and the most in the "fallow" treatment, which was heavily infested with weeds. However, the small plot size limits conclusions.

2020 Ground Cover Dry Times Demonstration: In a separate demonstration 8 summer fodder mixes were sown next to a monoculture of forage rape. Soil cores were taken, and soil macro fauna extracted using tullgren funnels. No differences in abundance or species number were observed between treatments: Note the high abundance in the AGF forage mix was due to collecting ants.

Treatment	Abundance	Species
Mix	394	10
Rape	106	13
SPS Econ	187	13
Pea Oat	499	14
Shed Special	727	14
SPS Bio Blend	369	17
Chicory treated	334	20
SPS Forage	167	13
AGF Forage	1268	14
AGF Bio Max	247	16

2019: Nearby paddocks sown to wheat following 2018/2019 mixed species cover crop were surveyed (Jul) using pitfalls and soil cores. A new endemic genus of springtail was recorded from this paddock.

Conclusions

Outcome 1:

Results from soil cores in 2019 suggested quite different communities in SA and Vic compared to previous studies from NSW. For example, Symphyla, Pauropoda, Centipedes, Oligochaeta, native earwigs were common. A novel native endemic genus was discovered at Bairnsdale Victoria with specimens provided to overseas taxonomists to describe. Sowing and what was applied to the seed did influence the communities extracted from the soil cores, hence sampling in the final year was delayed until the ground was moist well after sowing: i.e. Aug / Sep sampling.

This delay due to pesticide usage resulted on surveys at the demonstration sites being compromised by a dry spring. Overall, there was a low abundance and diversity of springtails with all sites infested with exotic species.

Unspent funds will be used to finalise interpretation of soil macroinvertebrate data linking it with soil analyses. The aim is to publish the combined data as a peer reviewed article. Findings will be communicated to individual collaborating growers.

Although springtails have been proposed as a bioindicator for soil function and health, there was no discernable detectable response in community diversity to multiple species cover crop treatments. Response may have been masked by pesticide usage, suggested by the lack of native fauna; limited time for communities to respond to the management changes imposed; plot size being sub optimal limiting methods used; and/or surveys not being repeated. Taking soil cores, extracting macro invertebrates and determining species may not be a good tool for land managers to quantify improved soil biodiversity.

Outcome 2:

The flexibility provided by the management team to deliver what individual growers requested from larger paddocks beyond the initial collaborators facilitated the development of new knowledge, as delivered through a crop science society article. The key learnings from testing the link of increased crop diversity to pest suppression or reduced risk of pest outbreaks were:

- 2021 - 20 demonstration sites 3 had pest threats: 2 no difference in pest numbers, nor damage.
- Red Legged Earth Mite may be prevalent following multi species crops. However, the opposite was also observed, with no damage recorded in clover being established after a mixed species summer cover crop when compared to a traditional summer fallow to control weeds.
- A diversity of results highlighted the complex responses invertebrates demonstrate to crop diversity.
- The growing of summer cover crops did not create a “green bridge” as some have suggested: in some cases, summer cover crops provide resources for generalist predators that regulate invertebrate communities including pest populations.
- Insecticide usage by collaborating growers was generally low, as quantified by a disruption index. However, due to no market tolerance to damage in pulses fungicide and insecticides were common in this part of the rotation.

This is one of very few studies that links increased crop diversity to pest suppression or reduced risk of pest outbreaks, however yield was rarely quantified and where it was no differences were detected.

The provision of increased crop diversity must be quantified to link the perceived benefits of pest control to both economic and environmental outcomes. The aim is to publish these data as a peer reviewed article.

Final Conclusions

The inclusion of polycultures, such as inter-cropping or cover cropping, may have multiple benefits under Australian conditions where fields are large, and there is a need to diversify crop cultivars, type and flowering time to minimise the risk of crop failures in dryland systems. What was clear is management needs to understand the context in which it is being applied and its tolerance to sporadic risk if pest threats are less where plant diversity is increased on farm.

Project Proponents

Project Funders