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Project Code	
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FINAL REPORT 2020

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PROJECT CODE	UA118
PROJECT TITLE	
Herbicide residue effects on soil microbial communities in alkaline soils	

PROJECT DURATION			
Project start date	**1/07/2018**		
Project end date	**30/06/2020**		
SAGIT Funding Request	**2020/21**	**2021/22**	**2022/23**
	$	**$**	**$**

PROJECT SUPERVISOR CONTACT DETAILS		
Title:	**First Name:**	**Surname:**
Dr	Matthew	Denton
Organisation:	The University of Adelaide	
Mailing address:	School of Agriculture, Food and Wine, PMB1 Glen Osmond SA 5064	
Telephone:		**Email:**
Mobile:		

ADMINISTRATION CONTACT DETAILS		
Title:	**First Name:**	**Surname:**
Ms	Chelsea	Dubois
Organisation:	The University of Adelaide	
Mailing address:		
Telephone:		**Email:**
Mobile:		

PROJECT REPORT:

Executive Summary

Mallee environments dominate the low rainfall farming zone of south-eastern Australia, and numerous soil constraints restrict crop productivity. Imidazolinone herbicides are increasingly applied by growers with the aim of providing long-term weed control but result in unwanted herbicide persistence that is affecting broadleaf crops and possibly key soil microbes. This project sought to evaluate the impact that imidazolinone herbicides (Intervix) have on soil microbes of alkaline soils in SA Mallee environments.

- Degradation of imidazolinone herbicides was greatly reduced in Mallee environments and was made worse by dry hot conditions in summer and autumn. Intervix applied to sandy alkaline soils persisted for more than 24 months, with imazapyr being more persistent than imazamox.
- Kord CL wheat and Hurricane XT lentils proved to be tolerant to Intervix residues, however, the non-Clearfield variety Gladius and the conventional lentil variety, Nipper, were sensitive to residues above 10% of the recommended field rate.
- The mean relative abundance of beneficial *Bacillus* and *Rhizobium* was reduced at high residue rates for both Gladius and Kord CL at both times of sampling.
- A trend of an increase in *Streptomyces* with increasing Intervix residues was observed.
- Of the two ingredients that make up Intervix, imazapyr residues in the lentil rhizosphere significantly reduced bacterial abundance and richness during both times of sampling. However, the relative abundance of *Rhizobium* was not affected by the presence of either imazamox or imazapyr residues at the recommended field rate.

Project objectives

This project sought to evaluate the impact that imidazolinone (imi) herbicides have on soil microbial populations of alkaline soils in SA Mallee environments. Intervix (imazamox/imazapyr) provides important chemical weed control for weeds like Brome grass (*Bromus diandrus and B. rigidus*) and barley grass (*Hordeum leporinum*) but residues can remain in soils for longer than intended, particularly in low rainfall, alkaline soils with low organic matter. These residues are responsible for poor growth of broadleaf crops and the impact of residues on reducing crop yield is not always recognized. Soil microbes have a role in maintaining nitrogen fixation of crops in these soils and potentially breaking down herbicide residues. The role of major microbes, (including beneficial bacteria such as N fixing rhizobia, phosphate solubilizing bacteria, and plant growth promoting rhizobacteria such as *Bacillus* and *Pseudomonas*, and functional fungi such as *mycorrhizal fungi* and plant pathogens) are predicted to be important to farming systems productivity.

Specific Aims:

•Determine the impact that imidazolinone residues have on soil microbial community diversity in low rainfall, alkaline soils,

•Identify the implications of imidazolinone residues on organisms of known economic significance such as nitrogen fixing rhizobia and phosphate solubilizing bacteria for crop production in these systems, and

•Determine the implications of herbicide residues are upon broadleaf crops and pasture growth and yields, with a particular focus on legume crops

Overall Performance

A broad objective of the project was to determine the impact of imi herbicide residues on soil microbes in alkaline soils. This was achieved by focusing on the impact that herbicide residues have on predominant plant growth-promoting bacteria (*rhizobia, bacillus, streptomyces, pseudomonas*), that have potential to enhance crop yield.

Difficulties: The impact of residues on soil bacteria were intended to be evaluated through a field and glasshouse pot experiment. However, due to budget constraints, only samples from the glasshouse pot experiment were submitted for DNA extraction and sequencing.

Project personnel:

University of Adelaide – Assoc Prof Matthew Denton, Dr Yi Zhou, Dr Thang Viet Lai

SARDI - Brian Dzoma, Dr Nigel Wilhelm, Kym Zeppel

KEY PERFORMANCE INDICATORS (KPI)		
KPI	**Achieved**	**If not achieved, please state reason.**
Establishment of pot trials with imidazolinone herbicide and pre-treatments for 2019 field trials	Yes ☒ No ☐	
Sampling of glasshouse pot experiment soils and processing for microbial sequencing, and crop metrics, including growth and yield.	Yes ☒ No ☐	
Annual report produced for SAGIT	Yes ☒ No ☐	
Implementation of field trials assessing the impacts of Intervix on crop performance	Yes ☒ No ☐	
Sampling of soils from field trials and processing for microbial sequencing, and crop metrics, including growth and yield.	Yes ☐ No ☒	Sequencing was not completed due to budget constraints. A request for additional budget was unsuccessful.
Annual report produced for SAGIT	Yes ☒ No ☐	
Final report produced	Yes ☒ No ☐	
	Yes ☐ No ☐	

TECHNICAL INFORMATION

Herbicide residues

Soil-applied imidazolinone herbicides should persist sufficiently long enough to provide an acceptable period of weed control during crop growth but not so long that soil residues after crop harvest affect the performance of subsequent crops. In alkaline soils, imidazolinone herbicide breakdown is primarily mediated by microbial degradation that may degrade the herbicide within one or two months under optimum conditions. However, dry hot conditions in summer and autumn, in moisture limited Mallee environments, degradation of these herbicides is greatly reduced, particularly when soils are alkaline and low in organic carbon (Beyer et al., 1988; Shaner and Connor, 1991; Cobb, 1992; Kah et al., 2007).

Our data show that from the Intervix (imazamox/imazapyr) applied in 2018 at the 1x recommended field rate (RFR), 18% imazamox and 31% imazapyr was detected at Peebinga, and 18% imazamox and 28% imazapyr was detected at Waikerie, two seasons later after the initial application (**Fig.1**). Our data also show that the percentage remaining imazapyr was higher at both sites, than imazamox. Imazamox has been reported as the least persistent imdazolinone with a half-life of 20 to 30 days (Vencill, 2002 and Quivet et al., 2004), while imazapyr was considered a more persistent herbicide with a half-life ranging from 90 to 730 days depending on soil type, environmental conditions, and the rate of application, Mangels (1991). It should, however, be noted that the dry hot conditions experienced in summer and autumn (2018/2019 and 2019/2020) in the Mallee did not promote degradation of these herbicides, and hence allowed their persistence.

Effect of Intervix soil residues on crop growth

Even though imidazolinone herbicides are effective on a broad range of weeds, low soil residue concentrations can have a sub-clinical impacts on crop productivity (Hollaway et al., 2005). From the pot experiments established, neither wheat nor lentils achieved optimum growth under the glasshouse conditions due to sowing completed after the ideal sowing window. However, significant responses of the different crop species to the different herbicide residue levels were recorded.

- Intervix did not affect shoot dry matter (DM) of Kord CL wheat which is tolerant to imi herbicides. However, shoot DM of Gladius was affected by Intervix residues at and above 0.5x of the RFR (**Fig.2**).
- Intervix residues did not affect shoot DM of Hurricane XT, a high yielding small red lentil variety with improved tolerance to residual levels of sulfonylurea and imi herbicides. However, nipper lentil shoot DM was significantly ($P<0.009$) affected at residues above 0.1x (**Fig.3**).

- Raptor (imazamox) and Warrant (imazapyr) did not affect early shoot DM of lentils at the RFR, however, at GS V1 (1st node) imazamox at the RFR significantly ($p<0.05$) reduced shoot DM of Nipper lentils by 67% when compared to the untreated control (**Table 1**).

Two replicated field trials were established in May 2018 at 2 contrasting sites in the northern Mallee (Waikerie) and southern Mallee (Peebinga). Herbicide treatments were imposed on 26 July 2018 by spraying different herbicide concentrations on plots sown to Scope barley at 3bar pressure, 4.5km/hr speed and 200l/ha water rate. This approach allowed 10 months for treatments to settle and move into the soil profile to simulate herbicide carryover. Prior to sowing in 2019, 0 – 10cm soil cores were sampled from each plot to determine the level of herbicides still present. Samples were prepared and analysed with liquid chromatography and mass spectrometry at the CSIRO lab (Waite). Trials were then sown on 21 May 2019 to wheat (Gladius and Kord CL) and lentils (Hurricane XT and Nipper).
From the two replicated field trials conducted

- Intervix residues did not affect crop establishment, early and late shoot DM, or grain yield of Kord CL Plus wheat at both sites (Table 2).
- Crop establishment of Gladius wheat was not affected by residues present at either site. However, there was a reduction in flowering shoot DM at 2x RFR residues, and grain yield at residues above 0.5x RFR at both sites
- Intervix residues did not affect crop establishment, early and late shoot DM, nodulation or grain yield of PBA Hurricane XT lentils at both sites (Table 3).
- Crop establishment and nodulation of Nipper lentils was not affected by Intervix residues. However, at Peebinga, there was a reduction in flowering shoot DM at 0.5x of RFR, and grain yield by 36% at Peebinga at the recommended field rate.

Effect on soil bacteria
Several beneficial free-living rhizobacteria have been identified to be plant growth-promoting rhizobacteria (PGPR), including, but not limited to, *Acinetobacter, Acetobacter, Alcaligenes, Arthrobacter, Azotobacter, Azospirillum, Bacillus, Burkholderia, Beijerinckia, Enterobacter, Flavobacterium, Methylobacterium, Pseudomonas, Rhizobium, Paenibacillus* and *Pantoea* (Kloepper at el., 1980; Chanway et al., 1988; Vessey, 2003; Sharma et al., 2010). Our experiment has focused on bacteria of importance to broadacre agricultural systems, like rhizobia, *Bacillus* and *Streptomyces*. Rhizobia is a group of biological nitrogen fixers that are of great importance as a primary source of N for agriculture. Phosphorus-solubilizing *Bacillus* strains have been reported to increase the plant biomass and yield of wheat through increased uptake of nutrients (Chen et al., 2006; Turan et al., 2012). *Streptomyces*, the most abundant genus, is associated with increased plant growth, and has can significantly improve the Fe, Mn and P content of wheat plants when compared with an uninoculated control (Sadeghi et al., 2012; Turan et al., 2012).

Effect on soil bacteria in a wheat production system
The objective of this experiment was to investigate whether Intervix soil residues affect wheat crop growth and soil bacteria. This was achieved by conducting a dose response pot experiment with two “times of sampling” (TOS), under glasshouse conditions at the Waite Research Precinct, comparing a tolerant Clearfield wheat variety (Kord CL) and a susceptible variety (Gladius). Each experiment had 2 times of sampling for crop growth and soil microbiology, to differentiate the effects of the residues at an early growth stage (GS). For wheat, sampling occurred at the 4 leaf stage (GS14) and at stem elongation (GS31), and for lentils at the first multifoliate leaf unfolding at the first node (GS V1) and at first bloom (GS R1). Six herbicide residues levels were simulated (0x, 0.001x, 0.01x, 0.1x, 0.5x, 1x, where x = recommended field rate of 500ml/ha Intervix).

- The results show that α-diversity (Shannon index) of bacterial communities was altered by the presence of Intervix soil residues (**Fig.4**). For TOS1, there was a temporary increase in bacteria species richness and diversity at the lowest residue level (0.001x) in the Gladius wheat rhizosphere. However, at TOS2, there was a significant reduction in the Shannon index at the 0.1x and 1x residue rate (1x) for Gladius wheat. The α-diversity was not altered in the Kord CL wheat rhizosphere in TOS1, however there was a significant reduction in TOS2 at the 0.01x residue rate.
- The Bray–Curtis distance index (β-diversity) varies between 0 and 1, where a distance of 0 means the treatments have the same composition as the control, and 1 means they do not share any species. Our results indicate that variety, herbicide residue rate and time of sampling all influenced the composition of bacteria in the presence of Intervix residues.

- Herbicide residue rate and TOS did not alter bacteria composition in the bulk soil, however, the Bray-Curtis distance index was higher in the highest Intervix residue treatment in Gladius TOS2 (**Fig.5**) indicating a change in bacteria composition. For Kord CL, there was a change in composition at residue rate 0.01x in TOS2. Residue rate 0.1x in TOS1 was closer to the control than all the other treatments.
- Our data show a reduction in relative abundance of *Bacillus* with increasing Intervix residues for Gladius and Kord CL at both times of sampling. (**Fig.6**). However, the data also shows an increase in *Bacillus* in the Gladius rhizosphere at the lowest residue rate (0.001x) for TOS2, and at 0.1x and 0.5x in the Kord CL rhizosphere in TOS1.
- Rhizosphere harboured more symbiotic *Rhizobium* than bulk soil, and *Rhizobium* abundance in bulk soil had no response to Intervix residues. However, there was a reduction in *Rhizobium* abundance for both Kord CL and Gladius at 0.001x, 0.01x, 0.5x and 1x Intervix residues for both TOS1 and TOS2 (**Fig.7**). *Rhizobium* rhizosphere significantly increased at the 0.1x residue rate for Gladius in TOS1
- Principal coordinate analysis PCoA (**Fig.8**) indicates that the bacterial communities were separated according to TOS and soil sample location. Bacterial communities of wheat rhizosphere were significantly different and more distant from bulk soil. PC1 and PC2 of the PCoA explained 42.60% and 32.06% respectively, of the total variance.

Effect on soil bacteria in a lentil production system

The objective of this experiment was to investigate whether Intervix soil residues have an effect on lentil crop growth and soil bacteria. This was achieved by conducting a dose response pot experiment with two “times of sampling” (TOS), under glasshouse conditions at the Waite Research Precinct, comparing a lentil variety bred with improved tolerance to imi herbicide residues (Hurricane XT) and a susceptible variety (Nipper). (0x, 0.001x, 0.01x, 0.1x, 0.5x, 1x, where x = recommended field rate of 500ml/ha Intervix).

- Results show that α-diversity (Shannon index) of bacterial communities was higher and not affected by residues in the bulk soil, than the lentil rhizosphere. Bacteria species abundance and richness in the lentil rhizosphere (TOS1) was significantly lower in the 0.1x, 0.5x and 1x residue rate for Hurricane XT, and at the 0.5x and 1x residue rate for Nipper as compared to the control (**Fig.9**). For TOS2, species abundance and richness was not affected in the Hurricane XT rhizosphere, however, there was a significant reduction at all residue rates in Nipper rhizosphere TOS2, when compared to the untreated control.
- Our results indicate that bacteria β-diversity in the bulk soil and lentil rhizosphere was altered by variety and Intervix herbicide residues rate. Time of sampling did not alter bacteria β-diversity, however, β-diversity was significantly higher in the lentil rhizosphere than the bulk soil. Bray-Curtis index was significantly higher for Hurricane XT at 0.01x, 0.5x and 1x residue rate and Nipper lentil rhizosphere at the higher residue rates 0.5x and 1x, than the untreated control. (**Fig.10**), indicating a shift in bacteria composition.
- Data shows a significant reduction in *Bacillus* relative abundance (%) at residue rate 1x for both TOS, and for lentil rhizosphere at residue rate 1x for TOS2 (**Fig.11**). Lentil rhizosphere also harboured less B*acillus* in TOS 2 than TOS1.
- Data also shows a general trend of an increase in *Streptomyces* relative abundance with increasing Intervix residues for TOS1. Lentil rhizosphere harboured less *Streptomyces* in TOS2 than in TOS1 (**Fig.12**).
- Rhizosphere harboured more symbiotic *Rhizobium* than bulk soil. Low residue levels of Intervix (0.001x) increased lentil symbiotic *Rhizobium* in TOS1, however, at 0.1x residue rate for Nipper rhizosphere, *Rhizobium* was significantly reduced for both TOS1 and TOS2 (**Fig.13**).
- PCoA (**Fig.14**) indicates that the bacterial communities were separated more by Variety (Bulk soil and lentil rhizosphere), than TOS. Axis1 and Axis2 of the PCoA explained 28.4% and 37.2% respectively, of the total variance.

Imazamox or Imazapyr?

The herbicide Intervix is made up of the active ingredients Imazamox (33g/L) and Imazapyr (15g/L). Of the two active ingredients, Imazamox is considered the least persistent, and this is attributed to non-reversible sorption to soil colloids (Vencill, 2002; Quivet et al., 2004). The objective of this experiment was to further ascertain the impact of imazamox and imazapyr soil residues reported on lentil and wheat rhizosphere bacteria, and to determine which one of these actives had more impact on soil bacteria richness and abundance. To achieve

this, the herbicides Raptor (imazamox) and Warrant (imazapyr) were used at rates equivalent to the original proportions in Intervix.

- The results demonstrate that the exposure of soil organisms to imazamox and imazapyr changed the bacterial communities in the lentil rhizosphere and not in the bulk soil. Imazapyr residues in the lentil rhizosphere significantly ($P<0.001$) reduced the α-diversity (Shannon index, **Fig.15**) in both TOS1 and TOS2 (Nipper) and TOS2 (Hurricane XT).
- PCoA (**Fig.16**) indicates that the bacterial communities of soil treated with imazamox and imazapyr were significantly different and more distant from the untreated control. Bacterial communities were more distant from the control in imazapyr treated soil in both TOS1 and TOS2 for Hurricane XT and Nipper lentil. Axis 1 and Axis 2 of the PCoA explained 46.1% and 26.2% respectively, of the total variance.
- Mean relative abundance of R*hizobium* (OTU11) was significantly higher in the Hurricane XT lentil rhizosphere by 114%, than in Nipper (**Fig. 17a**), however, relative abundance was not affected by the presence of either imazamox or imazapyr residues at the RFR (**Fig.17b**).

CONCLUSIONS REACHED &/OR DISCOVERIES MADE

Herbicides that remain active in the soil for weeks, months or years can be a useful tool in broadacre cropping as it ensures effective long-term weed control. However, if the herbicides remain in the soil longer than intended, they may damage sensitive crop or pasture species sown in subsequent years, or alter soil microbial dynamics. Growers are now faced with a challenge to identify low levels of herbicide residues before they affect crop performance, and potentially reduce profitability.

Our data have shown that on alkaline sands in moisture limited Mallee environments, applied Intervix is persisting in the soil longer than the optimal half-life. New imidazolinone-tolerant crop varieties are being developed to deal with weed issues and improve crop productivity in evolving farming systems. Therefore the problem of accumulating herbicides appears to be exacerbated by accumulating residues because of the frequent of use of imidazolinone herbicides. For the crops investigated, Kord CL wheat and Hurricane XT lentils proved to be tolerant to Intervix residues, however, the non-Clearfield variety Gladius and the conventional small red lentil variety, Nipper, were sensitive to residues above 10% of the recommended field rate. Sensitivity of susceptible crop types may alter rotations particularly in seasons of below average rainfall on poor performing alkaline sands in Mallee environments. Therefore necessary adjustments need to be made, particularly when the summer and autumn seasons are drier than normal; to ensure that herbicide residues have enough time to break down and not cause significant damage to sensitive crops.

The relationship between plants and microbes is well known, especially the interactions between plants and bacteria either from the soil or inside the plants that help to improve the plant health under adverse stress conditions. The role of these plant growth promoting bacteria and other core microbes, although poorly studied in these systems, could be important for farming system productivity. Our results have shown that bacteria of the *Bacillus* genus, present in wheat and lentil rhizosphere, are sensitive to Intervix herbicide residues in alkaline sandy soils, particularly under high residues. A reduction of *Bacillus* relative abundance was recorded in both lentil and wheat rhizospheres at the late vegetative growth stage. The increase in the relative abundance of *Streptomyces*, particularly at the high residue rate, was observed in both the tolerant and susceptible lentil variety rhizosphere. The *Streptomyces* genus, which is the most abundant, are efficient rhizosphere and rhizoplane colonizers.

Rhizobium–legume symbioses are of great ecological and agronomic importance, due to their ability to fix large amounts of atmospheric nitrogen, improving their production and subsequent non-legumes. Our data show that wheat and lentil rhizosphere harboured more symbiotic rhizobia than the bulk soil. However, rhizobial relative abundance was reduced by high Intervix residues in the wheat rhizosphere. In the lentil rhizosphere, the effect of Intervix residues was inconsistent, with a significant increase at the lower residue rates and a significant reduction at 0.1x for the susceptible Nipper lentil variety. The reduction in rhizobia shows that this group can be sensitive to Intervix residues, therefore adjusting crop rotations and rhizobial inoculation will have beneficial effects on nodulation, grain yield, and protein content of crops like lentils and peas. Change in abundance of these rhizobacteria may affect the availability of nitrogen, phosphorus and key micronutrients in soils that are already deficient. These impacts are therefore more likely to impact on the yields in low rainfall, low input environments, such as the Mallee.

INTELLECTUAL PROPERTY

No intellectual property generated, and project has no potential for commercialisation

APPLICATION / COMMUNICATION OF RESULTS

Key findings:

- Degradation of imidazolinone herbicides is greatly reduced in Mallee environments with alkaline soils that are low in organic carbon, and this is made worse by dry hot conditions in summer and autumn.
- Intervix applied to sandy alkaline soils can persist for more than 24 months, with imazapyr being more persistent than imazamox.
- Kord CL wheat and Hurricane XT lentils proved to be tolerant to Intervix residues, however, the non-Clearfield variety Gladius and the conventional small red lentil variety, Nipper, were sensitive to residues above 10% of the recommended field rate.
- Rhizosphere soil bacterial communities of the susceptible variety Gladius, were altered by the presence of high Intervix residues at GS31, however, variety, herbicide residue rate and time of sampling all influenced the composition of bacteria in the presence of Intervix residues.
- The mean relative abundance of organisms of economic significance like *Bacillus* and *Rhizobium* was reduced at the high residue rate for both Gladius and Kord CL at both times of sampling.
- Bacteria species abundance and richness in the lentil rhizosphere was lower in the high residue treatments during the early vegetative stage. However, Nipper was more sensitive to Intervix residues at GS V1 than Hurricane XT when compared to the untreated control.
- Lentil rhizosphere harboured less *Bacillus* and *Streptomyces* than the bulk soil. Mean relative abundance of Bacillus was reduced at high residue rates, while a general trend of an increase in Streptomyces with increasing Intervix residues was recorded.
- Of the two ingredients that make up Intervix, imazapyr residues in the lentil rhizosphere significantly reduced bacterial abundance and richness during both times of sampling. However, the relative abundance of *Rhizobium* was not affected by the presence of either imazamox or imazapyr residues at the recommended field rate.

Communication of results to farmers and industry was planned to be delivered through talks at appropriate field days and presentation at one national or international conference. This has not been achieved due to restrictions from the Covid-19 pandemic. Results will however be conveyed to other scientists through publications in internationally refereed scientific journals, which will be prepared in the next 6 months to one year. The involvement of SAGIT in funding and the contribution will be duly acknowledged in these publications.

POSSIBLE FUTURE WORK

The initial proposal was to investigate the impact of imazamox and imazapyr residues on soil microbes of known economic significance. The project only focused on bacteria, however, other microbes like *fungi*, *protozoa* and *nematodes* have different functions in boosting soil and plant health. Therefore, there is potential to investigate the impact on each of these other organisms, using the existing samples already collected. Further work will likely arise following additional data analysis and interpretation. It is intended that some of these outcomes will be captured in a future ARC linkage project, partly supported through SAGIT funding.