Final report

Advancing the agronomy package for tedera to fill feed-gaps

Project code: B.CCH.6621

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Date published: 31 March 2022

PUBLISHED BY
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NORTH SYDNEY NSW 2059

Meat & Livestock Australia acknowledges the matching funds provided by the Australian Government to support the research and development detailed in this publication.

Abstract

Tedera (*Bituminaria bituminosa* C.H. Stirton var. *albomarginata*) is a perennial drought tolerant forage legume that can grow all year round. This production can be accumulated and utilized to fill feed-gaps. For cropping/livestock farming systems with a defined winter and spring growing season, the two main feed-gaps are at the shoulders of the growing season: (a) from February/March to May/June after the stubbles lose their quality and before the annual species are ready to be grazed and (b) from mid-October when annual species start to senesce to mid-December when first stubbles become available. Tedera can reliably provide green forage at these two shoulders, thereby being a perfect fit for the needs of the red meat industry. The first tedera cultivar in the world, T15-1218 Lanza®, launched by DPIRD and MLA in 2018, was utilized in this project for grazing experiments, field plot experiments and glasshouse pot experiments in WA and for demonstration sites in WA, SA, Vic, NSW and Tasmania. An agronomy package has been developed to sow Lanza® in the right soil, at the right time, and following the right guidelines to maximize establishment success and maximize its production of the best quality biomass to be used at strategic times, complementing other feedbase options.

Executive summary

Background

Tedera is a perennial drought tolerant forage legume that was introduced to Australia for the first time in 2006. During the 16 years of domestication and breeding, parallel research programs developed strategies for agronomic management and animal production. The first cultivar in the world, T15-1218 Lanza® was launched by the Department of Primary Industries and Regional Development (DPIRD) and Meat & Livestock Australia (MLA) in October 2018. The agronomy package for a newly domesticated species needs to cover all aspects of adaptation, establishment and management: soil type, time of sowing, herbicide tolerance, fertilizer requirement, sowing depth, sowing density, row spacing, defoliation management, harvesting and hard-seed breakdown pattern. This information package is essential for farmers to successful integrate tedera in their farming operations by sowing in the right place, at the right time, in the right way to establish it successfully and then optimise management to strategically fill feed-gaps and maximise animal production over the whole farm. This final report presents 1) the experimental results from 2017 to 2022 for the critical components of the agronomy package for tedera and 2) the results of a grazing experiment at Kojonup, being the last of a series of animal production experiments conducted between 2013 to 2016 in project B.PBE.0027 *"Sheep production from tedera".*

Objectives

The overall objective of this project was to develop a robust agronomy package for the first cultivar of tedera to assist farmers to sow tedera in the right place, at the right time, with the right methodology and to apply the correct management to achieve the optimum strategy to fill feed gaps in Mediterranean-like climatic zones in southern Australia. Eight specific objectives were addressed by this project, and all were successfully achieved:

- to evaluate regional adaptation in different soils in WA, SA, and VIC with a focus on low- and medium-rainfall Mediterranean environments
- to research establishment techniques (time of sowing, sowing depth, seed density and spatial configuration)
- to identify fertilization requirements (P and K)
- to identify herbicide tolerance

- to understand population dynamics in a sward (original sown plants and recruits)
- to manage defoliation to maximize green leaf production in the out-of-season period
- to understand the pattern of hard-seed softening in the field
- to undertake summer and autumn grazing trials to validate livestock performance from previous trials

Methodology

Grazing experiments, field plot experiments and glasshouse pot experiments were conducted from 2017 to 2022 and each specific objective had its own methodology detailed in each of the respective sections below:

- Demonstration sites were sown from 2017 to 2019 in WA, SA, Vic, NSW and Tasmania to evaluate regional adaptation.
- Six field experiments were conducted to research the establishment techniques (time of sowing, sowing depth, row spacing and sowing rate).
- Three field experiments and one glasshouse experiment were conducted to evaluate the dose response to the macronutrients P, K, N and S.
- Seven field experiments and one glasshouse experiment were conducted to identify pre- and post-emergent herbicides tolerated by tedera to control grasses and broad-leaf weeds.
- Three field experiments were conducted to evaluate the best defoliation management to maximize biomass production and seasonal availability.
- Four experiments were conducted to evaluate the pattern of hard-seed softening in the field.
- One grazing experiment was conducted during summer and autumn to evaluate livestock performance.

Results/key findings

Establishment techniques

Key elements of a tedera agronomic establishment package have emerged from this study which involve early planting (close to the start of the growing season) and shallow sowing (2 cm depth) at 15 kg/ha of sowing rate with narrow row spacing (22 cm apart). Very similar practices are in common use for cereal establishment when grown in the same regions negating the need for any specialized equipment for tedera establishment (Real, 2022).

Defoliation management

The best overall management is to defoliate frequently when tedera is under stress and allow it to grow and accumulate biomass when there are good growing conditions.

Fertilization response to P, K, N and S

The optimum level in the soil and plant tissue for each nutrient to produce more than 90% of maximum biomass is reported. For the first time we have information on both soil nutrient requirements and/or plant tissue concentration of each of these nutrients and benchmarks for deficient, adequate, or toxic levels.

Hard seed break down pattern

While the extent of seed softening varied between sites, a general pattern emerged with slow initial softening, accelerating during early autumn (March-April), and then slowing into late autumn/winter. Such a pattern would offer some protection against false breaks of season.

Herbicide tolerance

A total of nine pre-emergent and 44 post-emergent herbicides were evaluated in eight experiments from 2017 to 2021. To control grasses such as annual ryegrass, propyzamide and carbetamide were identified for pre- or post-emergent applications and butroxydim, clethodim and haloxyfop for post-emergent applications. The broadleaf pre-emergent herbicides identified are clopyralid to control post-emergent capeweed, fomesafen to control pre-emergent radish and the double mix of fomesafen+diuron, flumetsulam+diuron and the triple mix of fomesafen+diuron+flumetsulam to control pre-emergent capeweed, pre and post-emergent radish and other broadleaf weeds. The most consistently well tolerated post-emergent herbicides by seedlings and adult plants were diflufenican, diuron, flumetsulam, fomesafen and their two- or three-way mixes that will provide good control of capeweed and radish. Desiccants such as paraquat or diquat were also well tolerated by adult tedera plants that recovered after being desiccated.

Herbicides identified that were well tolerated by tedera detailed above will need to be granted a special permit by the Australian Pesticide and Veterinary Medicines Authority (APVMA) or to be included in the herbicide label by the supplier. Once a permit is granted, their use will be recommended.

Demonstration sites

Demonstration sites were successfully established around Australia, with 3 in WA, 8 in Vic, 19 in SA, 18 in NSW and 1 in Tasmania.

Summer and autumn grazing trial

The grazing experiment at Kojonup during 2017 again demonstrated that tedera can be grazed to reduce or eliminate expensive hand-feeding in summer-autumn by using the simplest and least expensive grazing management, continuous grazing. Either continuous or rotational grazing of tedera increased animal live weight and condition and eliminated hand-feeding for 84 days during February to May. At equivalent stocking rates (5 DSE/ha), sheep grazing tedera could gain 5 to 6 kg/head more than sheep grazing a lucerne paddock. These results are consistent with the previous three summer and autumn grazing experiments conducted at Dandaragan and Kojonup (Real et al., 2018).

Benefits to industry

In Mediterranean-like climates with a winter/spring growing season two key feed-gaps were identified as the shoulders of the growing season. One shoulder arises from February/March after the stubbles lose their quality to May/June before the annual species are ready to be grazed. The second shoulder can be from the end of the season about mid-October when annuals start to senesce to mid-December when first stubbles become available. Tedera can grow all year round, but its production can be best utilized at these two strategic times, being a perfect fit for the needs of the red meat industry. Previous trials demonstrated that tedera grazing could maintain or increase animal live weight and condition, while reducing or eliminating expensive hand-feeding for around 3 months between December and May, using either continuous or rotational grazing. At stocking rates of 10 DSE/ha and from early February to late April, sheep grazing tedera could gain 5 to 10 kg/head more than sheep fed grain at a maintenance level. MIDAS modelling has previously indicated that shifting a Central Wheatbelt farm with 35 % annual pasture to a mix of 9 % annual and 26 % tedera pasture could increase mixed farm income by more than 30%, and that tedera may be suited to 6.3 M ha in WA alone. These benefits come from tedera’s out-of-season feed-base reducing supplementary feeding and allowing higher value ewe/prime lamb systems to replace wether/wool systems. This project developed the agronomy package to be able to sow tedera in the right soil, at the right time, following the right guidelines to maximize establishment success and maximize its biomass production of the best quality to be used at strategic times, complementing other feedbase options available to growers.

Future research and recommendations

All experiments and demonstration sites in this project were established with T15-1218 (Lanza®). This was the only tedera variety protected under the Plant Breeder's Rights (PBR) Act 1994 (Real, 2016), and commercially available since 2019. In 2021, PBR in Australia accepted a second cultivar of tedera with improved cold tolerance, also bred by DPIRD and co-owned with MLA. Seed increase of the new cultivar commenced in 2022. The expectation is that most of the agronomy package developed with Lanza® will be applicable to the new cultivar. However, regional adaptation and herbicide tolerance are two key areas for future research for the new cultivar once seed becomes commercially available.

Tedera needs to establish in a weed-free situation to develop a strong and deep root system to survive the first dry season. Herbicides are a very important tool to achieve this objective if it cannot be sown into a weed-free paddock. Several herbicides were identified that are well tolerated by tedera and that can be used pre- and/or post-emergent to control grasses and broadleaf weeds. When it became available in 2019, tedera was a completely new commercial species to worldwide agriculture, and therefore, there is no herbicide specifically registered for tedera use. It will be essential to continue the research work and to urgently seek tedera-specific use registration for the key herbicides identified in this project.

Tedera is a species that can be used strategically to fill feed-gaps and this project confirmed the value of that strategic use. Projects such as the "Summer / Autumn liveweight gain from Tedera" project hosted at the Producer Demonstration Site (PDS) of the Moora Milling Pasture Improvement Group (MMPIG) are key to support and to show the benefits of tedera at paddock scale using animal production data generated by farmers. This is a very powerful research model to drive adoption, and more PDS sites with Lanza® and the new cultivar in WA and eastern Australia will showcase the benefits of tedera to accelerate its wide-spread adoption. We suggest a network of demonstration sites should be established to provide growers and agronomists with confidence in the agronomy package of tedera and to demonstrate and measure the animal production/economic benefits of including tedera in the production system.

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1. Background

1.1 Forage species to fill feed-gaps in forage production systems in regions with Mediterranean-like climates in Australia

In Mediterranean-like climates of Australia, the quantity and quality of the forage available over late spring, summer, autumn, and early winter is limited due to the lack of effective rainfall, severely affecting the profitability and sustainability of livestock industries (Dear and Ewing, 2008, Young et al., 2011, Real et al., 2018). To fill these 'feed-gaps', the Plant-based Solutions for Dryland Salinity Cooperative Research Centre (Salinity CRC) compiled a collection of perennial legumes with potential adaptation to Mediterranean–like climates from around the world to be evaluated across southern Australia. Li et al. (2008) presented the results from the evaluation of 47 species in 21 genera at sites in New South Wales, South Australia and Western Australia from 2002 to 2005. Real et al. (2011) evaluated the same 47 species plus an extra 56 species making a total of 103 perennial legume and herb species from 32 genera evaluated across a diverse range of Mediterranean-like climatic environments in southern Australia from 2005 to 2008. One of the most promising species was tedera (*Bituminaria bituminosa* C.H. Stirton var. *albomarginata*) and it became the focus species for breeding, animal production and agronomy research.

1.1.1 Tedera

Tedera is a drought tolerant perennial forage legume native to the Canary Islands where it is traditionally utilized for direct grazing and/or cut-and-carry to produce high value goat's cheese (Méndez, 1993, Méndez and Fernández 1990, Méndez et al., 2006). Tedera became a priority species for domestication and breeding leading to commercial release in Australia. In October 2018, the first cultivar in the world, T15-1218 Lanza® was launched by DPIRD and MLA (Real et al., 2014, Pazos-Navarro et al., 2011, Pazos-Navarro et al., 2014, Pradhan et al., 2014, Castello et al., 2015). During the 16 years of domestication and breeding, parallel programs developed the animal production and agronomy packages.

1.1.2 Animal production for tedera

The animal production research concluded that:

(a) grazing tedera did not cause any ill-effect to the grazing animals even when grazed as a sole diet or in mixtures at different times of the year (Oldham et al., 2013, Oldham et al., 2015, Ghaffari et al., 2014)
(b) tedera can be grazed at strategic times of the year to fill feed-gaps with excellent animal production results (Real et al., 2018).

1.1.3 Agronomy package for tedera

The agronomy package for a newly domesticated species needs to cover all aspects of adaptation, establishment and management: soil type, time of sowing, herbicide tolerance, fertilizer requirement, sowing depth, sowing rate, row spacing, defoliation management, harvesting and hard-seed breakdown pattern. This information package is essential for farmers to successful integrate tedera in their farming operations by sowing in the right place, at the right time, in the right way to establish it successfully and then optimise management to strategically fill feed-gaps and maximise animal production over the whole farm. This final report presents 1) the experimental results from 2017 to 2022 of critical components of the agronomy package for tedera and 2) the results of a grazing experiment at Kojonup that is the last in a series of animal production experiments (Oldham et al., 2013, Real et al., 2018, Adriansz et al., 2017, Oldham et al., 2015, Ghaffari et al., 2014).

2. Objectives

The overall objective of this project was to develop a robust agronomy package for the first cultivar of tedera to assist farmers to sow tedera in the right place, at the right time, with the right methodology and to apply the correct management to achieve the optimum strategy to fill the summer, autumn, and early winter feed gaps in Mediterranean-like climatic zones in southern Australia. Eight specific objectives were addressed by this project, and all were successfully achieved:

- to evaluate regional adaptation in different soils in WA, SA, and VIC with a focus on low- and medium-rainfall Mediterranean environments
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3. Methodology

3.1 Summer and autumn grazing at Kojonup

A grazing experiment under continuous or rotational grazing by sheep during summer and autumn 2017 to fill the feed gap without supplementary feeding was conducted at 'Barrule' farm (Lat: S 33:55:19.88; Long: E 117:18:10.098), Kojonup, WA (site hereinafter referred as Kojonup).

3.1.1 Site description and plant material

The 2.4 ha Kojonup experimental site was established using a commercial air-seeder (Simplicity) in August 2013 with a mixture of seven accessions of tedera (T4, T27, T31, T42, T43, T48 and T52). A description of the seven accessions can be found in Real et al. (2014). The soil was a yellow/brown deep sandy duplex (WA Soil Group 407) (Schoknecht and Pathan, 2013). During the first four months of 2017, there was a total of 120 mm of rainfall at the site, measured with an automated weather station (AWS Junior, MEA 104, Australia).

3.1.2 Pasture management prior to start of experiment

In 2014 Kojonup was utilized for a grazing experiment. During the 2015 growing season, the pasture was chemically manipulated to suppress annual species and promote tedera growth as follows. On the 30 April 2015 it was sprayed with 500 mL/ha of clethodim (240 g/L), 40 g/ha flumetsulam (800 g/kg) and 100 mL/ha bifenthrin (25 g/L) to control grasses, clover, and insects. A second grass control was carried out on the 27 July 2015 using 2 L/ha of propyzamide (500 g/L). A second broad leaf application of 5 g/ha metosulam (100 g/L) and 90 g/ha metribuzin (750 g/kg) was sprayed on the 26 August 2015 providing good control of both capeweed (*Arctotheca calendula* (L.) Levyns) and subclover (*Trifolium subterraneum* L.), but tedera was also severely affected. There was limited herbicide tolerance information at that time, but current knowledge indicates metosulam and metribuzin are not recommended for tedera (further details reported in the Herbicide Section 4.6)., A maintenance level of fertiliser on 1 July 2015 was top-dressed at 60 kg/ha Agstar Extra™ (N:14.1, P:14.1, S:9.2, Cu: 0.10, Zn 0.20). From February 2016 to May 2016, this site was utilized for a similar summer and autumn grazing experiment (Real et al., 2018). After the 2016 grazing experiment, the total area (2.4 ha) was continuously grazed until the end of October when annual species started to senesce, and sheep were removed from the site. During the winter and spring grazing, stocking rate was increased to match annual pasture growth and to keep annual pasture and tedera between 5 cm and 10 cm of height. Tedera was allowed to recover without grazing until the start of the grazing experiment on the 7 February 2017.

3.1.3 Grazing treatments

Two methods of grazing management of a monoculture of tedera were assessed: (a) continuous grazing and (b) rotational grazing with 14 days of grazing followed by 70 days of recovery. The 2.4 ha site was divided into two plots of 1.2 ha that were judged to have a similar plant density of tedera. One plot was randomly selected for the continuously grazed treatment and the remaining 1.2 ha was divided into six sub-plots of 0.2 ha for the rotationally grazed treatment. The site was grazed from 7 February 2017 to 2 May 2017 and no supplementary feeding was provided to the sheep.

3.1.4 Experimental and commercial sheep

Approval to conduct animal research was granted by the Animal Ethics Committee of DPIRD - AEC 16-5-09. A stocking rate of 5 DSE/ha was utilized for the experiment. On the first day of grazing (7 February 2017), 10 Merino ewe hoggets that had never grazed tedera were drafted at random from a commercial flock of around 500 sheep. The selected sheep were randomly assigned to the two grazing treatments (five to each treatment), tagged, weighed (using Tru-Test load bars and Tru-Test XR3000 data logger) and condition scored (van Burgel et al., 2011) directly from their plots every 14 days. At the end of the experiment (2 May 2017), all the experimental sheep were returned to their flock of origin. The flock from which the experimental sheep had been randomly selected, was managed by the farmer, and rotationally grazed eight lucerne paddocks close to the experimental site with the same stocking rate of 5 DSE/ha. At the start of the experiment, 10 random ewe hoggets that remained with the flock were tagged, weighed and condition scored and at the end of the experiment, the same 10 sheep were weighed, and conditions scored.

3.1.5 Statistical analyses

Liveweight and condition score, at different times during the experiment were statistically compared based on measurements of each animal to assess differences between continuous grazing and rotational grazing. Standard errors and least significant differences ($p<0.05$) were calculated.

3.2 Plant material for agronomy package

All experiments and demonstration sites were established with T15-1218, a new tedera variety protected under the Plant Breeder's Rights Act 1994 (Real, 2016), and commercialized under the registered trade mark Lanza®. Lanza® tedera was bred by DPIRD as part of an initiative by the Future Farm Industries Cooperative Research Centre. Lanza® is co-owned by DPIRD and MLA and the commercial partner is Seednet / Nutrien Ag Solutions. Lanza® seeds in all experiments were inoculated with the specific rhizobium WSM 4083 (O'Hara et al., 2014, Yates et al., 2009, Farquharson et al., 2022).

3.3 Experimental and demonstration sites established in 2017, 2018 and 2019 in WA and eastern Australia

The experimental and demonstration site details established in 2017 and 2018 in WA, Vic and SA are presented in Tables 1 to 3. The demonstration experiments sown in 2017 at Stawell and in 2018 at Joel Joel and the Hart Field Day site, plus the information generated in WA, encouraged Seednet / Nutrien Ag solutions to distribute seed to sow 43 demonstration sites in autumn or spring of 2019: 18 sites in SA, 18 sites in NSW, 6 sites in Victoria and 1 site in Tasmania (Table 4).

Table 1. Experimental site details of tedera agronomy experiments sown in WA in 2017 and 2018.

Site	Dandaragan	Dandaragan	Three Springs	Cunderdin
Experiments	Establishment with companion grass (Panic)	Sowing depth, rate, row spacing and time; defoliation; fertilization; seed softening	Sowing depth, rate, row spacing and time; defoliation; fertilization; seed softening	Sowing depth, rate, row spacing and time; defoliation; fertilization; seed softening
State	WA	WA	WA	WA
Long term average annual rainfall (mm)	460	600	380	310
Crop rotation	2016 - Pasture	2017 – Fallow 2016 – Lupin 2015 - Wheat	2017 – Fallow 2016 - Wheat	2017 – Fallow 2016 – Field Peas 2015 - Wheat
Soil Type	White sand	Sandy Loam	Loamy sand	Loam
Soil $pH_{(Ca\ Cl2)}$		6.8	5.4	7.6
Sowing date	31 August 17	30 May 17 5 April 18 24 May 18 5 July 18 23 August 18	25 May 17 4 April 18 23 May 18 4 July 18 22 August 18	4 July 17 3 April 18 21 May 18 2 July 18 20 August 18
Sowing machine	Twin cone air-seeder	Hand-sown	Hand-sown	Hand-sown
Pre-emergent / knock down herbicide		None	None	None
Insecticide		None	None	None
Fertilizer	P 20 kg/ha	None	None	None

Table 2. Experimental site details of tedera agronomy experiments sown in WA in 2018.

Site	Manjimup	Merredin (Sandy salmon gum)	Merredin (Mallee duplex)
Experiment	Sowing rate and spacing	Sowing rate and spacing	Sowing rate and spacing
State	WA	WA	WA
Farm name	DPIRD - RSU	DPIRD - RSU	DPIRD - RSU
Location	28527 South West Highway	Great Eastern Hyw and Crooks Rd.	Great Eastern Hyw and Crooks Rd.
Long term average annual rainfall (mm)	990	310	310
Crop rotation	2016 and 2017 – Annual Ryegrass for Hay 2015 - Oats	2017 – Canola 2016 – Fallow 2015 - Oats	2017 – Canola 2016 – Fallow 2015 - Oats
Soil Type	Gravelly Loam	Loamy sand	Sandy Loam
Soil $pH_{(Ca\ Cl2)}$	5.3	5.1	4.5
Sowing date	30 May 18	11 June 18	11 June 18
Sowing machine	Twin cone air-seeder	Twin cone air-seeder	Twin cone air-seeder
Pre-emergent / knock down herbicide	2L/ha Propyzamide (500 g/L) + 1.5 L/ha Trifluralin (480 g/L)	2L/ha Sprayseed + 1.5 L/ha Trifluralin (480 g/L)	2L/ha Sprayseed + 1.5 L/ha Trifluralin (480 g/L)
Insecticide	200ml/ha Alpha-cypermethrin (100 g/L) (plus 200ml/ha Chlorpyrifos (500 g/L)	200ml/ha Alpha-cypermethrin (100 g/L) (plus 200ml/ha Chlorpyrifos (500 g/L)	200ml/ha Alpha-cypermethrin (100 g/L) (plus 200ml/ha Chlorpyrifos (500 g/L)
Fertilizer	P 20 kg/ha	P 20 kg/ha	P 20 kg/ha

Table 3. Demonstration site details of tedera sown in WA and eastern Australia in 2017 and 2018.

Site	Mingenew	Moora	Perenjori	Stawell	Hart Field Day	Joel Joel
State	WA	WA	WA	Vic	SA	Vic
Long term average annual rainfall (mm)	400	460	320	570	460	430
Area Sown	5 ha + strip of panic grass	5 ha	1 ha tedera and 1 ha tedera + perennials	0.5 ha	400 g for demo plots	200 g for demo plots
Crop rotation	2016 – non legume pasture 2017 – non legume pasture	2017 – Clover pasture		2016 – annual pasture 2015 – annual pasture		
Soil Type	White sand	Red loam		Clay loam		
Soil $pH_{(Ca\ Cl2)}$	5.5	6		5.0		
Sowing date	8 June 18 + strip of panic grass in early August 18	21 June 18	August 18	17 May 17	Autumn 2018	Autumn and Spring 2018
Sowing machine	Air seeder 30cm tyne	Air-seeder DBS 30cm tyne		Cone-seeder		
Pre-emergent / knock down herbicide	1L/ha Trifluralin + 1L/ha Propyzamide			Glyphosate 2L/ha, Trifluralin 2L/ha		

Table 4. Demonstration sites in eastern Australia - 2019

State	Site #	**Location**	**Seed quantity (Kg)**
SA	1	Kingscote, KI	5
	2	Murraybridge	1
	3	Murraybridge	1
	4	Murraybridge	1
	5	Cooke Plains	2
	6	Cleve	0.3
	7	Cummins	0.3
	8	Kapunda	1
	9	Clare	1
	10	Eudunda	1
	11	Orroroo	1
	12	Orroroo	1
	13	Port Broughton	1
	14	Tumby Bay	1
	15	Minnipa	20
	16	Kadina	1
	17	Cummins	1
	18	Booleroo	0.5
TAS	1	Boat Harbour	1
VIC	1	Glenisla	1
	2	Birchip	1
	3	Birchip	1
	4	Colbinabbin	1.75
	5	Echuca	1
	6	Telopea Downs	1
NSW	1	Deniliquin	1
	2	Temora	1
	3	Coonamble	1
	4	West Wyalong	20
	5	Coolamon	1
	6	Cootamundra	1
	7	Warren	1
	8	Warren	1
	9	Narrabri	1
	10	Wee Waa	1
	11	Dunedoo	
	12	Armidale	2
	13	Deniliquin	0.6
	14	Barham	
	15	Tocumwal	
	16	Deniliquin	
	17	Finley	1
	18	Womboota	5

3.4 Establishment techniques (Real, 2022)

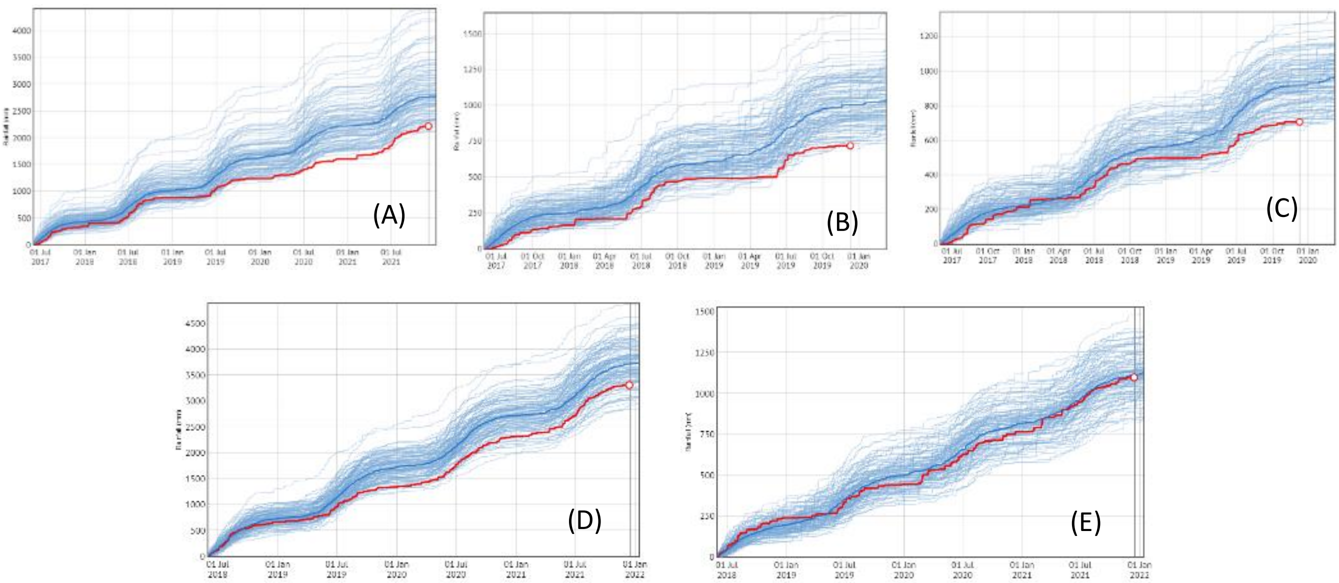
3.4.1 Experimental site details

The tedera establishment experiments were sown in 2017 and 2018 at Dandaragan, Three Springs, Cunderdin, Manjimup, Merredin (Malley duplex) and Merredin (Sandy Salmon Gum) in Western Australia. Details of each site are presented in Tables 1 and 2.

3.4.2 Experimental site rainfall

Accumulated rainfall for the experimental period and a historical comparison for the period from 1900 to the present are shown for the five experimental sites (Fig. 1) using CliMate software developed by the International Centre for applied Climate Sciences, University of Southern Queensland (https://climateapp.net.au/) accessed 10 December 2021.

Figure 1. Accumulated rainfall at Dandaragan (A); Three Springs (B); Cunderdin (C); Manjimup (D), and Merredin (E). The red line shows observed cumulative rainfall for each experimental site, the dark blue line is long-term average rainfall since 1900 and light blue lines are for each individual year of record contributing to the mean.



Dandaragan, Three Springs, and Cunderdin were extremely dry during the experimental period in comparison to the long-term average, with falls in the frequency percentiles of 2%, 1%, and 5% respectively. The Manjimup and the two Merredin sites also had below average rainfall, with frequency percentiles of 9% and 29%.

3.4.3 Time of sowing (2018)

The time-of-sowing experiments were established in 2018 at Dandaragan, Three Springs, and Cunderdin (Table 1). Establishment success was assessed. The experimental design was a randomized complete block with four times of sowing (April - dry sowing before the break of season, May - early sowing just after the break of season, July - late sowing after cereal crop program was completed, and August - early spring sowing) with nine replicates. Tedera was sown in 2 m rows (0.5 m apart) by hand at 10 kg/ha into furrows of 2 cm depth and then covered. At the three sites, the

April 2018 and May 2018 treatments were sown before the first winter rains and both germinated at the same time in late May following the first rains. Assessments at the three sites took the form of three seedling counts: early October 2018, mid-December 2018, and July 2019 after their first full dry season. The percentage of summer survival was calculated as ((Count in July 2019/Count in October 2018) × 100). The experiments were hand-weeded from sowing to July 2019 to remove any effect of weed competition during the establishment phase.

3.4.4 Sowing depth, row spacing, and sowing rate (2017)

The sowing depth, row spacing, and sowing rate experiments were established in 2017 at Dandaragan, Three Springs, and Cunderdin (Table 1). Establishment success and dry matter production were assessed. The experimental design was a split-plot with the main plot (6 treatments) comprising a combination of row spacings and depths (22 cm, 44 cm, or 66 cm of distance between rows and sowing depths of 2 cm or 6 cm) with subplots as sowing rates (5, 10, or 15 kg/ha) with 4 replications and plot size 1.54 × 10 m. The field plan is presented in Fig. 2.

Figure 2. Field plans of establishing methods at Cunderdin, Dandaragan and Three Springs, WA.

			48.1 m																								
				Rep 1						Rep 2						Rep 3						Rep 4					
				1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	
	10m	1	Buffer	22cm x 6cm x 5 kg/ha	44cm x 6cm x 10 kg/ha	66cm x 2cm x 5 kg/ha	22cm x 2cm x 15 kg/ha	66cm x 6cm x 5 kg/ha	44cm x 2cm x 5 kg/ha	66cm x 2cm x 5 kg/ha	44cm x 2cm x 10 kg/ha	22cm x 6cm x 10 kg/ha	66cm x 6cm x 10 kg/ha	44cm x 6cm x 15 kg/ha	22cm x 2cm x 15 kg/ha	44cm x 6cm x 10 kg/ha	66cm x 2cm x 10 kg/ha	22cm x 2cm x 10 kg/ha	66cm x 6cm x 10 kg/ha	22cm x 6cm x 15 kg/ha	44cm x 2cm x 15 kg/ha	66cm x 2cm x 15 kg/ha	22cm x 2cm x 5 kg/ha	44cm x 2cm x 10 kg/ha	66cm x 6cm x 5 kg/ha	44cm x 6cm x 5 kg/ha	22cm x 6cm x 10 kg/ha	Buffer
			Buffer	3001	3002	3003	3004	3005	3006	3007	3008	3009	3010	3011	3012	3013	3014	3015	3016	3017	3018	3019	3020	3021	3022	3023	3024	Buffer
30m	10m	2	Buffer	22cm x 6cm x 15 kg/ha	44cm x 6cm x 15 kg/ha	66cm x 2cm x 10 kg/ha	22cm x 2cm x 5 kg/ha	66cm x 6cm x 15 kg/ha	44cm x 2cm x 10 kg/ha	66cm x 2cm x 15 kg/ha	44cm x 2cm x 15 kg/ha	22cm x 6cm x 5 kg/ha	66cm x 6cm x 5 kg/ha	44cm x 6cm x 10 kg/ha	22cm x 2cm x 5 kg/ha	44cm x 6cm x 5 kg/ha	66cm x 2cm x 5 kg/ha	22cm x 2cm x 15 kg/ha	66cm x 6cm x 15 kg/ha	22cm x 6cm x 5 kg/ha	44cm x 2cm x 5 kg/ha	66cm x 2cm x 10 kg/ha	22cm x 2cm x 10 kg/ha	44cm x 2cm x 15 kg/ha	66cm x 6cm x 10 kg/ha	44cm x 6cm x 15 kg/ha	22cm x 6cm x 15 kg/ha	Buffer
			Buffer	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023	2024	Buffer
	10m	3	Buffer	22cm x 6cm x 10 kg/ha	44cm x 6cm x 5 kg/ha	66cm x 2cm x 15 kg/ha	22cm x 2cm x 10 kg/ha	66cm x 6cm x 10 kg/ha	44cm x 2cm x 15 kg/ha	66cm x 2cm x 10 kg/ha	44cm x 2cm x 5 kg/ha	22cm x 6cm x 15 kg/ha	66cm x 6cm x 15 kg/ha	44cm x 6cm x 5 kg/ha	22cm x 2cm x 10 kg/ha	44cm x 6cm x 15 kg/ha	66cm x 2cm x 15 kg/ha	22cm x 2cm x 5 kg/ha	66cm x 6cm x 5 kg/ha	22cm x 6cm x 10 kg/ha	44cm x 2cm x 10 kg/ha	66cm x 2cm x 5 kg/ha	22cm x 2cm x 15 kg/ha	44cm x 2cm x 5 kg/ha	66cm x 6cm x 15 kg/ha	44cm x 6cm x 10 kg/ha	22cm x 6cm x 5 kg/ha	Buffer
			Buffer	1001	1002	1003	1004	1005	1006	1007	1008	1009	1010	1011	1012	1013	1014	1015	1016	1017	1018	1019	1020	1021	1022	1023	1024	Buffer

3.4.4.1 Plant counts and establishment percentage

Established seedlings were counted on 25 October, 26 October, and 8 November 2017 at Cunderdin, Dandaragan, and Three Springs, respectively. Counts were made in every row of every plot in a 1 m wide central strip across the plots. A total of 3, 4, or 7 rows were counted per plot depending on whether they had 66, 44, or 22 cm row spacing. Plant counts/m^2 and establishment percentage (seedlings counted/total seeds sown) were calculated.

3.4.4.2 Biomass cuts

Dry matter (DM) production was evaluated by cutting with a 21-inch-wide self-propelled lawn mower at a height of 5 cm for the full length of the tedera plots. The weight of the mower bag with the cut sample was taken for each plot. The empty mower bag weight was also made for each cut. A subsample from the mower bag was taken for each plot, bagged in calico, weighed in the field, and used, following drying, to calculate the total sample dry weight and estimated DM kg/ha for each plot. The sub samples were oven dried at DPIRD, South Perth, for 72 h at 60°C. After cutting, the remainder of the plot was also mowed to the sampling height. The three experimental sites were assessed for the first time at the end of the first summer in April 2018 and then 3-monthly in July 2018, October 2018, January 2019, July 2019, and October 2019. At Cunderdin, in June 2019, just prior to the scheduled evaluation cut, the whole site was heavily defoliated accidentally by livestock, therefore no measurements were taken in July 2019, but measurements resumed in October 2019. Three Springs recorded the second driest two years since 1900 (percentile 1%) during the experimental period, so recovery after the January 2019 cut was very poor due to the extremely dry conditions, and the experiment was terminated due to low number of surviving plants.

3.4.5 Row spacing and sowing rate at Manjimup and Merredin (2018)

Row spacing and sowing rate experiments were conducted at a high rainfall site at Manjimup and two low rainfall sites at Merredin (Table 2). Establishment success and dry matter production were assessed. The experimental design for Manjimup was a split-plot with the main plot of spatial configurations of 17 cm or 34 cm of distance between rows, sub-plots were sowing rates (5 or 10 kg/ha). Treatments were replicated four times and the plot size was 1.8 × 10 m. The experiment was sown 30 May 2018, and seedlings were counted 26 July 2018.

The experimental design for the two Merredin experiments was a split-plot with the main plot of spatial configurations of 22, 44, or 66 cm of distance between rows, subplots as sowing rates (5 or 10 kg/ha). Treatments were replicated 4 times, and plot size was 1.54 × 10 m. Experiments were sown 11 June 2018, and seedling counts were taken on the 20 July 2018.

The biomass evaluation cuts in Manjimup were conducted in April, July, and October 2019 and in Merredin in July and October 2019, following the same methodology as described in Section 3.4.4.2.

3.4.6 Sowing depth in screenhouse (2018)

On 13 July 2018, a controlled experiment was set up to evaluate six sowing depths (2, 4, 6, 8, 10, and 12 cm) with 20 seeds per treatment and 3 replicates. The experiment was set up in a plastic box (with holes for drainage) filled with a commercial potting mix, inside a naturally lit screenhouse without temperature control. Plant counts were taken to assess the germination percentage of the six sowing depths.

3.5 Defoliation management

3.5.1 Experimental details

Defoliation management experiments were conducted at Cunderdin, Dandaragan and Three Springs and sown in 2017. Experimental site details, rainfall and plant material details are reported in Sections 3.4.1, 3.4.2 and 3.2. The experimental design was a split-plot with the main plot (allowing seed set and recruitment and not allowing seed set) with sub-plots as three defoliation managements (cut two, four and eight times per year). Row spacing was at 44 cm, sown at 2 cm of depth at a rate of 10 kg seed/ha, with 4 replicates and plots of 1.54 m x 10 m. Biomass cuts were taken following the same methodology described in Section 3.4.4.2. The field plan for all sites is presented in Fig. 3.

Figure 3. Field plan of defoliation and seedling recruitment experiment at Cunderdin, Dandaragan and Three Springs, WA.

			←		16.65 m	→					
				1	2	3		1	2	3	
40m	10m	Rep 4	Buffer	2	8	4	Buffer	8	4	2	Buffer
			Buffer	4001	4002	4003	Buffer	4004	4005	4006	Buffer
	10m	Rep 3	Buffer	8	4	2	Buffer	4	2	8	Buffer
			Buffer	3001	3002	3003	Buffer	3004	3005	3006	Buffer
	10m	Rep 2	Buffer	4	2	8	Buffer	2	8	4	Buffer
			Buffer	2001	2002	2003	Buffer	2004	2005	2006	Buffer
	10m	Rep 1	Buffer	8	4	2	Buffer	4	2	8	Buffer
			Buffer	1001	1002	1003	Buffer	1004	1005	1006	Buffer

3.6 Fertilization experiments

3.6.1 Field experiments (2017)

Fertilization experiments were sown in 2017 at the Dandaragan, Three Springs and Cunderdin sites. The experimental design for the three sites was a randomized complete block design with seven levels of P fertilizer (0, 5, 10, 15, 20, 25 and 30 kg/ha), seven levels of K fertilizer (0, 5, 10, 20, 40, 60 and 80 kg/ha) and two treatments with P and K at medium (P 15 + K 20) and high level (P 30 + K 80) (Fig. 4).

Figure 4. Field plan of fertilization response experiments at Cunderdin, Dandaragan and Three Springs, WA.

											33.3 m									
	10m	Rep 4	Buffer	P 25	P 0	P 30	P 10	P 15	P 5	P 20	P 30 + K 80	P 15 + K 20	K 60	K 0	K 80	K 10	K 20	K 5	K 40	Buffer
			Buffer	4001	4002	4003	4004	4005	4006	4007	4008	4009	4010	4011	4012	4013	4014	4015	4016	Buffer
40m	10m	Rep 3	Buffer	P 15 + K 20	P 30 + K 80	K 5	K 80	K 40	K 20	K 10	K 60	K 0	P 5	P 30	P 20	P 15	P 10	P 25	P 0	Buffer
			Buffer	3001	3002	3003	3004	3005	3006	3007	3008	3009	3010	3011	3012	3013	3014	3015	3016	Buffer
	10m	Rep 2	Buffer	P 10	P 5	P 15	P 20	P 25	P 0	P 30	K 10	K 5	K 20	K 40	K 60	K 0	K 80	P 15 + K 20	P 30 + K 80	Buffer
			Buffer	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	Buffer
	10m	Rep 1	Buffer	K 20	K 0	K 10	K 60	K 80	K 5	K 40	P 15 + K 20	P 30 + K 80	P 15	P 0	P 10	P 25	P 30	P 5	P 20	Buffer
			Buffer	1001	1002	1003	1004	1005	1006	1007	1008	1009	1010	1011	1012	1013	1014	1015	1016	Buffer

For all experiments, the row spacing was at 44 cm, 2 cm of depth, sowing rate at 10 kg/ha, 4 replicates and plots of 1.54 m x 10 m. The site location, characterization and soil analysis for Dandaragan, Three Springs and Cunderdin is presented in Tables 1 and 5. The accumulated rainfall from sowing up to end of June 2020 is presented in Section 3.4.2.

Table 5. Soil analysis for Dandaragan, Three Springs and Cunderdin fertilizer response experiment sites.

Site	Dandaragan	Three Springs	Cunderdin
Soil Type	Sandy Loam	Loamy sand	Loam
0 - 10cm			
Soil pH$_{(Ca\ Cl2)}$	6.8	5.4	7.6
EC (dS/m)	0.143	0.225	0.139
Organic C (%)	2.03	0.75	1.45
NO_3 (mg/kg)	36	8	10
NH_4 (mg/kg)	3	1	0
Colwell P (mg/kg)	30	35	22
PBI	19	23	120
Colwell K (mg/kg)	47	170	291
S (mg/kg) KCl 40	12	19	23
11 - 30 cm			
Soil pH$_{(Ca\ Cl2)}$	5.1	5.2	5.7
EC (dS/m)	0.040	0.230	0.073
Organic C (%)	0.77	0.48	1.38
NO_3 (mg/kg)	7	5	19
NH_4 (mg/kg)	0	0	2
Colwell P (mg/kg)	11	18	6
PBI	26	20	49
Colwell K (mg/kg)	18	247	414
S (mg/kg) KCl 40	16	17	15

3.6.1.1 Sampling times and biomass cuts

The three experimental sites were assessed for the first time at the end of the first summer in April 2018 and then every three months. Dandaragan had nine defoliations up to July 2020 when the experiment was terminated. Cunderdin had five defoliations up to October 2019. In June 2019, just prior to the scheduled evaluation cut, the whole site was heavily defoliated accidentally by livestock, therefore no measurements were taken in July 2019. Three Springs had only four defoliations due to the extremely dry conditions (see Section 3.4.4.2). Recovery after the January 2019 cut was very poor, and the experiment was terminated due to low number of surviving plants. Biomass cuts were taken following the same methodology described in Section 3.4.4.2.

3.6.2 Glasshouse experiment 2021

3.6.2.1 Growth conditions

Two genotypes of tedera (T21 and cv. Lanza®) were grown alongside lucerne cv. SARDI Grazer in an air-conditioned glasshouse at DPIRD South Perth (Latitude: 31°59’22’’ S; Longitude: 115°53’2’’ E) between 31 August 2021 and 30 November 2021. Plants were grown in washed play sand (RICHGRO) with nutrients added to provide basal nutrients and 40 treatments with 10 discrete levels each of P, K, N and S. The soil used had a Phosphorus Buffering Index (PBI) of 2.5. Nutrients added for the 40 treatments are provided in full in Table 6. Each nutrient by genotype treatment combination was replicated twice, and the experiment was managed as two randomised blocks, with all pots completely randomised every two weeks within each discrete block.

Table 6. Quantity of nutrients added to play sand to create the 40 treatments, 10 levels each of P, K, N and S. All values are in mg/kg.

	P (mg/kg)	K (mg/kg)	N (mg/kg)	S (mg/kg)
P treatments	0, 1, 2, 4, 8, 16, 32, 64, 128, 256	24.3	18.9	23.7
K treatments	23.8	0, 1, 2, 4, 8, 16, 32, 64, 128, 256	18.9	111-119
N treatments	26.6	33.6	0, 0.42, 0.83, 1.67, 3.33, 6.67, 13.3, 26.7, 53.3, 106.7	14.4
S treatments	26.6	41.4 - 72.8	17.4	0, 0.06, 0.13, 0.25, 0.50, 1.01, 2.01, 4.02, 8.04, 16.1

	Mg	Na	Ca	Cl	Cu	Zn	Co	Mn	Mo	B	Fe
P treatments	7.39	0.54 - 190	15.3	20.1	0.74	0.57	0.035	2.33	0.53	0.012	1.01
K treatments	7.39	18.23	137 - 15.3	20.1	0.74	0.57	0.035	2.33	0.53	0.012	1.01
N treatments	7.39	0.54	16.1	20.1	0.74	0.57	0.035	2.33	0.53	0.012	1.01
S treatments	15.1	0.54	12.8	31.6 - 24.5	0.74	0.57	0.035	2.33	0.53	0.012	1.01

The pots used were 8 L sealed pots measuring 200 mm height and 250 mm diameter, and each pot contained 6 kg of dry sand. Basal and treatment nutrients were added from stock solutions and mixed in a cement mixer in batches large enough to fill the six pots needed for each nutrient treatment (three genotypes x two replicates). No further nutrients were provided during the experiment.

The glasshouse was set to cool the environment to 24^{o}C daytime (6 am to 6 pm), and 20^{o}C at night. Glasshouse temperatures exceeded the cooling capacity of the air conditioners on several occasions in late November, but temperatures did not exceed 35^{o}C at any time.

Seeds were prepared for sowing by scarification and inoculation with appropriate strains of root nodule bacteria. Lucerne received Group AL inoculum and WSM 4083 was used for tedera. Approximately 15 seeds of the allocated genotype were initially sown in each pot, and seedlings were thinned to five healthy and uniform plants within three weeks of germination. Pots were watered to 100% field capacity weekly, with additional unweighed top up watering every 2 to 3 days. High Density Polyethylene (HDPE) beads (200 g) were added to the surface of each pot in week 4 of the experiment, providing a layer approximately 15 mm deep to limit soil evaporation.

3.6.2.2 Biomass components

Plant components were separated at harvest for measurement of dry biomass and later nutrient analysis. After thoroughly washing all sand from roots, the above- and below-ground components were separated, and the roots were dried and measured on a whole-pot basis. Above-ground shoots were treated on a per-plant basis, and the leaf, stem and flower components were separated for individual drying and measurement. Throughout the experiment, any leaves dropped in pots were collected for later weighing.

3.6.2.3 Soil and plant nutrient analysis

Samples of un-amended soil, and soil prepared with nutrients prior to planting were analysed for colour, texture, ammonium and nitrate nitrogen using the Rayment and Lyons Method 7C2b (Rayment and Lyons 2011), Colwell phosphorus and potassium (Colwell 1965), KCl 40 sulfur (Blair et al. 1991), organic carbon (Walkley and Black 1934), and electrical conductivity and pH (Rayment and Lyons Method 4A1 (pH water); 4B4 (pH CaCl2); 3A1 (Conductivity)) by CSBP laboratories (Bibra Lake, WA, CSBPlab.com.au). An estimate of available soil N was calculated by summing the ammonium and nitrate nitrogen results.

Leaf, stem, and flower samples were recombined on a whole pot basis and analysed for nutrient content by CSBP laboratories. Chloride and nitrate were analysed via an in-house colorimetric method; P, K, S, Cu, Zn, Mn, Na, Fe and B were measured using ICP (McQuaker et al. 1979); and total N was measured using Rayment and Lyons Method 9G2 (Rayment and Lyons 2011). In some cases, the weight of plant samples was insufficient to perform all tests.

3.6.2.4 Statistical analysis

Models were created to smooth variability within CSBP analysis of soil nutrient levels, by regressing CSBP results against the amount of nutrients added to soil. In the case of lower levels of P, K and N, the CSBP testing returned results below the minimum detectable limit, and so the smoothing models were extrapolated to provide estimated soil nutrient concentrations in these lower levels. The CSBP results and estimated soil nutrient levels are presented in Table 7.

Table 7. CSBP nutrient content analysis and estimated nutrient concentrations based on smoothing models for all treatments. All values are in mg/kg.

	Play sand	P treatment	K treatments	N treatments	S treatments
P Colwell	<2	<2, <2, 4, 6, 12, 20, 32, 39, 87, 147	33.3	20.1	22.9
Smoothed P Colwell		0, 2, 4, 7, 11, 18, 30, 50, 83, 138			
K Colwell	<15	18.3	Levels 1 to 6 <15, 20, 39, 77, 138	20.9	23 - 69
Smoothed K Colwell			0, 1, 2, 3, 6, 11, 20, 39, 74, 142		
NH_4 N	<1	3.7	4.0	Levels 1 to 5 <1, 2, 3, 6, 11, 14	<1
Available Soil N	<2	7.8	8.9	Levels 1 to 5 <2, 3, 5, 11, 19, 24	4.7
Smoothed Available N				0, 0.2, 0.4, 0.78, 1.5, 3.0, 5.9, 11, 19, 24	
Soil S	2.35	13.5	254	9.4	1.1, 1.2, 1.1, 0.8, 1.6, 1.8, 2.8, 3.8, 6.6, 13
Smoothed Soil S					1.3, 1.4, 1.4, 1.5, 1.7, 2.0, 2.7, 4.1, 6.9, 12.5
Conductivity (dS/m)	<0.01	0.052	0.37	0.038	0.032
pH (CaCl2)	6.1	5.6	6.3	6.1	6.1

Plant responses to the soil nutrient concentrations were assessed in two ways. First, means of treatments or nutrient levels were compared using anova ('aov') and Fisher's LSD method ('LSD.test' in the agricolae package) in R Studio ver. 2022.02.0 with alpha set to 10%. Results were tabulated. The optimal nutritional level where productivity was 90% of the peak productivity was then estimated by extracting and tabulating the lowest treatment level and soil nutrient concentration where productivity was not significantly different to the maximum productivity. Second, plant responses in shoot biomass and shoot nutrient concentrations in response to soil nutrient levels were summarized using quadratic models. These modelled plant responses in shoot biomass and shoot nutrient concentrations to soil nutrients are presented in Table 8. Quadratic models were used for simplicity in all cases. The soil nutrient concentrations were log_{10} transformed prior to fitting. Where a significant relationship existed, soil nutrient levels at which the models predicted > 90% peak biomass production and the coincident shoot nutrient concentrations were extracted from the models and tabulated. Bearing in mind the extremely low PBI of the experimental soil, and the shoot sampling method used, these critical levels can be used by growers to establish if there is likely to be a profitable response to additional fertiliser in tedera pastures by using a tool such as the Five Easy Steps tool provided by MLA (https://www.mla.com.au/extension-training-and-tools/tools-calculators/phosphorus-tool).

Table 8. Model descriptions for curves fitted to shoot biomass (BM) and shoot nutrient concentrations ([K], [P], [N] or [S]) in response to CSBP soil test results (Colwell P, Colwell K, Total Soil N, Soil S). Model fits are indicated by their significance (Pr(>F)), quadratic ($y=ax^2+bx+c$) and used a log10 transformation of soil fertility as the x variable. In three cases, no significant fit (NS) was available for shoot BM in response to soil nutrient levels, and no genotype had a significant fit between shoot [N] and Total Soil N.

Variable (y)	Genotype	x	Model terms			Model fit Pr(>F)
			Quadratic (a)	Linear (b)	Constant (c)	
Shoot BM	Lanza®	Log_{10}(Colwell P)	-2.42	4.28	2.00	$4.1e^{-03}$
Shoot BM	Lucerne	Log_{10}(Colwell P)	-9.83	26.3	-7.30	$3.2e^{-07}$
Shoot BM	T21	Log_{10}(Colwell P)	-7.41	16.1	-0.027	$2.6e^{-04}$
Shoot BM	Lanza®	Log_{10}(Colwell K)	-1.83	3.98	4.59	$8.1e^{-03}$
Shoot BM	Lucerne	Log_{10}(Colwell K)	-3.22	9.20	6.97	$1.3e^{-03}$
Shoot BM	T21	Log_{10}(Colwell K)	-1.03	2.60	7.72	$2.5e^{-01}$ NS
Shoot BM	Lanza®	Log_{10}(Avail. Soil N)	5.88	0.58	-1.03	$1.6e^{-08}$
Shoot BM	Lucerne	Log_{10}(Avail. Soil N)	-0.44	0.76	10.6	$5.1e^{-01}$ NS
Shoot BM	T21	Log_{10}(Avail. Soil N)	4.57	2.29	3.49	$8.6e^{-09}$
Shoot BM	Lanza®	Log_{10}(Soil S)	0.52	1.50	3.49	$4.1e^{-02}$
Shoot BM	Lucerne	Log_{10}(Soil S)	-9.22	17.4	3.80	$4.0e^{-05}$
Shoot BM	T21	Log_{10}(Soil S)	4.20	-3.42	9.20	$1.5e^{-01}$ NS
Shoot [P]	Lanza®	Log_{10}(Colwell P)	0.25	0.70	-0.34	$3.40e^{-08}$
Shoot [P]	Lucerne	Log_{10}(Colwell P)	1.31	-1.55	0.47	$1.45e^{-12}$
Shoot [P]	T21	Log_{10}(Colwell P)	0.58	-0.080	-0.070	$1.02e^{-06}$
Shoot [K]	Lanza®	Log_{10}(Colwell K)	1.16	-0.39	0.43	$6.37e^{-14}$
Shoot [K]	Lucerne	Log_{10}(Colwell K)	1.20	-1.06	0.41	$4.44e^{-12}$
Shoot [K]	T21	Log_{10}(Colwell K)	0.91	-0.27	0.30	$2.47e^{-13}$
Shoot [N]	Lanza®	Log_{10}(Avail. Soil N)	0.075	-0.079	1.57	$9.13e^{-01}$ NS
Shoot [N]	Lucerne	Log_{10}(Avail. Soil N)	-0.30	-0.15	3.47	$5.86e^{-02}$ NS
Shoot [N]	T21	Log_{10}(Avail. Soil N)	-0.41	0.35	2.00	$1.22e^{-01}$ NS
Shoot [S]	Lanza®	Log_{10}(Soil S)	0.0012	0.12	0.12	$5.24e^{-05}$
Shoot [S]	Lucerne	Log_{10}(Soil S)	0.23	-0.055	0.074	$6.54e^{-09}$
Shoot [S]	T21	Log_{10}(Soil S)	0.058	0.15	0.076	$1.09e^{-12}$

3.7 Pattern of hard seed softening in the field

3.7.1 Hard seed test methodology

Newly ripened seeds of Lanza® tedera were hand-harvested from mature plants growing at Cunderdin, Dandaragan and Three Springs in 2018 and from Dandaragan again in 2019. For each site/year, six sets of four replicate seed lots, each with 100 seeds, were prepared. One set was used for a 21-day germination test to measure the initial level of hard seed. The remaining five sets were placed into flywire mesh pockets and pinned to the ground at each respective site at the beginning of summer (Fig. 5). A set of four replicate seed lots were recovered every 45 days over the summer and autumn period for analysis of germination. Each replicate of 100 seeds was placed into four Petri dishes with two layers of Whatman No. 1 filter paper and watered with de-ionized water. All Petri dishes were placed in a dark room at a 15°C constant temperature. Germinated seeds were counted weekly for 21 days with final hard seeds counted on day 21.

Figure 5. Five sets with four replicates of 100 seeds each at Cunderdin in December 2018.

The monthly rainfall, and maximum and minimum temperatures at Three Springs, Cunderdin and Dandaragan from December 2018 to June 2019 and for Dandaragan from December 2019 to June 2020 are shown in Table 9.

Table 9. Monthly rainfall, maximum and minimum temperatures at Three Springs, Cunderdin and Dandaragan during seed softening experiments.

	Three Springs 2018/19			Cunderdin 2018/19			Dandaragan 2018/19			Dandaragan 2019/20		
	Rainfall (mm)	Max. Temp. (°C)	Min. Temp. (°C)	Rainfall (mm)	Max. Temp. (°C)	Min. Temp. (°C)	Rainfall (mm)	Max. Temp. (°C)	Min. Temp. (°C)	Rainfall (mm)	Max. Temp. (°C)	Min. Temp. (°C)
Dec	1	41.8	11.5	2.4	41.2	6.7	5.4	41.7	10	1.2	43.1	9.3
Jan	0.2	44.4	12.3	0	44.3	11.3	2	44.2	11.6	1.0	43.8	10.3
Feb	0	42.9	11.5	0.4	42.9	11.3	0.2	42.0	12.2	84.2	42.6	13.6
March	5.4	39.4	12.9	1.6	39.1	9.7	1.2	38.4	11.5	0.0	37.9	11.3
April	4.4	37.3	3.5	21.6	37.5	3	16.6	37.4	5.1	0.0	38.3	8.6
May	0.4	30.6	0	7.6	29.5	-0.8	4.6	30.4	-0.3	32.8	29.9	4.5
June	114.2	25.8	1.7	67.6	23.5	0.2	138.0	25.7	4	63.0	27.5	6.4

The summer and autumn were very dry for the three sites in 2018/19, but extremely dry for Three Springs with only 11.4 mm of rainfall in 6 months (December to May). In 2020, at Dandaragan there was an unusual summer storm in February.

3.8 Herbicide tolerance

Eight herbicide tolerance experiments were conducted from 2017 to 2021. General experimental details are presented in Table 10 and specific details for each experiment are presented in sections 3.8.1 to 3.8.8. Unless stated otherwise, spraying was performed using Teejet AIXR11002 (coarse droplet size) nozzles and a boom output of 96 L/ha.

Table 10. General experiment details for eight tedera herbicide tolerance experiments.

Location	Dandaragan	Northam	Northam
Experiments	Exp 1, 2, 3 & 7	Exp 4, 5 & 8	Exp 6
Annual average rainfall (mm)	480	430	430
Irrigation	Rain-fed	Yes	Yes
Soil Type	Sandy Loam	Gravelly Loam	Soil/sand mix
Soil $pH_{(Ca\ Cl2)}$	6.8	5.8	
Type of Experiment	Field	Field	Glasshouse

3.8.1 Experiment 1 (2017). Post-emergent herbicides on a 2-year-old tedera seed crop

On the 15 June 2017, a section of a tedera seed crop established at Dandaragan in July 2015 was sprayed with 15 post-emergent herbicides alongside an un-sprayed control (Table 11) in a randomized complete block design with plots of 3 m x 20 m and three replicates. The wind speed was 11 km/h, temperature of 20°C and a relative humidity of 54% (Fig. 6).

Figure 6. Spraying a two-year-old stand of tedera with 15 experimental post-emergent herbicides at Dandaragan on 15 June 2017.

The effect on the 2-year-old tedera was evaluated as biomass reduction in comparison to the un-sprayed control and the percentage of yellowing, chlorosis and/or necrosis observed on the 14 July

2017 (1 month after application). Measurements were on a scale of 0-100% where 0% means no effect and 100% means dead plants.

3.8.2 Experiment 2 (2018). Post-emergent herbicides on a 1-month-old tedera stand

A post-emergent experiment in the Dandaragan 2018 seed crop was sprayed on the 5 August 2018, five weeks after sowing, to test 11 post-emergent herbicides (Table 11) in a strip-plot design with plots of 3 m x 5 m and three replicates. Eight broad-leaf selective herbicides treatments (Diflufenican, Flumetsulam, Flumetsulam + Diuron, Imazamox, Imazethapyr, Oxyfluorfen, Prometryn and Pyraflufen-ethyl) plus an un-sprayed control were applied in strips east-west, while three grass selective herbicides (Butroxydim, Haloxyfop and Propyzamide) plus an un-sprayed control were applied in strips north-south, both randomised within each replicate. The wind speed was 10 km/h, temperature of 19°C and a relative humidity of 45%. The experiment was visually assessed after 8 days and after almost 6 weeks on the 13 August 2018 and 14 September 2018 for biomass reduction percentage in comparison to un-sprayed control.

3.8.3 Experiment 3 (2018). Post-emergent herbicides on a 1-year-old tedera stand

A section of the Dandaragan 2017 seed crop was sprayed on the 28 June 2018 with 12 post-emergent herbicides (Table 11) in a strip-plot design/criss-cross with plots of 3 m x 5 m and three replicates. Nine broad-leaf selective herbicides treatments (Diflufenican, Flumetsulam, Flumetsulam + Diuron, Imazamox, Imazethapyr, Oxyfluorfen, Prometryn, Pyraflufen-ethyl and Saflufenacil) were applied in strips east-west plus an un-sprayed control, while three grass selective herbicides (Butroxydim, Haloxyfop and Propyzamide) plus an un-sprayed control were applied in strips north-south at right angle to broadleaf herbicide application direction, both randomised within each replicate. The wind speed was 10 km/h from the South, temperature was 19°C and a relative humidity of 45%. The experiment was visually assessed on the 13 August 2018 and 14 September 2018 for biomass reduction percentage in comparison to un-sprayed control.

3.8.4 Experiment 4 (2020). Pre-emergent and post-emergent herbicides on 1 month old seedlings

On the 27 March 2020 a strip-plot design was sown at Northam with tedera at a sowing rate of 10 kg/ha and a depth of 2 cm with a row spacing of 22 cm. The strips in one direction had two treatments: pre-emergent applications and post-emergent applications. In the perpendicular direction were seven herbicide treatments (Table 11) plus an un-sprayed control. Each experimental unit was 2 m x 3.08 m, with four replicates. The pre-emergent herbicides were sprayed on the 26 March 2020 and the post-emergent herbicides on the 29 April 2020 (1 month post sowing). Seedling counts (four 1 m rows were counted in the middle of each 7-row plot) were taken about one month after herbicide application either pre- or post-emergent experiments. Visual biomass reduction estimates were taken on the 29 May 2020. On the 25 August 2020, two 50 cm x 50 cm quadrats in each plot were cut to 5 cm of height to assess the biomass.

3.8.5 Experiment 5 (2020). Post-emergent herbicides on 5-month-old plants

The plots of Experiment 4 sown at Northam on the 27 March 2020 that were unaffected by the herbicide treatments were allowed to grow for 5 months. Eleven herbicide treatments plus an un-sprayed control were then applied on the 31 August 2020 (Table 11). The experimental design was a randomized complete block design, each experimental unit was 2 m x 3.08 m, with four replicates. Visual assessment of the effect on flowering was assessed on the 21 September 2020 and a biomass cut of one 50 cm x 50 cm quadrat was taken in each plot on the 18 December 2020.

3.8.6 Experiment 6 (2020). Post-emergent herbicides on 1 month old seedlings

A post-emergent herbicide experiment was conducted in a glasshouse at Northam. On the 5 October 2020, 99 1 L pots were filled with a mixture of soil and river sand, sown with 12 tedera seeds each and placed in a naturally lit glasshouse. One week after sowing, tedera rhizobium inoculant (WSM 4083) was watered into the pots. Pots were irrigated three times a week. One month after sowing (3 November 2020), when plants were in the 5-leaf stage, they were sprayed with 31 herbicide treatments (Table 11) and two un-sprayed controls in a spray-cabinet with an output equivalent to 100 L/ha. The 33 treatments were completely randomized in the glasshouse and replicated three times. Eight weeks after spraying the experiment was harvested. Roots attached to the shoot were carefully washed several times manually using a water jet and a sieve (0.7 mm mesh size) to remove debris and soil particles while preventing root damage and losses. The detached roots were collected from the sieve and added to the main root mass. Root portion was separated from the shoot portion and roots were stored in water in a cold room at 4°C until the subsequent root image analysis, which commenced immediately afterwards. Fine cleaning of roots using forceps/tweezers was done before scanning. All the material that was not live roots, especially dead roots which can be identified from their darker colour and lack of elasticity was removed. Then roots were spread into a thin layer (2-3 mm) of distilled water in a transparent plastic tray. Care was taken to fully submerge, spread roots, and minimize overlapping of roots. Roots were cut into small segments and spread with a paintbrush wherever appropriate to facilitate the above. For each sample, one or several 400 dpi resolution images were taken with a flatbed scanner (Epson Perfection V800 Photo; Epson, Nagano, Japan). When the root sample was too large to complete in one scan, the sample was divided into two or more sub-samples and images were taken for each sub-sample. The images were analysed with the software package WINRHIZO™ Pro 2007a (Regent Instruments, Québec, Canada) for total root length, average diameter, and surface area using the Global Threshold Method where a single threshold value was chosen automatically to classify all pixels of an analysed region. After scanning the roots, samples were oven dried at 60°C for one week and root biomass assessed. The shoot length of each plant in each pot was measured, cut and oven dried at 60°C for one week and shoot biomass production assessed.

3.8.7 Experiment 7 (2021). Post-emergent herbicides on a 3-year-old tedera stand

On the 24 June 2021, a section of a 3-year-old tedera seed crop at Dandaragan was sprayed with 22 herbicide treatments plus three un-sprayed controls in a randomized complete block design (Table 11). Each experimental unit was 2 m x 30 m, with three replicates. Visual assessments of biomass reduction percentage were conducted on 22 July 2021 and 24 August 2021. A biomass cut was taken on 31 August 2021 for each plot with a self-propelled lawnmower with a cutting width of 0.53 m, a cutting height of 5 cm and a length of the cut of 5 m. Samples were oven dried for 72 hours at 60 °C and tedera material was separated from other species and weighed.

3.8.8 Experiment 8 (2021). Pre-emergent and post-emergent herbicides on 1-month-old seedlings

On the 8 October 2021 a randomised complete block design with 4 replications was sown at Northam with tedera at sowing rate of 10 kg/ha and a depth of 2 cm with a row spacing of 22 cm. All the experimental area was sprayed with propyzamide as a pre-emergent (IBS) just prior to imposing the treatments to the plots. The plots had six pre-emergent treatments incorporated by sowing (IBS), three post-sowing pre-emergent (PSPE) treatments, two un-sprayed controls and on the 11 Nov 2021, 10 post-emergent treatments were applied (Table 11). On the 14 December 2021, plants were counted in each plot, and a central 50 cm x 50 cm quadrat was cut to ground level. Samples were oven dried for 72 hours at 60°C and weighed.

Table 11. The herbicide products and rates (grams of active ingredients per hectare) used in herbicide tolerance experiments

Product	Group	Exp. 1	Exp 2.	Exp. 3	Exp. 4	Exp. 5	Exp 6	Exp 7	Exp 8
Pre-emergent herbicides									
Aclonifen+Diflufenican+ Pyroxasulfone (IBS[A])	32+F+K								400+66+ 100
Fomesafen (IBS)	G								360; 720
Fomesafen (PSPE[B])	G								300; 600
Fomesafen+Diuron (IBS)	G+C								240+450
Fomesafen+Diuron+ Flumetsulam (IBS)	G+C+B								240+450 +40
Flumetsulam+Diuron (IBS)	B+C								40+450
Clopyralid (PSPE)	I								90
Propyzamide (IBS)	D				1000;2000				1000
Prosulfocarb+S-Metolachlor (IBS)	K				2000+300; 4000+600				
Terbuthylazine (IBS)	C				900;1800				
Post-emergent herbicides									
2,4-DB	I							500; 1000; 2000	
2,4-DB+Flumetsulam	I+B							1000+20	
Aclonifen+Diflufenican+ Pyroxasulfone	32+F+K								400+66+ 100
Bentazone	C	1440							
Bromoxynil	C	400					250; 500; 1000		
Bromoxynil+Diflufenican	C+F	250+ 25				250+25; 750+75	250+25; 500+50		
Butroxydim	A	45	45	45					
Carbetamide	E						2070; 4140	2070	
Clethodim	A	120							
Clopyralid	I								45
Cyanazine	C	1080							
Diflufenican	F	100	100	100		100;300	50; 100; 200; 400	100	
Diflufenican+Pyraflufen	F+						100+8; 200+16		
Diflufenican+Flumetsulam+ Diuron	F+B+C						50+20+90; 100+40+ 180	100+20+ 90; 200+40+ 180; 400+80+ 360	100+20+ 90
Diuron	C						450; 900		
Flumetsulam+Diuron	B+C		20+ 50	20+ 50	20+90; 40+180	40+180	20+90; 40+180	40+180	20+90
Flumetsulam	B	32	20	20					
Flumetsulam+Diflufenican	B+F						20+100	20+100	
Flumetsulam+Picolinafen	B+F							20+37.5	
Flumetsulam+Diuron+ Picolinafen	B+C+F							20+90+ 37.5; 40+180+75	
Flumioxazin	G						90;180		
Fluroxypyr	I						50;100		
Fomesafen	G						180; 360	180; 360	180; 360
Fomesafen+Diuron	G+C								240+90
Fomesafen+Clopyralid	G+I								240+30
Glyphosate	M							450	
Haloxyfop	A		104	104					
Imazamox+Imazapyr	B+B	24.75+ 11.25					10+4.5; 20+9		12.4+5.6; 24.8+11. 2
Imazamox	B	35	35	35					
Imazethapyr	B	98	98	98					
Linuron	C	500							
Clopyralid	I								45
MCPA+Bromoxynil	I+C					250+250; 750+750			
MCPA+Diflufenican	I+F					250+25; 750+75			
MCPA+Bromoxynil+ Diflufenican	I+C+F					250+250 +25; 750+750 +75			
MCPB+MCPA+ Flumetsulam	I+I+B							600+40+20; 1200+80+40; 2400+160+80	
Mesotrione	H						96; 192		
Oxyfluorfen	G		120	120					
Picolinafen	F							37.5	

Prometryn	C		400	400				
Propyzamide	D	1000	1000	1000	1000;2000			
Prosulfocarb + S-Metolachlor	K				2000+300; 4000+600			
Pyraflufen-ethyl	I		8	8			16; 32	
Saflufenacil	G	23.8		23.8				
Saflufenacil+Paraquat	G+L	23.8+ 375						23.8+375
Terbuthylazine	C				900;1800			

[A]IBS – Incorporated by Sowing
[B]PSPE – Post-Sowing Pre-Emergent

3.8.9 Statistical analysis

Analysis of variance using Genstat was undertaken for most of the data analysis, with blocking and treatment structures appropriate for the randomized block or strip plot designs. Significance lettering was determined based on the least significant difference (l.s.d.) at P≤0.05. With experiment 2, the analysis was repeated without Pyraflufen-ethyl to confirm no significant interaction without this herbicide. For experiment 4, the application of propyzamide followed by flumetsulam + diuron was considered the same treatment pre-and post-herbicide despite different rates. This was done to give a balanced strip plot design and only after checking the results supported this adjustment. With experiment 6, shoot dry weight per plant, root dry weight per plant and total root volume per plant were square root transformed prior to analysis to give more constant variance. Results were back transformed and presented on the original scale.

4. Results

4.1 Summer and autumn grazing at Kojonup

4.1.1 Sheep production

The liveweight and condition score of the sheep for the duration of the experiment for the continuously and rotationally grazed treatments is presented in Figs. 7 and 8. Sheep liveweights and condition scores for the continuous and rotational grazing throughout the experiment were not significantly different, except for liveweight on the 7 March 2017, where continuous grazing was higher. Over 84 days, sheep gained 10.2 kg/head in rotational grazing treatments and 11.3 kg/head in the continuous grazing treatments. The sheep of the same flock used to source the experimental sheep that were managed by the farmer stocked at the same 5 DSE/ha grazing lucerne paddocks gained less weight (5.2 kg/head) during the same 84 days (Fig. 7). There was no difference in condition score among the experimental and control sheep (Fig. 8).

Figure 7. Liveweights for rotational (dashed lines) and continuous (solid lines) grazing. The solid circles represent a control group. The average LSD (5%) for comparing between grazing method is 3.5 kg.

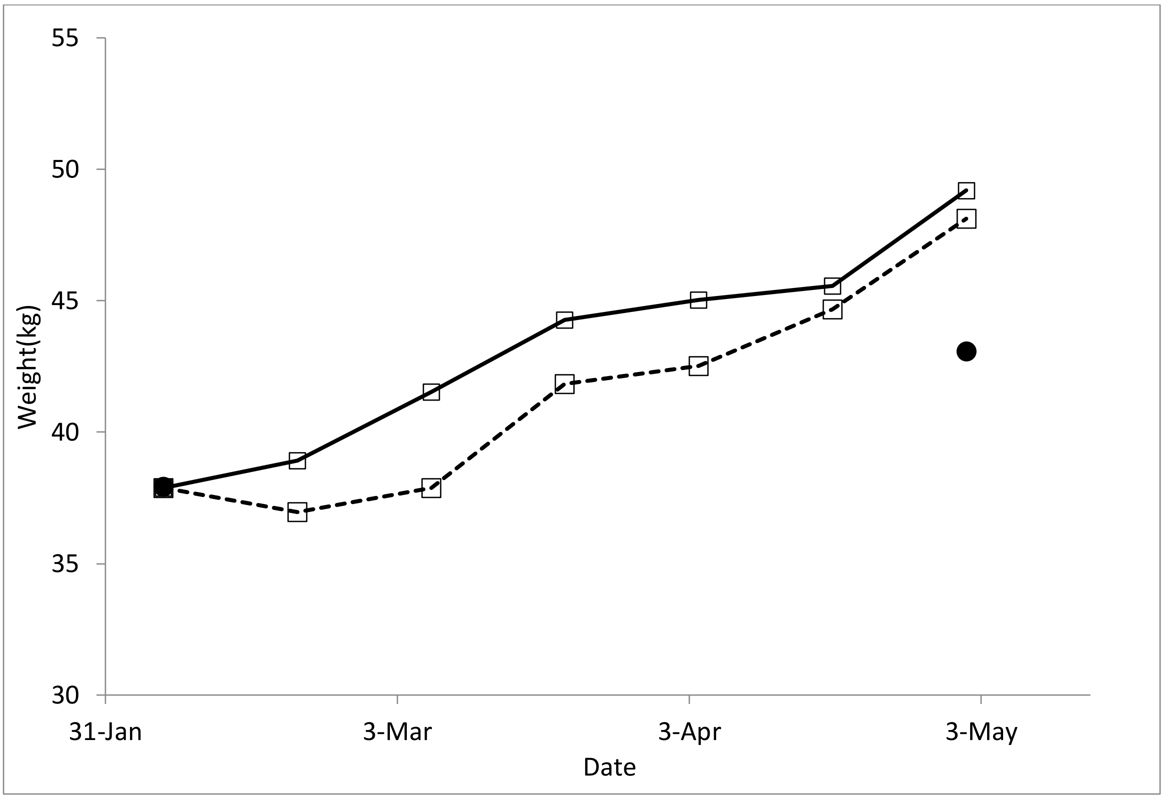
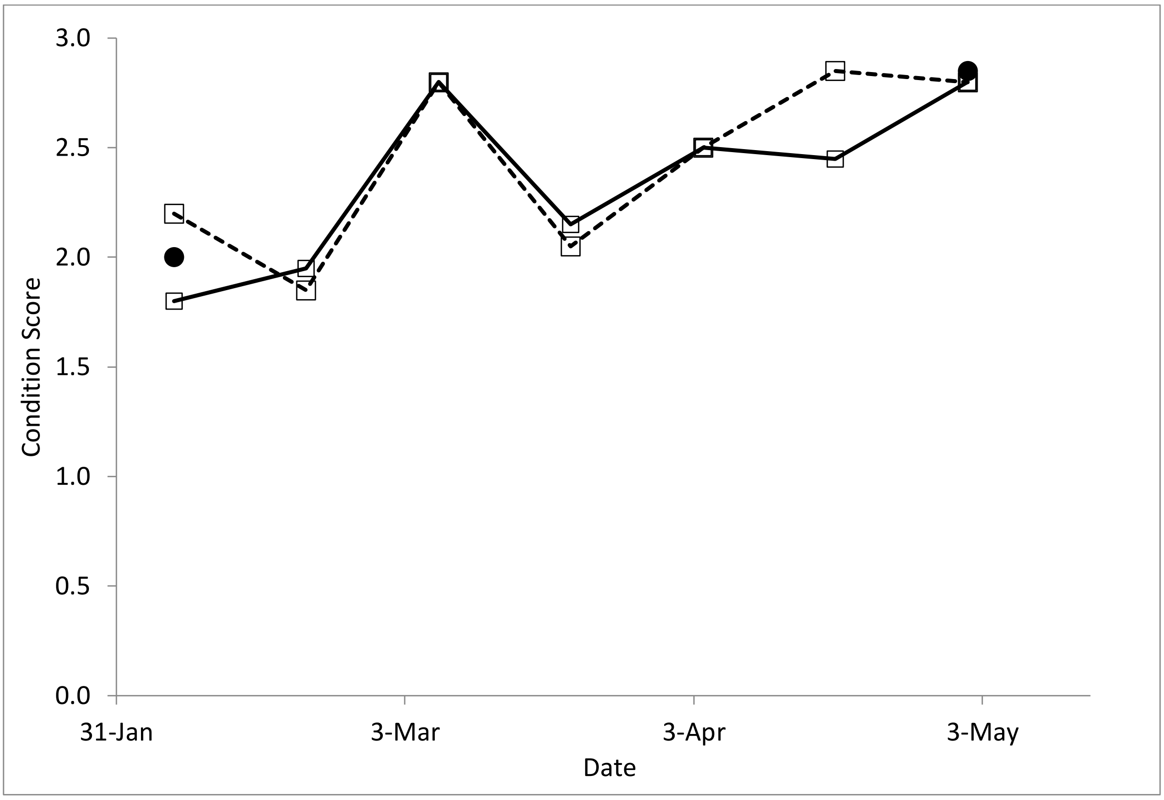


Figure 8. Condition scores for rotational (dashed lines) and continuous (solid lines) grazing. The solid circles represent a control group. The average LSD (5%) for comparing between grazing method is 0.6.



This experiment demonstrated that tedera can be reliably grown as a monoculture and preserved as green pasture to be grazed to reduce or eliminate expensive hand-feeding in summer-autumn using the simplest and least expensive method, continuous grazing management. These results are in agreement with the previous three summer and autumn grazing experiments conducted at Dandaragan and Kojonup (Real et al., 2018).

4.2 Establishment techniques

4.2.1 Time of sowing (2018)

Time of sowing experiments were established at three sites and with four sowing times. The main site effect was not significant for seedling counts in October 2018 (20.0 plants/m row) and December 2018 (10.6 plants/m row). However, for July 2019 counts, the site effect was highly significant (mean = 7.4 plants/m row). Dandaragan and Cunderdin were statistically the same (l.s.d. = 3.32) with 10.6 and 8.2 plants/m row; however, there was significantly less plants at Three Springs with 3.3 plants/m row.

The time-of-sowing effect was highly significant (Table 12) with 1 July 2018 the best sowing time for the first two plant counts, likely due to high soil moisture at sowing. For the final count in July 2019, the May 2018 and July 2018 sowing times were best and statistically the same.

Table 12. Mean seedling counts/m from three time-of-seeding sites.

Time of Sowing	Early October 18	Mid-December 2018	July 2019
1 April	15.84c [A]	7.94c	6.98b
15 May	18.94b	8.49c	9.65a
1 July	24.08a	13.85a	8.78a
15 August	20.87b	12.22b	3.93c
l.s.d. (5%)	2.21	1.53	1.61

[A] Figures in the columns that share a common letter are not significantly different ($p < 0.05$).

For the percentage of survival over the first summer (final counts on July 2019 compared to initial counts on October 2018), there was a significant site effect, with Dandaragan being the site that had the best survival over the first summer, followed by Cunderdin and then Three Springs (Table 13).

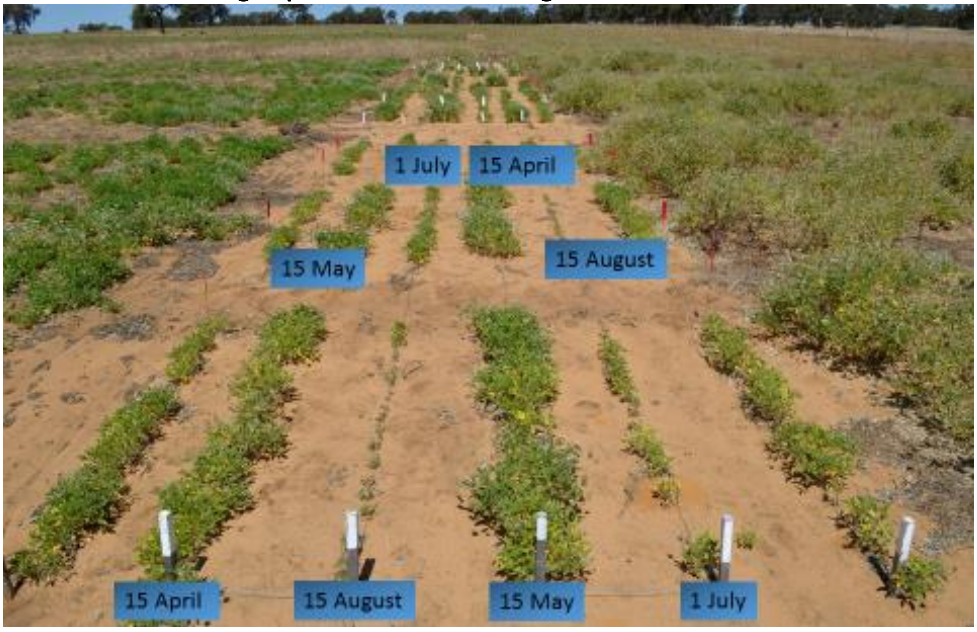
Table 13. Percentage of survival over the first summer for four sowing times at Dandaragan, Cunderdin, and Three Springs.

Time of Sowing	Dandaragan	Cunderdin	Three Springs	Mean
1 April	70.4a [A]	47.7a	25.9a	47.6a
15 May	73.3a	50.2a	27.2a	49.8a
1 July	65.7a	29.0b	13.3b	36.0b
15 August	29.5b	17.0c	10.1b	18.8c
l.s.d. (5%)	13.85	10.99	8.22	5.82
Mean	59.7A [B]	35.4B	19.1C	38.1

[A] Figures in the columns that share a common letter are not significantly different ($p < 0.05$).
[B] Figures in the row for the means that share a common letter are not significantly different ($p < 0.05$).

The rainfall at Cunderdin, Dandaragan, and Three Springs for the six typically dry months from December 2018 to the end of May 2019 was 33.6 mm, 30.0 mm, and 11.4 mm, respectively.
At Dandaragan, the percentage of survival over the first summer for the first three sowing times was similar, while the August time of sowing had a shorter period to develop a deep root system before the extended dry period and had a significantly poorer survival percentage of only 29.5% of germinated seedlings surviving in comparison with 73.3% for the May sowing. A photo of the Dandaragan experiment on the 10 December 2018 at the start of the dry season is presented as Figure 9 and demonstrates the differences in plant size and vigour that affected survival over the first summer from various sowing times.

Figure 9. Time of sowing experiment at Dandaragan on the 10 December 2018.



For Cunderdin and Three Springs, establishment of seedlings from the first two sowing times were similar, while July 2018 and August 2018 had lower percentage of survival over the first summer. At Three Springs, all four times of sowing had poor survival percentage with the best being 27.2% for the May 2018 sowing treatment.

4.2.2 Sowing depth, row spacing, and sowing rate (2017)

4.2.2.1 Plant counts and establishment percentage

The analysis of plant counts/m^2 and establishment percentage for Cunderdin, Dandaragan, and Three Springs resulted in significant differences (5%) for both characters for the “Site” effect (Table 14).

Table 14. Plant counts/m^2 and establishment percentage for Cunderdin, Dandaragan, and Three Springs in 2017.

Site	Plant Counts/m^2	Establishment Percentage
Cunderdin	15.1b [A]	18b
Dandaragan	23.5a	27a
Three Springs	6.7c	8c
l.s.d. (5%)	4.04	3.8

[A] Figures in the columns that share a common letter are not significantly different ($p < 0.05$).

Dandaragan was the site with most plants and highest establishment percentage, followed by Cunderdin and Three Springs. Due to the significant differences between sites, results are presented separately. The significance of the main effects and their interactions for plant counts/m^2 and establishment percentage per site are presented in Table 15.

Table 15. Significance of main effects and their interactions for plant counts/m² and establishment percentage per site.

	Plant Counts/m²			Establishment Percentage		
	Cunderdin	**Dandaragan**	**Three Springs**	**Cunderdin**	**Dandaragan**	**Three Springs**
Row Spacing	n.s. [A]	n.s.	n.s.	n.s.	n.s.	n.s.
Sowing depth	*** [B]	* [C]	***	***	*	***
Sowing rate	***	***	***	n.s.	n.s.	n.s.
Spacing × Depth	n.s.	n.s.	*	n.s.	n.s.	*
Spacing × S. rate	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Depth × S. rate	* [3]	n.s.	n.s.	n.s.	n.s.	n.s.
Spacing × Depth × S. rate	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

[A] Not significant. [B] ***, Highly significant (Pr. < 0.001). [C] *, Moderately significant (0.01 < Pr. < 0.05).

Sowing depth was moderately or highly significant for plant counts/m² and establishment percentage for each of the three sites. Row spacing was not significant and sowing rate was significant for all three sites for plant counts/m² but not for establishment percentage (establishment percentage was largely independent of sowing rate). The two-way interactions were not significant with the exceptions of row spacing × sowing depth at Three Springs and sowing depth × sowing rate at Cunderdin. Both these moderately significant two-way interactions included sowing depth, and difference in sowing depth was the main cause for the significant differences. The three-way interaction was not significant.

- Sowing depth

Plant counts/m² and establishment percentage for the sowing depths of 2 cm and 6 cm for Cunderdin, Dandaragan, and Three Springs are presented in Table 16.

Table 16. Plant counts/m² and establishment percentage for the sowing depths of 2 cm and 6 cm at the three sites.

	Plant Counts/m²			Establishment Percentage		
Depth	**Cunderdin**	**Dandaragan**	**Three Springs**	**Cunderdin**	**Dandaragan**	**Three Springs**
2 cm	23.0a [A]	27.5a	11.6a	27a	31a	14a
6 cm	7.2b	19.6b	1.7b	8b	23b	2b
l.s.d. (5%)	2.78	4.73	1.90	4.1	7.7	1.9

[A] Figures in the columns that share a common letter are not significantly different ($p < 0.05$).

The 2 cm sowing depth was consistently better than 6 cm, with significantly more plants and higher establishment percentage for all three sites. The difference between depths was not as marked at the Dandaragan site where the soil type is a sandy loam and as a result, seedlings were able to germinate from depth better than in the loamy sand soil type of Three Springs or the loam soil type at Cunderdin. However, even at the least stressful site (Dandaragan), sowing at 6 cm reduced the plant count. At Three Springs, the plant counts in the deep sown treatments were extremely low. In the combined analysis of the three experimental sites, the overall mean establishment percentage for the 2 cm and 6 cm of sowing depth were 24% and 11%, respectively. This is a reduction in establishment of 54% due to the increase in sowing depth. It is unclear how this soil-type effect is related to texture, and may represent interactions between soil moisture, temperature and gas exchange: high soil moisture is likely to help seedlings emerge from heavy soils, but soils with low gas-exchange or cold temperatures may reduce germination.

- Row spacing

There was no significant difference in plant counts or establishment percentage among the row spacings of 22 cm, 44 cm, or 66 cm.

- Sowing rate

The plant counts/m^2 for the three sowing rates of 5, 10, and 15 kg/ha at the three sites is presented in Table 17. The plant counts/m^2 were significantly different for the three rates within site. The higher the sowing rate was, the higher the number of plants/m^2. The establishment percentage was not significantly different for the three sowing rates within each site. The mean establishment percentages were 18%, 27%, and 8% for Cunderdin, Dandaragan, and Three Springs, respectively.

Table 17. Plant counts/m^2 for the three sowing rates of 5, 10, and 15 kg/ha at the three sites.

	Plant Counts/m^2		
Rates (kg/ha)	**Cunderdin**	**Dandaragan**	**Three Springs**
5	9.0c[A]	13.2c	3.6b
10	15.8b	21.7b	7.4a
15	20.5a	35.6a	9.1a
l.s.d. (5%)	3.89	5.25	1.74

[A] Figures in the columns that share a common letter are not significantly different ($p < 0.05$).

4.2.2.2 Biomass cuts

Measurement of the 6 cm sowing depth treatment was terminated due to the poor plant establishment across all three sites. Biomass cuts were taken from the 2 cm sowing depth treatment for each site every three months from April 2018.

In April 2018, ANOVA indicated that the DM production of the three sites (Dandaragan, Three Springs, and Cunderdin) and the three sowing rates (5, 10, or 15 kg/ha) were not significantly different. Row spacing was the only significant effect, and none of the interactions was significant. In July 2018 and October 2018, the three main effects were significant (site, sowing rate and row spacing) while in October 2018, the interactions of site by row spacing and site by sowing rate were also significant (Table 18).

Table 18. Dry matter production (kg/ha) in April, July, and October 2018 for significant treatments in the establishment methods experiments at Cunderdin, Three Springs, and Dandaragan.

April 2018					
Row spacing		22 cm	44 cm	66 cm	l.s.d.
		891a [A]	804a	635b	123.6
July 2018					
Site		Cunderdin	Three Springs	Dandaragan	
		686a	635a	536b	70.7
Sowing rate		15 kg/ha	10 kg/ha	5 kg/ha	
		647a	634ab	577b	58.6
Row spacing		22 cm	44 cm	66 cm	
		698a	583b	577b	74.2
October 2018					
Site		Dandaragan	Cunderdin	Three Springs	
		2268a	1522b	1348b	253.3
Row spacing		22 cm	44 cm	66 cm	
		2021a	1688b	1429c	196.0
Sowing rate		15 kg/ha	10 kg/ha	5 kg/ha	
		1958a	1680b	1500c	160.3
Interactions					
Site	Row Spacing	22 cm	44 cm	66 cm	
Dandaragan		2793a	2274b	1736c	359.2
Cunderdin		1788c	1558cd	1221d	
Three Springs		1481cd	1233d	1329d	
Site	Sowing rate	15 kg/ha	10 kg/ha	5 kg/ha	
Dandaragan		2831a	2156b	1816c	321.4
Cunderdin		1569cd	1584cd	1414d	
Three Springs		1474d	1300d	1270d	

[A] Figures within a row or within an interaction block that share a common letter are not significantly different ($p < 0.05$).

The effect of site on DM production was initially small, but the effect of consistently drier conditions at Three Springs became apparent as the experiment continued. In April 2018, dry matter production of the three sites was similar. By July 2018, Cunderdin and Three Springs were the same and better than Dandaragan, respectively, but by October 2018 Dandaragan exceeded Cunderdin and Three Springs, which were similar.

Again, as the experiment progressed, the effect of row spacing on DM production changed, with the advantage of a 22 cm spacing becoming more apparent. In April 2018, across the three sites, the 22 cm and 44 cm spacing were similar and both better than the 66 cm spacing. By July 2018, the 22 cm treatment was the best, and 44 cm and 66 cm were the same, but by October 2018, 22 cm was the highest yielding, followed by 44 cm with 66 cm showing a 30% reduction in yield in comparison to 22 cm spacing.

The effect of sowing rate on DM production was initially negligible, with no significant differences in April 2018, but higher sowing rates produced more biomass at later measurements. The 15 kg/ha treatment was better than 10 kg/ha by July 2018, and the 10 kg/ha better than 5 kg/ha by October 2018.

In January 2019, the overall analysis of variance for the three sites indicated that the DM production was highly significant ($p < 0.007$) for the site effect. The most productive sites were Dandaragan and

Three Springs followed by Cunderdin (Table 19). The analysis of variance within site, showed no significant effects for row spacing, sowing rate, or their interactions.

In July 2019, only Dandaragan was evaluated. Due to a very late start of the season in June 2019, the plants were just conserving moisture during the long summer, autumn, and early winter following the January 2019 cut. The DM production grand mean was 325 kg/ha (Table 19). There were no significant effects of row spacing, sowing rate, or their interaction.

In October 2019, Dandaragan and Cunderdin were evaluated. The site effect was highly significant with production at Dandaragan being more than triple that of Cunderdin. Low production at Cunderdin was not only due to dry conditions but also due to the extreme grazing event in June 2019. At Dandaragan, the sowing rate was highly significant with higher rates producing more DM than lower rates (Table 19). Row spacing and the interaction were not significant. At Cunderdin, there was no significant effect for row spacing, sowing rate, or their interactions.

For total production in 2019 at Dandaragan, the DM grand mean was 2621 kg/ha and the sowing rate was the only significant variable, with the highest sowing rate being the best performer with 2921 kg DM/ha (Table 19).

Table 19. Dry matter production (kg/ha) in January, July, and October 2019 for the establishment methods experiments at Cunderdin, Three Springs, and Dandaragan.

January 2019				
Site	Cunderdin	Three Springs	Dandaragan	l.s.d. (5%)
	260b [A]	388a	472a	112.9
July 2019				
Site	Cunderdin	Three Springs	Dandaragan	
	N.A.	N.A.	325	
October 2019				
Site	Cunderdin	Three Springs	Dandaragan	l.s.d. (5%)
	551b	N.A.	1824a	206.1
October 2019 Dandaragan				
Sowing Rate	15 kg/ha	10 kg/ha	5 kg/ha	l.s.d. (5%)
	2052a	1811b	1609c	174.3
Total for 2019 (January + July + October) Dandaragan				
Sowing Rate	15 kg/ha	10 kg/ha	5 kg/ha	l.s.d. (5%)
	2921a	2614b	2329c	269.0

[A] Figures in a row that share a common letter are not significantly different ($p < 0.05$).

At Dandaragan, on 9 January 2020 and 26 March 2020, the effect of sowing rate, row spacing, and their interaction were not significant, with a grand mean for the two dates of 460 kg/ha and 250 kg/ha, respectively. On 6 July 2020, sowing rate had significant effects on DM production but row spacing and its interaction with sowing rate were not significant. On 15 October 2020, both row spacing, and sowing rate had independent significant effects on DM production, but their interaction was not significant. Row spacing and sowing rate treatments results for 6 July 2020, 15 October 2020, and the total DM production of 2020 are presented in Table 20.

Table 20. Establishment method treatment results for Dandaragan in 2020 including sowing rate, October 2020 DM production, and the total DM production of 2020.

6 July 2020				
Sowing rate (kg/ha)	15	10	5	l.s.d. (5%)
	687a [A]	613a	514b	81.1
15 October 2020				
Row spacing (cm)	22	44	66	l.s.d. (5%)
	2239a	1995a	1483b	277.7
Sowing rate (kg/ha)	15	10	5	
	2102a	1851ab	1764b	266.2
Total Production for 2020				
Row spacing (cm)	22	44	66	l.s.d. (5%)
	3730a	3303ab	2628b	830.0
Sowing rate (kg/ha)	15	10	5	
	3533a	3195ab	2933b	366.0

[A] Figures in the rows that share a common letter are not significantly different ($p < 0.05$).

For 6 July 2020, the 10 and 15 kg/ha sowing rates produced more DM than the 5 kg/ha treatment. For the October 2020 cut, the grand mean was 1906 kg/ha, equivalent to a daily growth of 19 kg/ha. The 22 and 44 cm row spacing were significantly better than 66 cm. The sowing rate of 15 kg/ha was significantly better than 5 kg/ha while the 10 kg/ha was not significantly different to either the 5 or 15 kg/ha treatments.

For the 2020 total annual production, the grand mean was 3220 kg/ha, equivalent to an average daily growth of 8.8 kg/ha. The significance for the main effects and their interactions were the same as for the October 2020 cut, reflecting this cut's large contribution to the overall production. Overall, the best treatment combination came from the highest density planting; the row spacing of 22 cm sown at a rate of 15 kg/ha yielded 4068 kg/ha, 25% more than the average of the experiment.

4.2.3 Row spacing and sowing rate at Manjimup and Merredin (2018)

4.2.3.1 Manjimup

- Plant counts and establishment percentage

For the seedlings counted on 26 July 2018, the higher sowing rate had significantly more seedlings, while row spacing, and all the interactions were not significant. The treatment of 5 kg/ha had 19.7 seedlings/m^2, and the 10 kg/ha had 32.2 seedlings/m^2 (l.s.d. = 9.93). There were no significant main or interaction effects for establishment percentage. The grand mean was 38% establishment, irrespective of the sowing rate.

- Biomass cuts

The biomass evaluation cuts conducted in April 2019, July 2019, and October 2019 had a grand mean biomass of 397 kg/ha, 987 kg/ha, and 1030 kg/ha, respectively. There were no significant effects of sowing rate, row spacing, or any interaction, except for the sowing rate in July 2019, where the higher rate produced more biomass. The total biomass produced in 2019 was 2414 kg/ha with the sowing rate being the only significant effect (Table 21).

Table 21. Manjimup DM cuts in April 2019, July 2019, and October 2019.

July 2019			
Sowing rate (kg/ha)	5	10	l.s.d. (5%)
	837b [A]	1137a	196.9
April 19 + July 19 + October 2019			
Sowing rate (kg/ha)	5	10	l.s.d. (5%)
	2136b	2692a	542.7

[A] Figures in the rows that share a common letter are not significantly different ($p < 0.05$).

In January 2020 and April 2020, the experiment was evaluated, and 360 kg/ha and 1140 kg/ha of DM were harvested, respectively. Neither the main effects nor their interactions were significant on either date. Only row spacing was moderately significant (F pr. 0.069) in April 2020 with a higher mean of 1215 kg/ha at a 17 cm spacing, compared to 1065 kg/ha at a 34 cm row spacing. On 16 June 2020, biomass cuts were taken with a grand mean of 1064 kg/ha, and there was no significant main effect or interactions.

4.2.3.2 Merredin—Mallee duplex and sandy salmon gum

- Plant counts and establishment percentage

For seedling counts taken on 20 July 2018, there was no significant site effect. The sowing rate was the only significant main effect. The treatment of 5 kg/ha had 17.8 seedlings/m^2, and the 10 kg/ha had 37.3 seedlings/m^2 (l.s.d. = 4.99). None of the interactions were significant except for the interaction of site by row spacing, where the sandy salmon gum soil at a 44 cm row spacing had higher seedling counts than all the other five treatment interactions. There was no significant main or interaction effect for establishment percentage except where the Mallee duplex soil at 44 cm spacing was significantly better than the other five site by row spacing interactions. The grand mean was 39% establishment irrespective of the number of seed sown.

- Biomass cuts

The experiments were mowed on 8 July 2019 and 14 October 2019. The sites were not significantly different for total biomass produced but were significantly different at each of the two mowing times (Table 22).

Table 22. Biomass production on 8 July 2019, 14 October 2019, and total production on Mallee duplex and sandy salmon gum soils at Merredin.

Soil Type	**Mallee duplex**	**Sandy salmon gum**	**l.s.d. (5%)**
8 July 2019	361a [A]	205b	65.5
14 October 2019	626b	890a	84.2
Total Production	987n.s	1095n.s.	138.1

[A] Figures in the rows that share a common letter are not significantly different ($p < 0.05$).

For the Mallee duplex on 8 July 2019, the grand mean was 361 kg/ha. The only significant effect was the interaction of sowing rate by row spacing (Table 23). All other main effects and interactions were not significant.

Table 23. Biomass production on 8 July 2019 on a Mallee duplex soil at Merredin.

8 July 2019				
Row spacing (cm)	Sowing rate (kg/ha)	5	10	l.s.d. (5%)
22		387ab[A]	317b	
44		326b	431a	78.6
66		346b	360ab	

[A] Figures in the rows that share a common letter are not significantly different ($p < 0.05$).

On the 14 October 2019 biomass assessment, the grand mean was 626 kg/ha, and neither the main effects nor the interactions were significant.

For the sandy salmon gum site, on 8 July 2019 and 14 October 2019, the grand mean of biomass production was 205 kg/ha and 890 kg/ha, respectively. There was no significant main effect or interaction.

In 2020, the first biomass cut was expected to be in January, but due to dry conditions and lack of plant growth, this cut was not undertaken. Despite the dry conditions, the tedera plants remained green and healthy, but were small. The first cut for 2020 was taken on 7 May, and the second cut was taken on 22 July.

The sites produced significantly different biomass on 7 May 2020 and 22 July 2020 in that the sandy salmon gum site was more productive on both dates than the Mallee duplex soil (Table 24).

Table 24. Biomass production on 7 May 2020 and 22 July 2020 on Mallee duplex and sandy salmon gum soils at Merredin.

Soil Type	**Mallee duplex**	**Sandy salmon gum**	**l.s.d. (5%)**
7 May 2020	206b [A]	261a	39.2
22 July 2020	479b	526a	41.0

[A] Figures in the rows that share a common letter are not significantly different ($p < 0.05$).

For the Mallee duplex soil, on 7 May 2020 there was no significant treatment effect for either the main treatments or the interactions due to the low level of production of all treatments. On 22 July 2020, the only significant effect was the row spacing, with 44 cm the most productive treatment. For the sandy salmon gum, on 7 May 2020, the only significant effect was caused by row spacing, where again, 44 cm spacing was the most productive treatment. On 22 July 2020, the only significant effects were row spacing and sowing rate, with a biomass production of 560 kg/ha for a sowing rate of 10 kg/ha and 492 kg/ha for 5 kg/ha (l.s.d. = 52.9) (Table 25).

Table 25. Biomass production on 22 July 2020 on a Mallee duplex and on 7 May 2020 and 22 July 2020 on a Sandy Salmon Gum soil at Merredin.

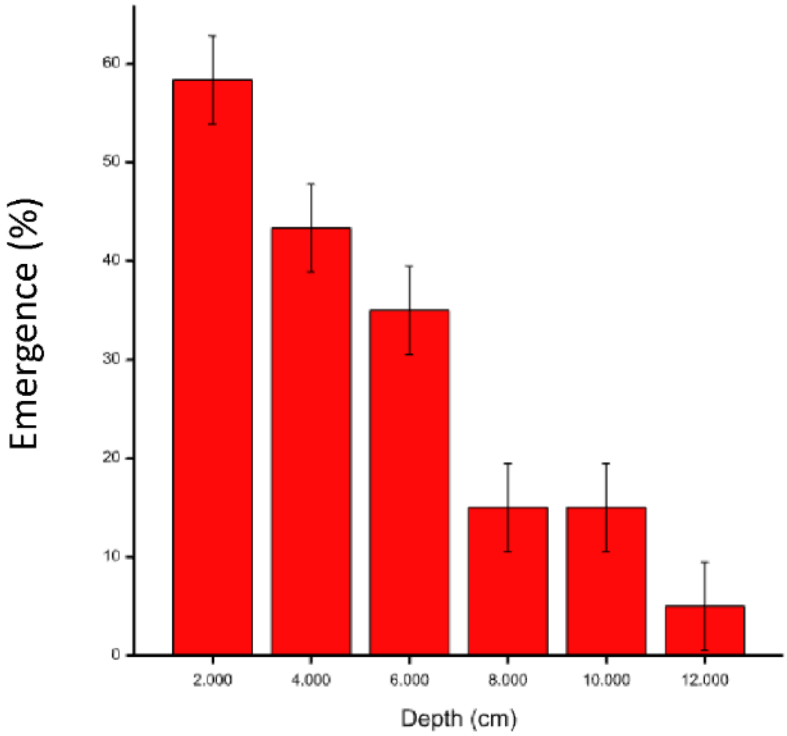
	22 July 2020	7 May 2020	22 July 2020
Soil type	Mallee duplex	Sandy salmon gum	Sandy salmon gum
Row spacing (cm)			
22	423b [A]	228b	476b
44	567a	316a	599a
66	447b	240b	504b
l.s.d. (5%)	95.6	63.9	67.9

[A] Figures in the columns that share a common letter are not significantly different ($p < 0.05$).

4.2.4 Sowing depth in screenhouse (2018)

Amongst the six sowing depths from 2 to 12 cm evaluated, the first seedlings emerged after 8 days in the 2 cm depth treatment. The first seedlings emerged from the 4 cm and 6 cm depth 11 days after sowing, while the first seedlings to emerge from 8, 10, and 12 cm took 14 days, 18 days, and 25 days, respectively. After 75 days, the sowing depth that achieved the best total emergence of 58% was 2 cm, followed by 4 cm (43%), and 6 cm (35%) with similar emergence, while 8 cm (15%), 10 cm (15%), and 12 cm (5%) had similarly poor emergence (Figure 10).

Figure 10. Emergence percentage of tedera seeds sown at 2, 4, 6, 8, 10, and 12 cm of depth.



4.3 Defoliation management

We tested defoliation treatments with two (2x), four (4x) or eight (8x) cuts per year. To compare among treatments, six months was the minimum time required to have 1 cut in the 2x treatment, 2 cuts in the 4x treatment and 4 cuts in the 8x treatment.

In April 2018, before treatments were imposed, there were no treatment differences and the only significant effect was the site effect, with Dandaragan the most productive site, followed by Cunderdin and then Three Springs (Table 26).

From May 2018, the seedling recruitment and defoliation treatments were imposed. Tedera seedlings were hand-weeded from the “no recruitment” treatment and tedera seedlings in the “with recruitment” treatment were allowed to establish. The recruitment management treatment was not significant for any of the assessments over the April 2018 to October 2018 growing season, but significant effects were seen in the biomass production over the dry season.

From April 2018 to October 2018, the 8x treatments were defoliated 4 times (every 45 days), 4x treatments were defoliated twice (every 90 days) and the 2x treatments were defoliated once, 6 months after the April cut. In October 2018 as all treatments were cut, the sum of the 4 cuts in the

8x treatment, the sum of the two cuts in the 4x treatment and the single cut of the 2x treatment produced comparable biomass (Table 26).

Table 26. Dry matter production (kg/ha) in April 2018 and from April 2018 to October 2018 (cumulative) for significant treatments in the defoliation experiments at Cunderdin, Three Springs, and Dandaragan

April 2018					
Site		Dandaragan	Cunderdin	Three Springs	l.s.d.
		823a[A]	708b	435c	87.4
April 2018 to October 2018 (cumulative)					
Site		Dandaragan	Cunderdin	Three Springs	
		2777a	2058b	1951b	404.1
Defoliation/year		2x	4x	8x	
		2629a	2113b	2045b	198.2
Interaction					
Site	Defoliation/year	2x	4x	8x	
Dandaragan		3149a	2418cd	2765b	469.6
Cunderdin		2169cde	2076def	1929ef	
Three Springs		2568bc	1640f	1644f	

[A]Figures in the rows that share a common letter are not significantly different ($p<0.05$).

The site, defoliation/year and their interaction were all significant for the cumulative April 2018 to October 2018 production. The most productive site was Dandaragan while Cunderdin and Three Springs were statistically the same. The best defoliation treatment was 2x, while 4x and 8x were not significantly different. The best treatment combination was produced at Dandaragan with 3,149 kg/ha in the 2x treatment, with the worst biomass production from Three Springs in either 4x or 8x treatments, with 1,640 and 1,644 kg/ha, respectively.

The results of cumulative dry season production in the second year from November 2018 to April 2019 are presented in Table 27.

Table 27. The effect of defoliation frequency and seedling recruitment treatments on cumulative biomass production (kg/ha) from November 2018 to April 2019 for Dandaragan, Three Springs, and Cunderdin.

November 2018 to April 2019					
Site		Dandaragan	Cunderdin	Three Springs	l.s.d.
		852a[A]	361c	506b	100.4
Recruitment		No	Yes		
	Three Springs	486n.s.	526n.s.		186.9
	Cunderdin	399a	323b		45.7
	Dandaragan	907a	797b		110.1
Defoliation/year		2x	4x	8x	
	Three Springs	645a	342c	531b	100.8
	Cunderdin	318b	241c	523a	64.9
	Dandaragan	565c	678b	1313a	90.7
Interaction	Dandaragan				
Recruitment	Defoliation/year	2x	4x	8x	
No		527d	727c	1467a	127.6
Yes		603cd	629cd	1159b	

[A]Figures in the rows that share a common letter are not significantly different ($p<0.05$).

For the first half of the second year, during the dry season period from November 2018 to April 2019, Dandaragan was the most productive site, followed by Three Springs and then Cunderdin. The recruitment effect (allowing recruitment = Yes; not allowing recruitment = No) was significant only for Cunderdin and Dandaragan, with the no recruitment treatment more productive. A possible explanation for this effect is that by removing recruits, there was less competition to the adult plants which are the ones contributing mostly to the biomass harvested with the mower. The effects of defoliations/year behaved differently in Cunderdin and Dandaragan in comparison with Three Springs. Eight times per year was the best treatment at Dandaragan and Cunderdin, while 2x was best at Three Springs. The interaction effect of defoliation/year and recruitment was only significant at Dandaragan, with the best treatment combination again being with no recruitment, but only in the 8x treatment that produced a DM of 1,467 kg/ha.

For the growing season in the third year, from May 2019 to October 2019, and for the full year from November 2018 to October 2019 (Table 28), only Dandaragan has a full set of data to compare the defoliation and recruitment treatments. The only significant effect was the defoliation/year, where the 2x treatment was best in both the growing season and for the full year. Recruitment and interaction effects were not significant.

Table 28. Defoliation and seedling recruitment treatment biomass results (kg/ha) from May 2019 to October 2019 and for November 2018 to October 2019 at Dandaragan.

May 2019 to October 2019 (growing season)					
Site		Dandaragan			
		1965			
Defoliation/year		2x	4x	8x	
	Dandaragan	2491a[A]	1940b	1465c	191.9
November 2018 to October 2019 (full year)					
Site		Dandaragan			
		2817			
Defoliation/year		2x	4x	8x	
	Dandaragan	3055a	2618b	2778b	238.8

[A]Figures in the rows that share a common letter are not significantly different (p<0.05).

For the first half of the fourth year, the dry season from November 2019 to March 2020, the recruitment effect and its interaction with defoliations/year were not significant. However, the effect of defoliations/year was highly significant, with 4x the best treatment followed by 8x and then 2x (Table 29).

Table 29. Defoliation and seedling recruitment treatment biomass results (kg/ha) from November 2019 to March 2020 at Dandaragan.

November 2019 to March 2020				
Recruitment	No	Yes		l.s.d.
	610n.s.	591n.s		100.0
Defoliation/year	2x	4x	8x	
	443.1c[A]	731.4a	627.5b	80.0

[A]Figures in the rows that share a common letter are not significantly different (p<0.05).

Overall, it seems the best defoliation treatment was dependent on site and season, however the 2x treatments (a single cut at the end of the season) were consistently best in the growing season measurements across all sites tested. The effect of defoliation frequency during dry seasons was likely affected by rainfall events at individual sites, but more frequent cuts were often the best treatment. It is likely that the best overall defoliation management for tedera was not evaluated in these experiments, as our results indicate that plants are likely to be most productive when defoliated frequently if they are under stress and allowed to grow and accumulate biomass when conditions are cooler and there is no moisture stress. The results also highlight that the productivity of tedera was highly seasonal, with many treatments accumulating 2 to 3 t/ha biomass over the growing seasons, and generally less than 1 t/ha over dry seasons.

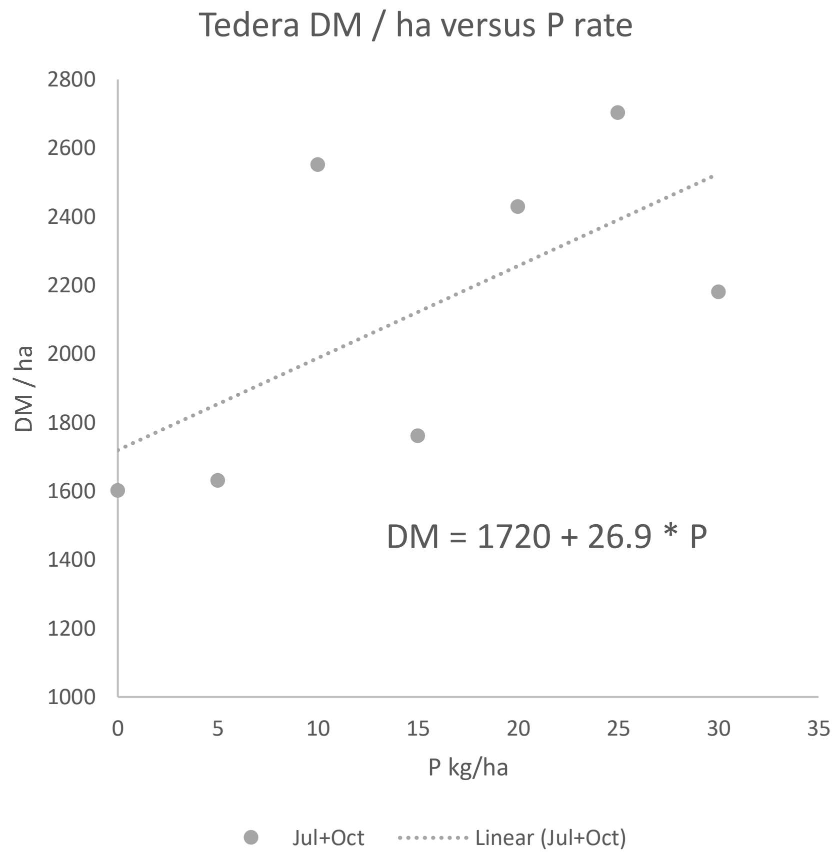
4.4 Fertilization experiments

4.4.1 Field experiment 2017 - 2020

At Three Springs (April 2018 to January 2019) and at Cunderdin (April 2018 to October 2019) there were no significant responses to either P or K for any of the evaluation times.

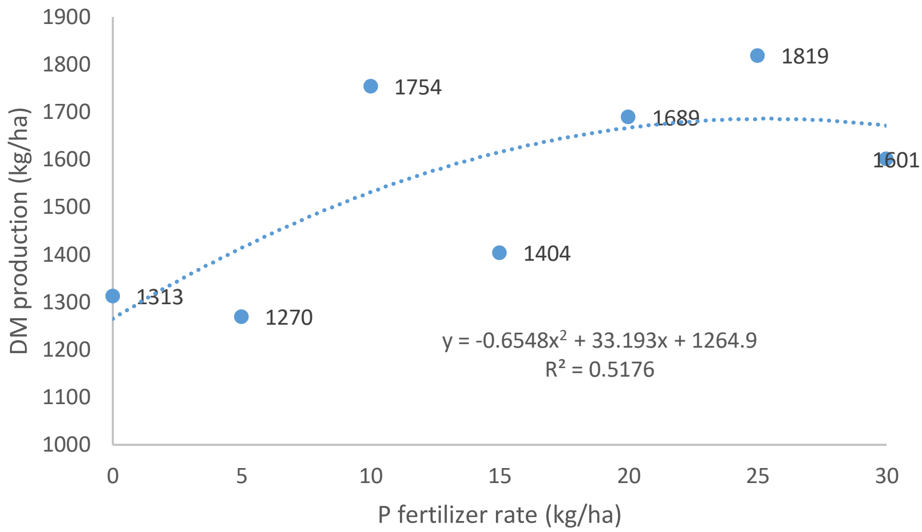
At Dandaragan, there was no response to K for any of the nine evaluations times from April 2018 to July 2020. P response was only significant for July 2018 and October 2018 and October 2019 when growing conditions were best and allowed differences in response to P doses to be expressed. The cumulative biomass of the July 2018 and October 2018 cuts is presented in Fig. 11 and the linear trend was significant (F pr. = 0.011).

Figure 11. Biomass response to P application in tedera for July and October 2018 cuts at Dandaragan.



At Dandaragan in October 2019, there was a significant response to P doses (F pr. = 0.006) (Fig. 12).

Figure 12. Biomass response to P application in tedera in October 2019 at Dandaragan.



4.4.2 Glasshouse experiment 2021

ANOVA results indicated that all genotypes had significant (p<0.1) differences in shoot biomass among the levels tested in all four nutrient sub-experiments (Table 30). Overall, the higher N treatments elicited the highest shoot biomass production across the four sub-experiments.

Table 30. Shoot biomass means (g/pot) and significant differences (P<0.1) in Lanza®, tedera T21 and lucerne SARDI Grazer in response to soil nutrient levels (mg/kg).

Level	1	2	3	4	5	6	7	8	9	10
				Shoot Biomass (g/pot) in response to Colwell P						
P (mg/kg)	**0.0**	**2.4**	**4.0**	**6.6**	**-**	**18.2**	**30.2**	**50.2**	**83.4**	**138.5**
Lanza®	0.37e[A]	2.2cd	3.9b	6.2a	-	2.5c	2.5cd	1.7cd	1.6d	0.46e
Tedera T21	1.5d	4.5c	6.3bc	9.6a	-	10.1a	6.9b	4.3c	1.8d	2.3d
Lucerne	0.19f	1.9ef	3.9d	7.7c	-	9.9ab	10.6a	8.5bc	7.8c	3.2de
				Shoot Biomass (g/pot) in response to Colwell K						
K (mg/kg)	**0.0**	**0.8**	**1.5**	**2.9**	**5.6**	**10.6**	**20.3**	**38.8**	**74.2**	**141.7**
Lanza®	4.8cde	4.4de	5.1bcde	6.5abc	6abcd	6.2abcd	6.7ab	6.6ab	6.9a	3.7e
Tedera T21	7.4d	7.9cd	6.8d	9bc	10.3ab	10.3ab	7.5cd	8.3cd	10.7a	8.1cd
Lucerne	5.5g	7fg	8.2ef	9ef	11.9cd	14.7ab	12.9bc	11.6cd	16.7a	9.9de
				Shoot Biomass (g/pot) in response to Available Soil N						
N (mg/kg)	**0.0**	**0.2**	**0.4**	**0.8**	**1.5**	**3.1**	**5.9**	**11.0**	**18.7**	**24.3**
Lanza®	0.26e	0.26e	0.23e	0.2e	0.25e	0.68de	1.5d	4.2c	8.5b	13.2a
Tedera T21	2.5e	3.8e	3.2e	3.7e	4.2e	7.1d	6.8d	9.7c	13.1b	17a
Lucerne	9.5bc	10.9ab	8.6c	10abc	11.5a	11.8a	11.1ab	9.5bc	11.2ab	11ab
				Shoot Biomass (g/pot) in response to Soil S						
S (mg/kg)	**1.3**	**1.4**	**1.4**	**1.5**	**1.7**	**2.0**	**2.7**	**4.1**	**6.9**	**12.5**
Lanza®	5.1ab	3.9bc	3.3c	3.4c	2.7c	4.3bc	4bc	4.2bc	6.4a	5.2ab
Tedera T21	9.1bc	8.3cde	9.9ab	8.7bcd	9.3bc	7.1e	7.4de	8.8bcd	11.1a	9.7abc
Lucerne	7cd	4.6e	7.4cd	6de	8.1c	8c	7.2cd	11.2b	14.4a	10.7b

[A]Figures in rows that share a common letter are not significantly different (p<0.1).

The shoot biomass and shoot concentration in response to soil nutrient levels of either P, K, N or S for Lanza®, tedera T21 and Lucerne are presented in Figs. 13, 15, 17 and 19, respectively. Images of pots from the different treatments taken at 12 weeks of age are shown in Figs. 14, 16, 18 and 20. Critical nutrient concentrations in soil and shoot to provide 90% of peak productivity, and the levels at which peak productivity occurred are given in Table 31. These critical levels can be used by growers to establish if there is likely to be a profitable response to additional fertiliser in tedera pastures by using a tool such as the Five Easy Steps tool provided by MLA (https://www.mla.com.au/extension-training-and-tools/tools-calculators/phosphorus-tool). The soil nutrient concentrations identified here should be read in the context of the soil type used (a highly leaching soil type with PBI=2.5). Similarly, shoot nutrient concentrations should be read in the context of the sampling method used in this experiment (the entire shoot biomass, including thicker stems was sampled and analysed).

Figure 13. a) Shoot biomass response (g/pot), and b) shoot P concentration (%) in response to increasing levels of Colwell P in soil. Circles, triangles, diamonds represent Lanza®, lucerne and tedera T21, respectively. Dashed, dotted and solid lines are fitted models for Lanza®, lucerne and tedera T21, respectively. An asterisk on the x axis indicates that the soil nutrient concentration was estimated from other soil test results.

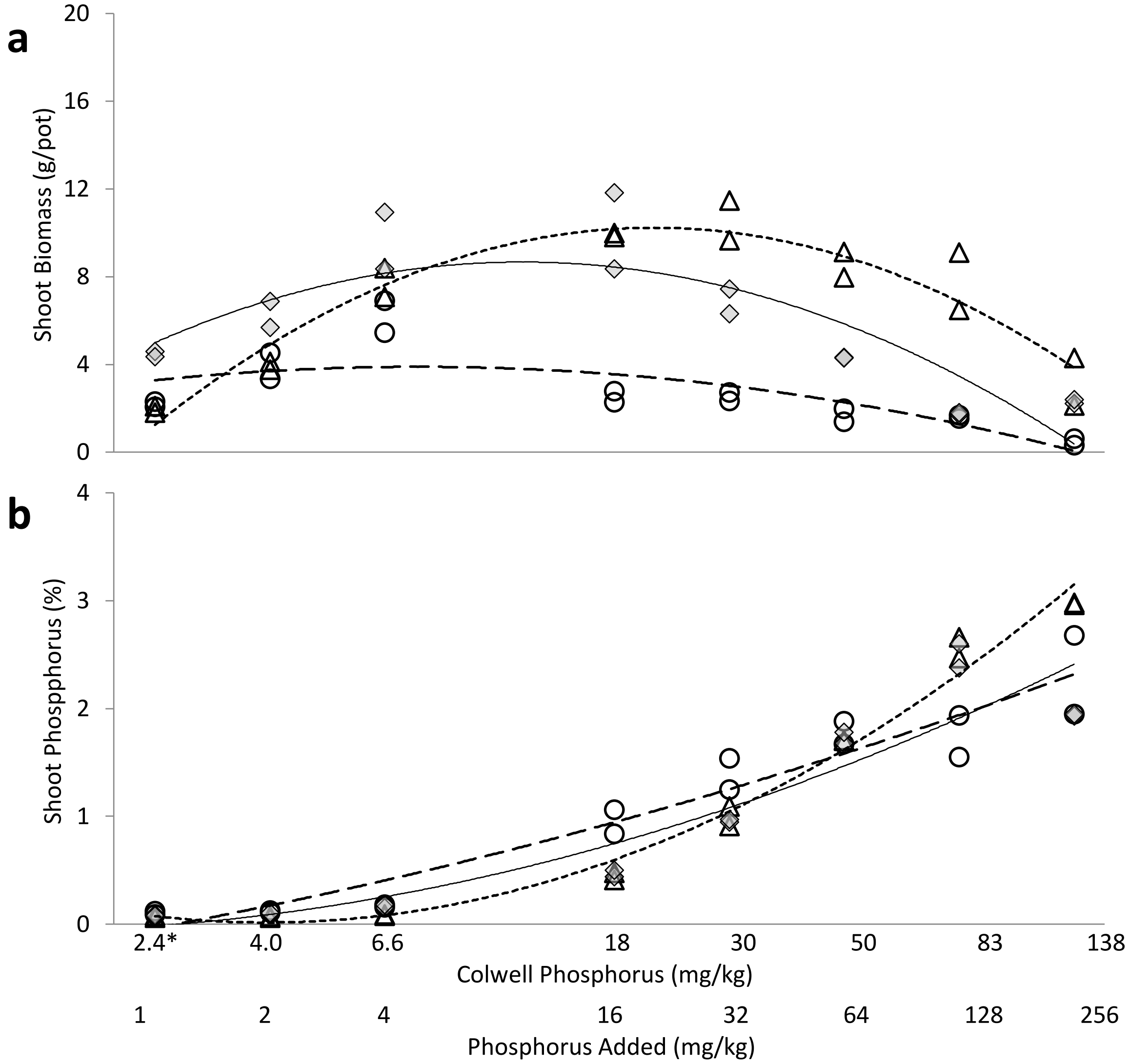
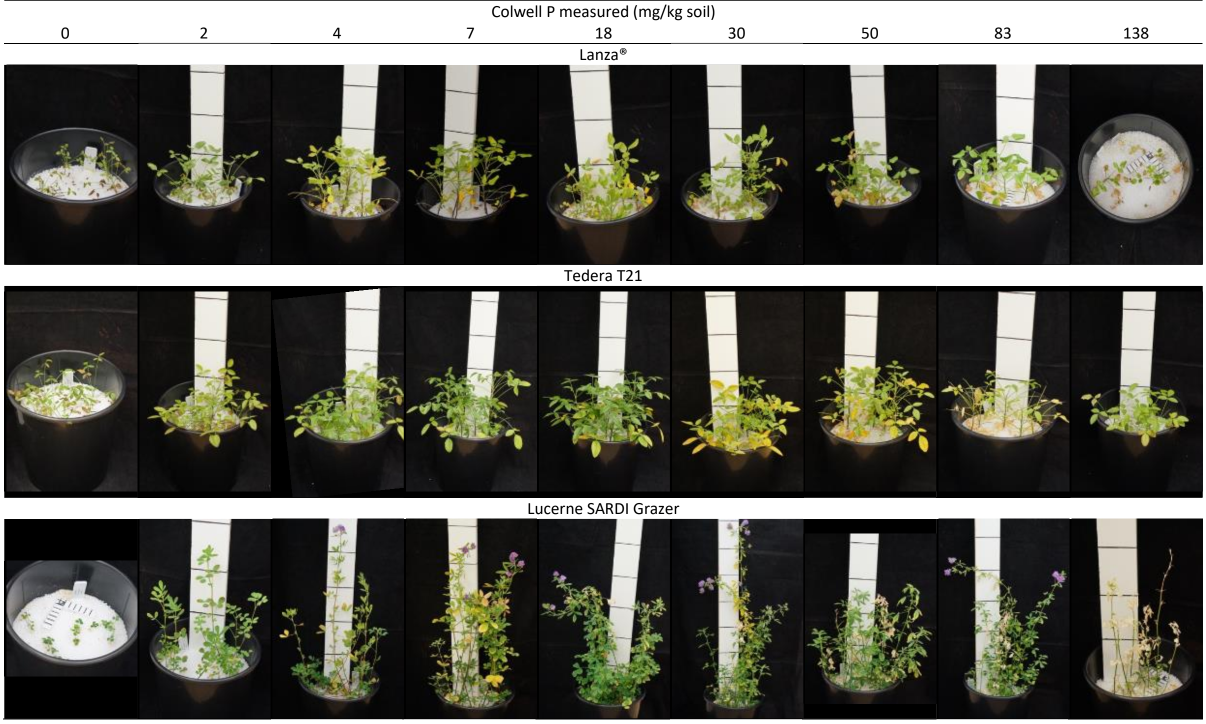


Figure 14. Images of pots containing 12-week-old Lanza®, tedera T21, and lucerne grown in the glasshouse with differing levels of soil Colwell P. Black lines on large scale bars are 10 cm intervals.



All three genotypes showed similar shoot biomass responses to Colwell P, with a rise in productivity as Colwell P increased and then a fall in productivity as Colwell P, and shoot P concentration reached higher levels and shoot P toxicity occurred (Fig. 14). However, the two tedera genotypes reached 90% peak productivity with lower Colwell P levels (3 - 19 mg/kg for Lanza® and 5 – 27 mg/kg for T21) (Table 31), compared to lucerne (10 - 45 mg/kg). The tedera genotypes showed a more severe toxicity response to high P compared to lucerne, with the productivity of tedera being reduced to almost zero, whereas lucerne productivity at the highest P level was reduced to roughly 50% of peak productivity. Once again, all three genotypes had similar responses in shoot P concentration for the bulk of the treatments. Shoot P concentration rose from very low levels (<0.1 %), to levels ca. 2 % in the highest P treatment. Lanza®, T21 and lucerne reached 90% peak productivity with shoot P concentrations of 0.06, 0.19 and 0.24%, respectively. For both tedera genotypes, the decline to 90% of peak productivity set in with shoot P concentrations just below 1.0 %, whereas lucerne tolerated a higher internal P, dropping below 90% productivity at 1.5 % shoot P. The Five Easy Steps tool provided by MLA (https://www.mla.com.au/extension-training-and-tools/tools-calculators/phosphorus-tool) will be useful for growers to interpret these results in the context of their systems.

Figure 15. a) Shoot biomass response, and b) shoot K concentration in response to increasing levels Colwell K in soil. Circles, triangles, diamonds represent Lanza®, lucerne and tedera T21, respectively. Dashed, dotted and solid lines are fitted models for Lanza®, lucerne and tedera T21, respectively. Biomass response of tedera T21 showed no significant fit for added K. An asterisk on the x axis indicates that the soil nutrient concentration was estimated from other soil test results.

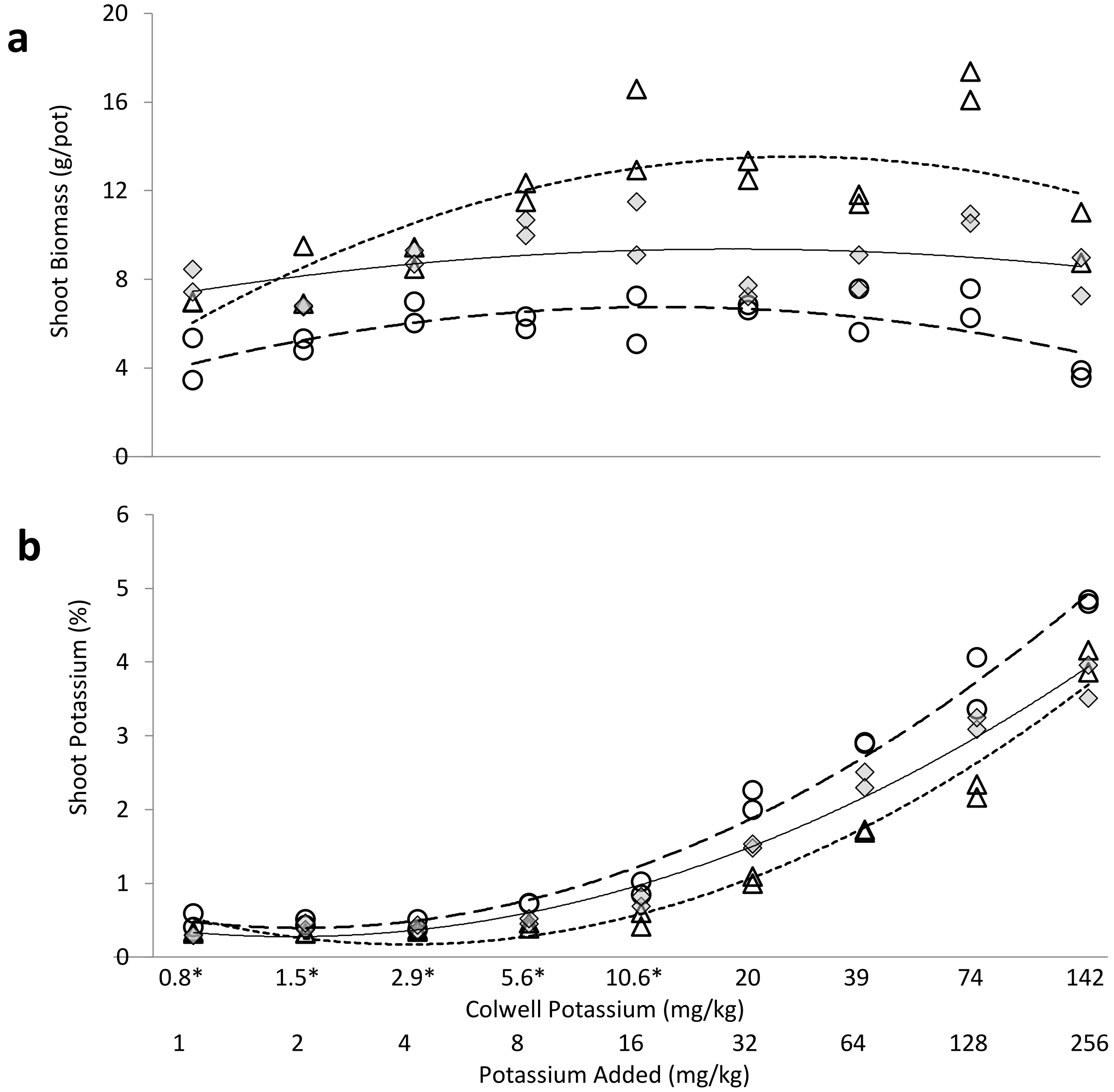
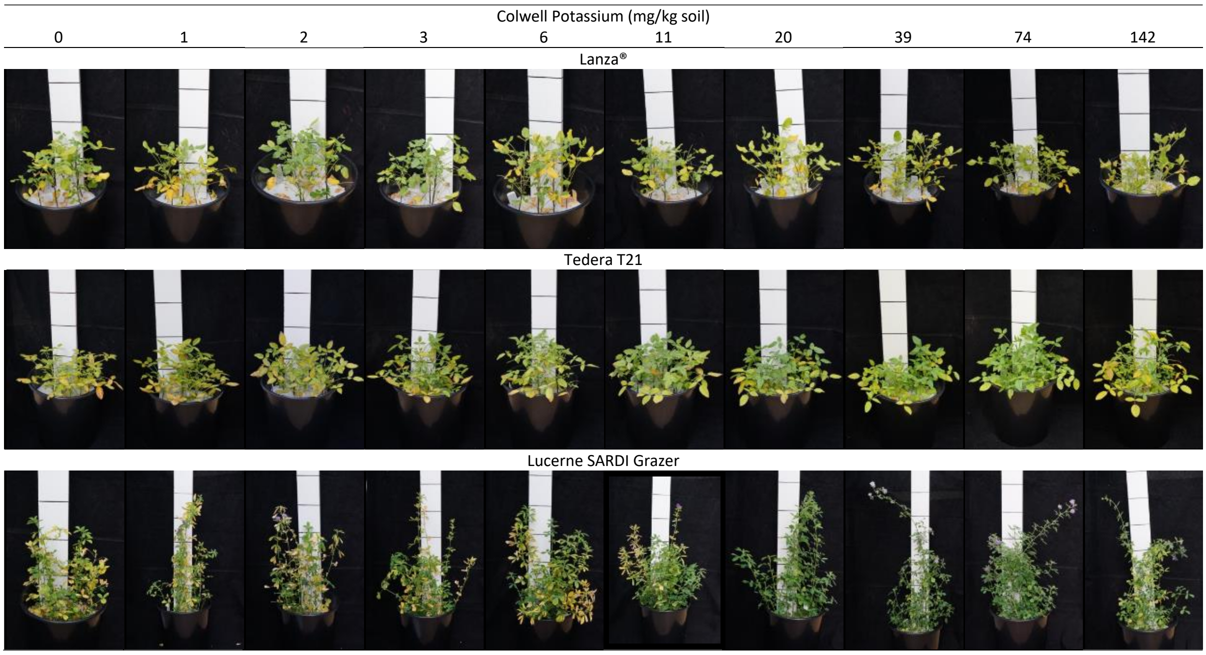


Figure 16. Images of pots containing 12-week-old Lanza®, tedera T21, and lucerne grown in the glasshouse with differing levels of potassium added to soil. Black lines on large scale bars are 10 cm intervals



Lanza® and lucerne showed significant responses to added K, with 90% of peak productivity occurring across a broad range of Colwell K for both genotypes, although lucerne did require a higher Colwell K (3 – 50 mg/kg for Lanza® and 6 to 119 mg/kg for lucerne). The overall biomass benefit of K in Lanza® was less compared to lucerne. In Lanza®, peak productivity was 6.8 g/pot at 12.2 mg/kg Colwell K, roughly a 60% improvement on the productivity at 0.8 mg/kg Colwell K, whereas lucerne produced 13.5 g/pot at peak Colwell K (27 mg/kg) which was roughly a 125% productivity improvement compared to 0.8 mg/kg Colwell K. The shoot K concentration response of the three genotypes to added K followed a similar curve and a similar shoot K concentration was required for to obtain 90% peak productivity (0.5 – 3.1 % Shoot [K] for Lanza® and 0.3 to 3.4 % Shoot [K] for lucerne). T21 appeared to be insensitive to low and high soil K as it did not show a significant relationship between shoot biomass and K added to soil. T21 was also more productive overall than Lanza® at all levels of added K.

Figure 17. a) Shoot biomass response, and b) shoot N concentration in response to increasing levels of Available Soil N. Circles, triangles, diamonds represent Lanza®, lucerne and tedera T21, respectively. Dashed, dotted and solid lines are fitted models for Lanza®, lucerne and tedera T21, respectively. Biomass response of lucerne showed no significant fit for Available Soil N, and no genotype had a significant fit between Available Soil N and Shoot N concentration.

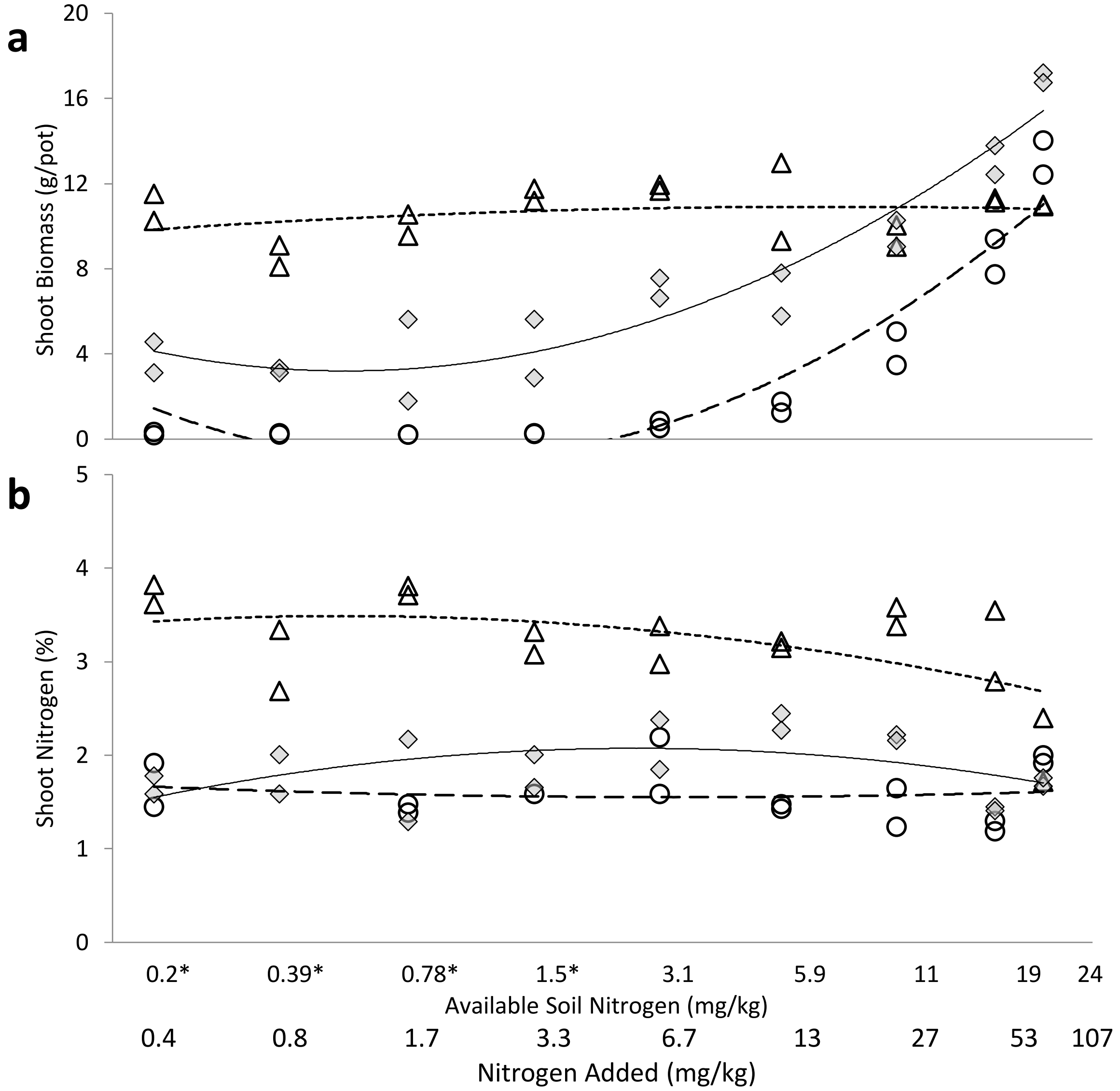
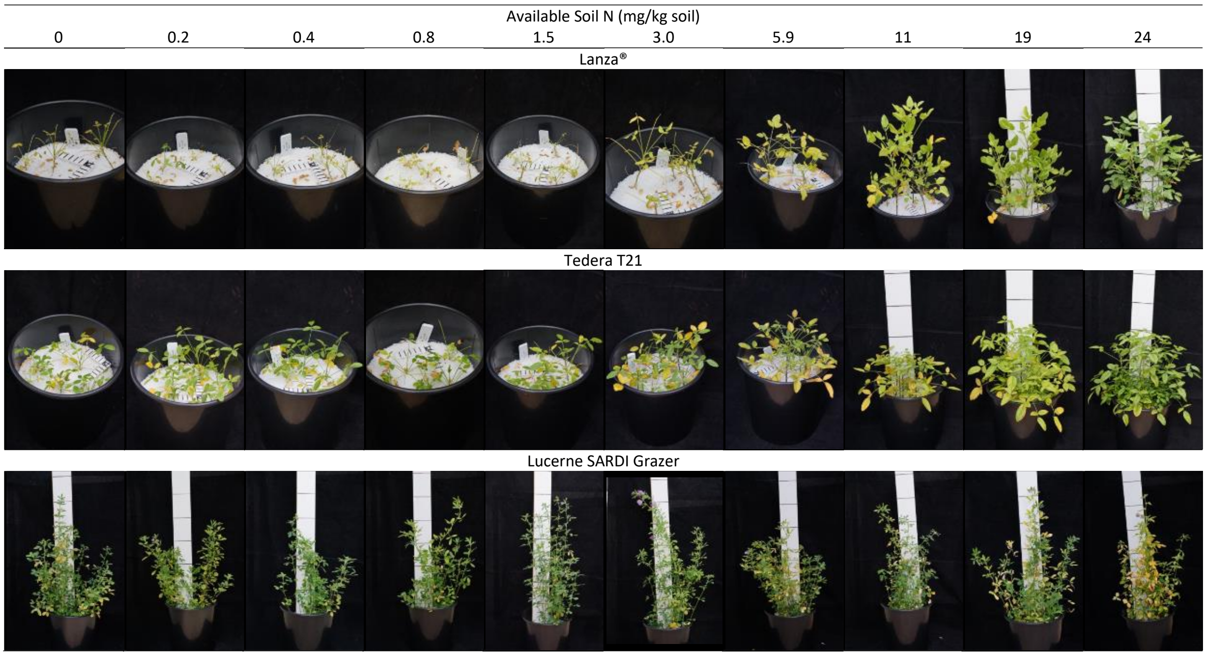


Figure 18. Images of pots containing 12-week-old Lanza®, tedera T21, and lucerne grown in the glasshouse with differing levels of nitrogen in pots. Black lines on large scale bars are 10 cm intervals.



Added nitrogen led to significant responses in shoot biomass for the two tedera genotypes, but not for lucerne. Both tedera genotypes continued to increase biomass up to the highest rate of available soil N, and at the highest rate the productivity of T21 exceeded that of lucerne. The highest level of N also resulted in the highest productivity for the tedera genotypes over the 4 nutrient sub-experiments, possibly indicating that N levels used in the other three sub-experiments was sub-optimal. Assuming modelled shoot biomass at the highest N rate was peak productivity, 90% of the peak productivity coincided with similar levels of total soil N for the tedera genotypes: 20.8 mg/kg for Lanza® and 19.0 mg/kg for T21. However, at these high levels of added N, we observed markedly reduced nodulation in all three genotypes, indicating the plants were accessing added N to satisfy demand, rather than setting up effective nodulation. We also observed that tedera took longer than lucerne to set up nodulation in the lower rates of added N and this provides some explanation for the lower productivity at low soil N levels. However, tedera has been shown to nodulate quickly and effectively in the past (Yates et al., 2009, O'Hara et al., 2014), so our observation may be related to the quality of inoculum used in this experiment. Conclusions regarding shoot N concentration required for peak productivity cannot be drawn from our results as there was no significant model fit between shoot N concentration and soil N for any genotype. Overall, the tedera genotypes appeared to maintain a reasonably stable shoot N concentration that was notably lower than lucerne. This result agrees with nutritive value analysis showing protein content of tedera is lower than lucerne (Adriansz et al., 2017, Real et al., 2018, Oldham et al., 2015, Oldham et al., 2013).

Figure 19. a) Shoot biomass response, and b) shoot S concentration in response to increasing soil S level. Circles, triangles, diamonds represent Lanza®, lucerne and tedera T21, respectively. Dashed, dotted and solid lines are fitted models for Lanza®, lucerne and tedera T21, respectively. Biomass response of tedera T21 showed no significant fit for soil S level.

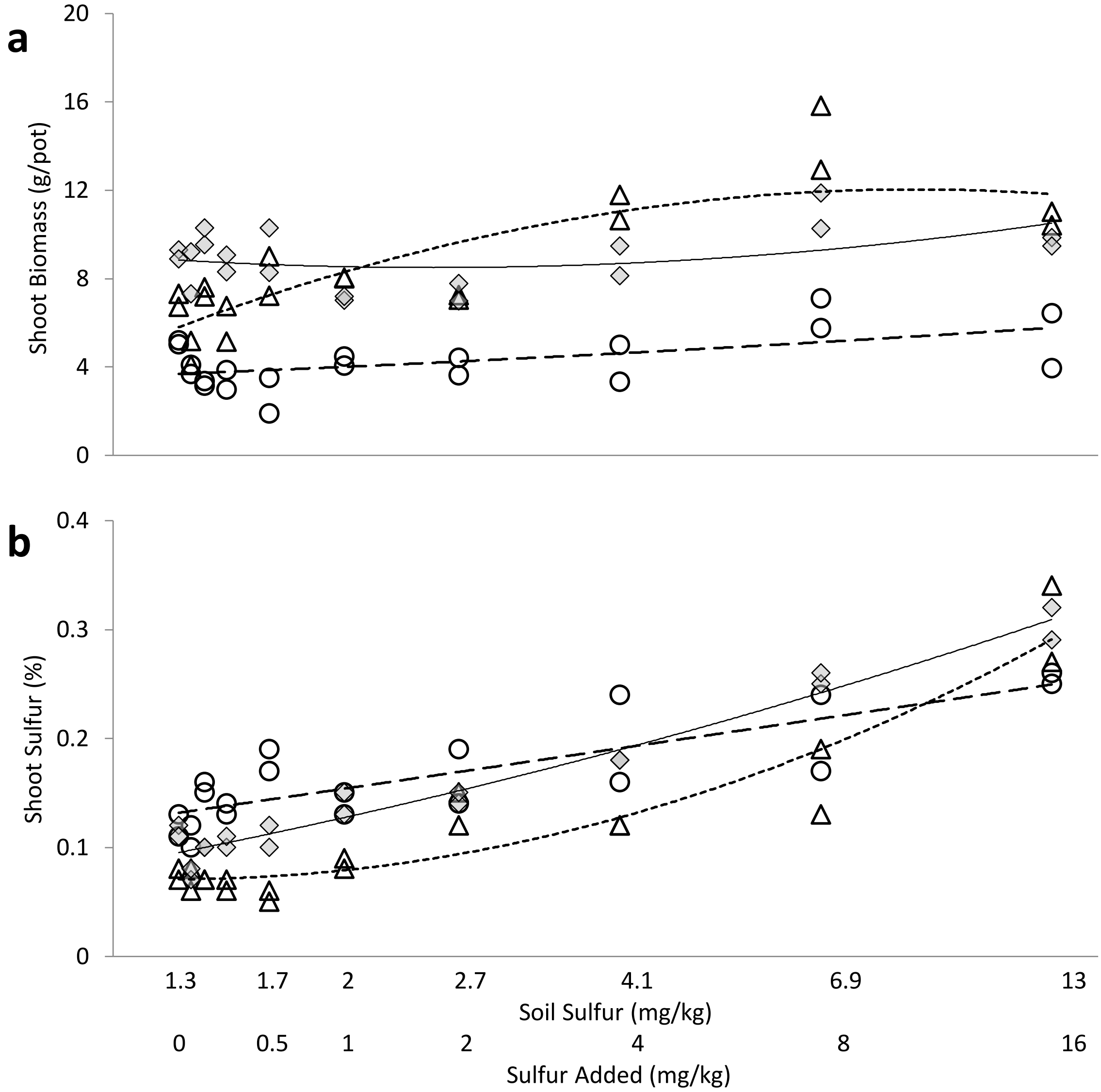
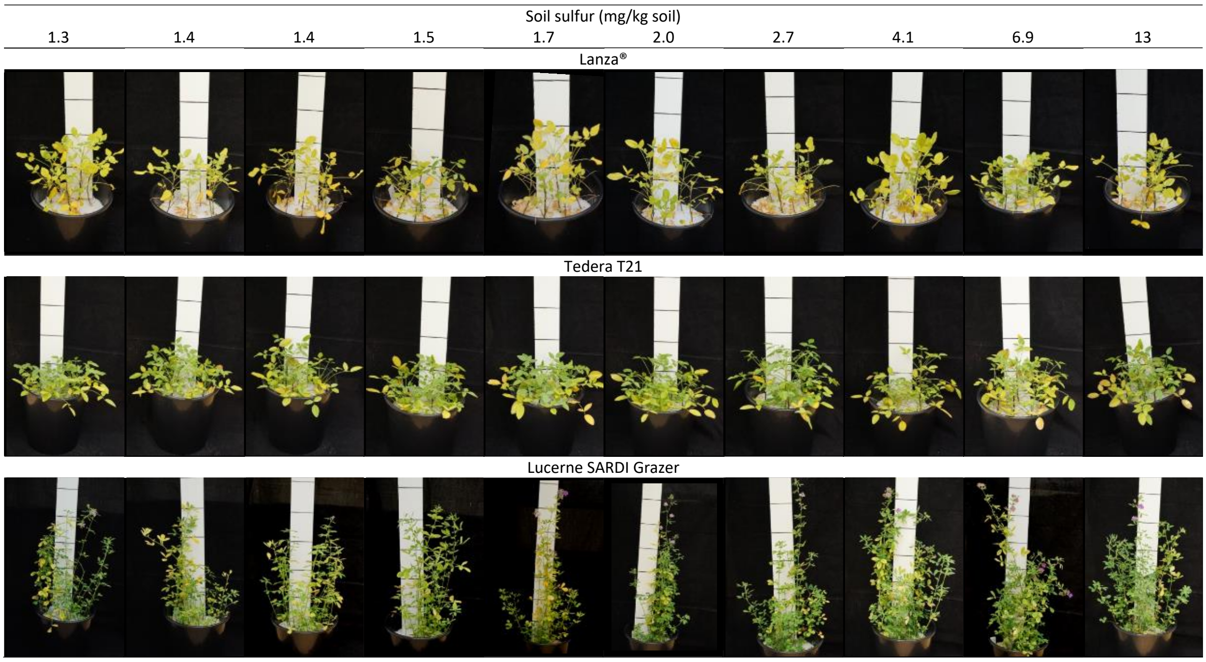


Figure 20. Images of pots containing 12-week-old Lanza®, tedera T21, and lucerne grown in the glasshouse with differing levels of soil S. Black lines on large scale bars are 10 cm intervals.



Tedera genotypes demonstrated little shoot biomass response to soil sulfur, compared to lucerne. Indeed, increasing soil S had no significant effect on T21. Increasing soil S did lead to a significant increase in shoot S concentrations for all genotypes up to the maximum soil S tested here. Assuming the highest soil S produced peak productivity, 90% of peak productivity for Lanza® occurred above 7.4 mg/kg Soil S, and lucerne achieved 90% peak productivity between 3.8 and 20.2 mg/kg soil S (NB. this higher value was extrapolated beyond the values tested here). Within the soil S values tested here, 90% of peak productivity coincided with shoot S concentrations of 0.22 and 0.12 % for Lanza® and lucerne, respectively. The peak productivity of Lanza® among the soil S values tested was 5.8 g/pot and occurred at 13 mg/kg soil S (the highest S level tested). This was a 57% increase over the productivity of Lanza® at the lowest soil S concentration. In contrast, the peak productivity of lucerne was 12 g/pot at 8.8 mg/kg soil S and represented a 112% increase over the productivity at the lowest soil S.

Table 31. P, K, N and S nutrient concentrations in soils (mg/kg) and shoots (%) at which two tedera genotypes and lucerne SARDI Grazer produced greater than 90% of peak biomass based on quadratic models. Measurements outside these figures could indicate deficiency or toxicity.

mg/kg	Lanza®			Tedera T21			Lucerne		
	Lower 90%	Peak	Upper 90%	Lower 90%	Peak	Upper 90%	Lower 90%	Peak	Upper 90%
Colwell soil P	3.0	7.6	19	5.5	12	26.6	10	22	46
Shoot [P]	0.06	0.48	0.98	0.19	0.52	0.99	0.24	0.74	1.5
Colwell soil K	3.0	12	50	NS	NS	NS	6.0	27	120
Shoot [K]	0.50	1.36	3.1	NS	NS	NS	0.31	1.3	3.4
Soil N[A]	21	24[B]	No max	19	24[B]	No max	NS	NS	NS
Shoot [N]	NS	NS	NS	NS	NS	NS	NS	NS	NS
Soil S	7.4	12[B]	No max	NS	NS	NS	3.8	8.8	20[C]
Shoot [S]	0.22	0.25[B]	No max	NS	NS	NS	0.12	0.23	0.39[C]

[A] High levels of soil N reduced nodulation in all three genotypes

[B] Peak productivity was not reached within the soil nutrient concentrations tested (No max) and so the peak productivity level is taken as the maximum productivity

[C] These figures are extrapolated from beyond the range of tested soil S concentrations

NS indicates the model fitted did not show a significant fit between soil nutrient levels and shoot biomass or shoot nutrient concentration

A comparison between the two methods used to understand shoot biomass responses to soil nutrition is presented in Table 32. The lower rates of Colwell P, Colwell K and available soil N required to ensure around 90% optimum production identified by the two methods were similar for all genotypes; likely a reflection of the clear shoot biomass response to these nutrients. This comparison lends confidence that the fitted models are adequately representing the true nature of the soil nutrition responses of these genotypes within the ranges tested.

Table 32. Comparison of two methods (Fisher LSD test vs. Curve Fitting) to identify optimal soil nutrition levels (P, K, N and S) for shoot biomass production in Lanza®, tedera T21 and lucerne SARDI grazer.

Nutrient	Genotype	Fisher LSD test results		Curve Fitting
		Minimum level not significantly different to maximum production	Minimum rate not significantly different to maximum production (mg/kg)	Lower rate at 90% peak (mg/kg)
P	Lanza®	4	6.6	3
P	Tedera T21	4	6.6	5.5
P	Lucerne	6	18.2	10
K	Lanza®	4	2.9	3
K	Tedera T21	5	5.6	NS
K	Lucerne	6	10.6	6
N	Lanza®	10	>24.2	21[A]
N	Tedera T21	10	>24.2	19[A]
N	Lucerne	2	0.4	NS
S	Lanza®	1	1.3	7.4
S	Tedera T21	3	1.4	NS
S	Lucerne	9	6.9	3.8

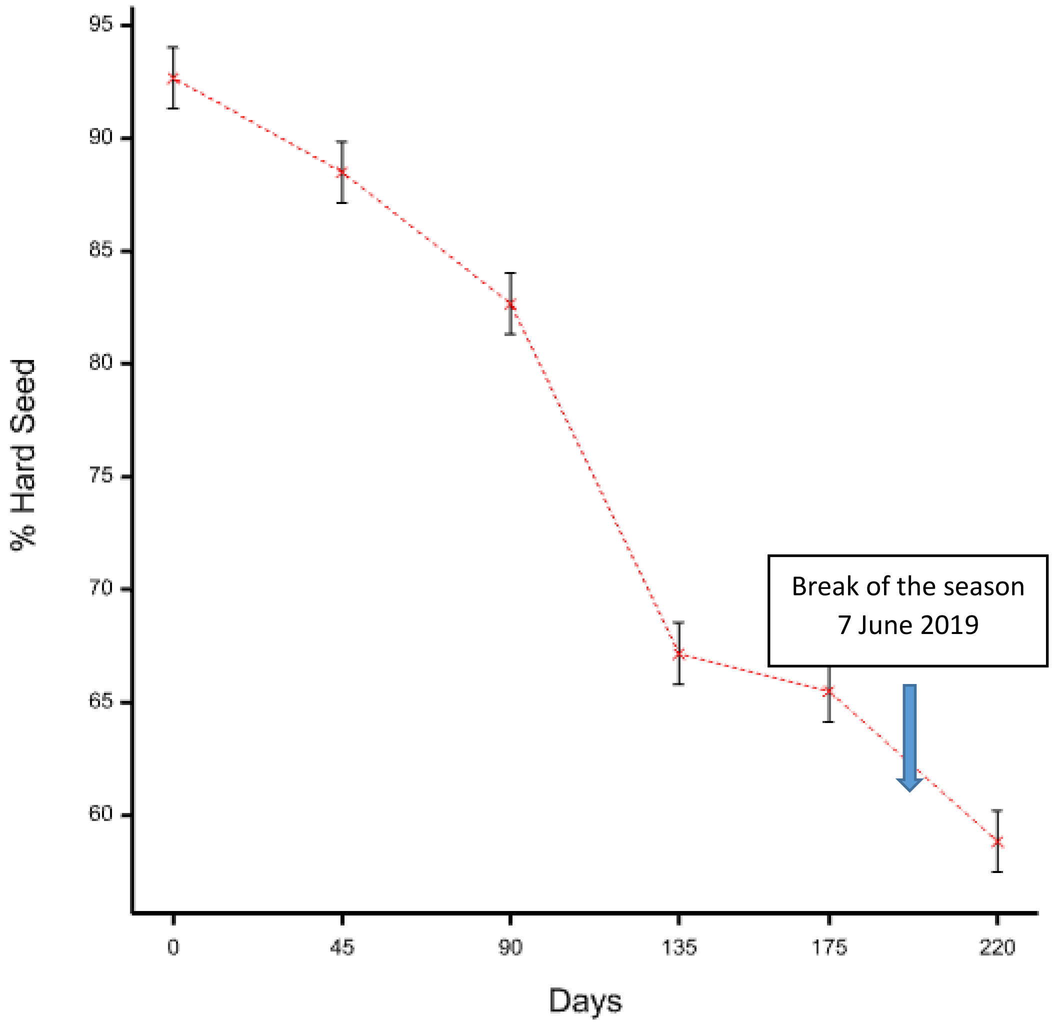
[B] Peak productivity was not reached within the soil nutrient concentrations tested and so the peak productivity level is taken as the maximum productivity

NS indicates the model fitted did not show a significant fit between soil nutrient levels and shoot biomass or shoot nutrient concentration

4.5 Pattern of hard seed softening in the field

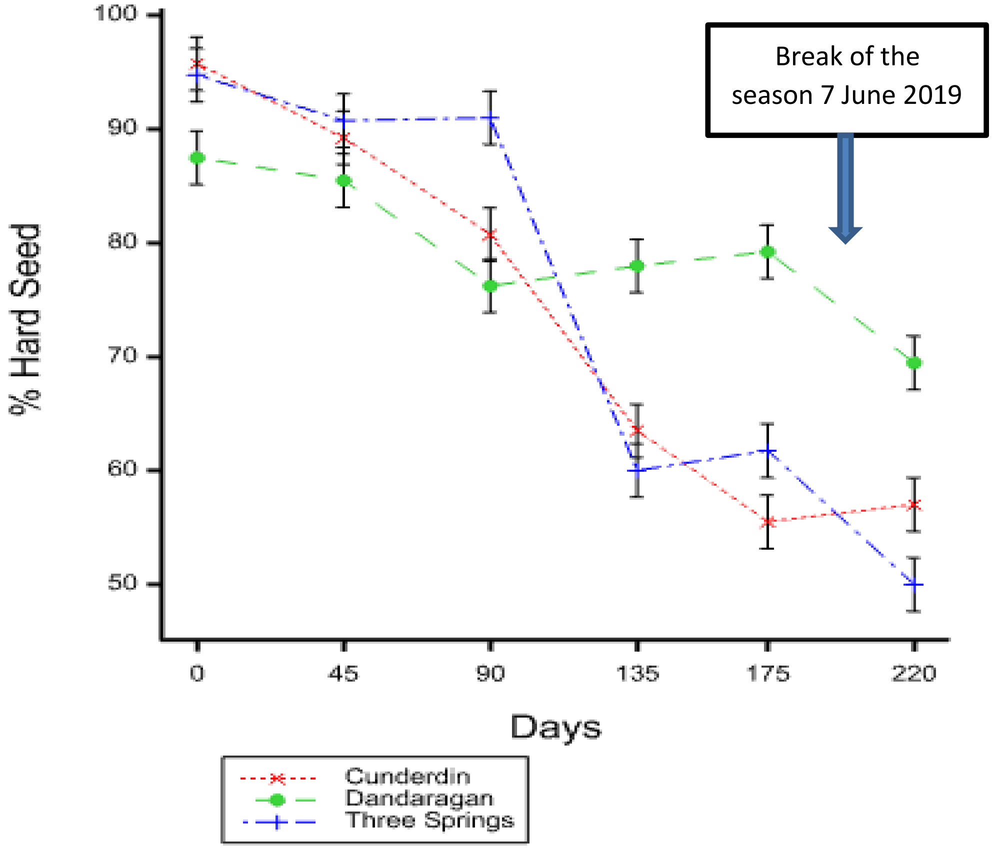
The percentage of hard seed was highly significant for the main effects of site (Cunderdin, Dandaragan, and Three Springs) and days (0 to 220 days), and the interaction of sites x days. The main days effect is presented in Fig. 21. All dates were significantly different except days 135 and 175.

Figure 21. Percentage of hard seed for the main effect of days spent softening in the field from 0 to 220.



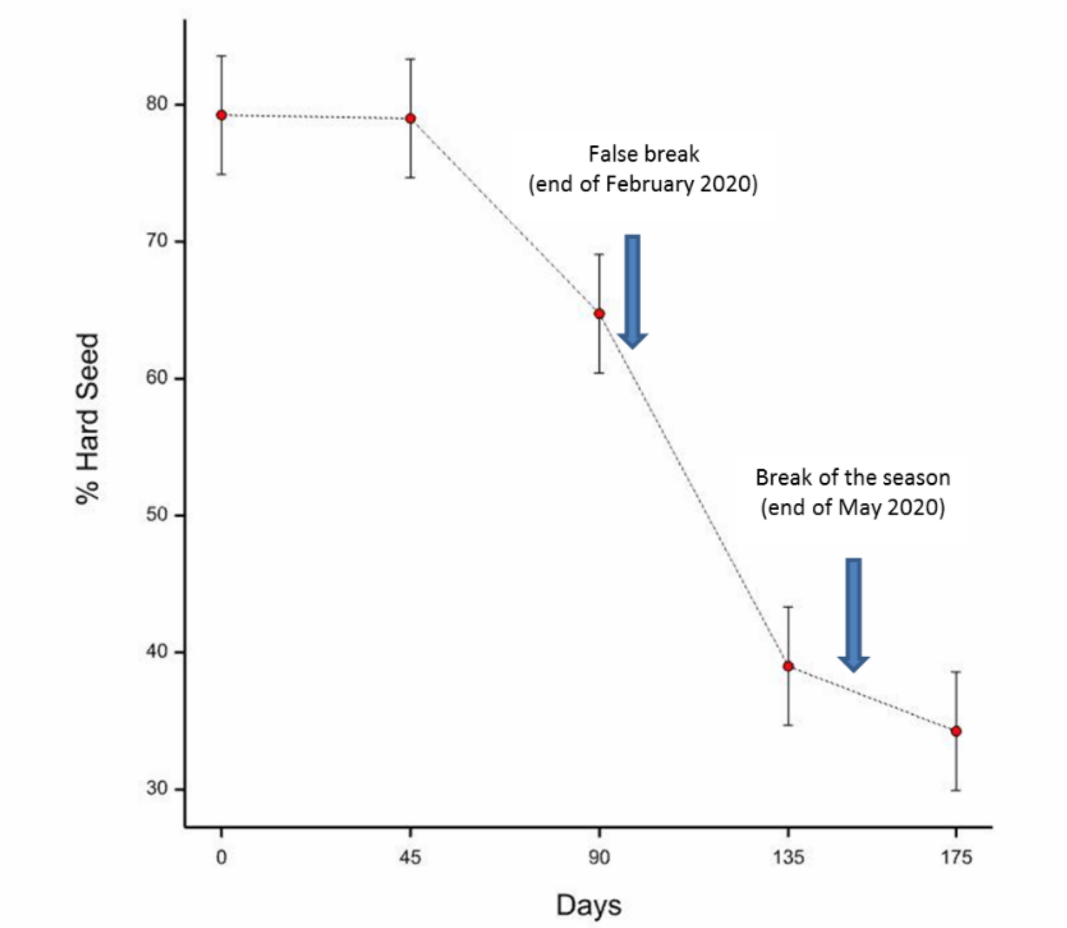
The three sites did not perform in the same way. The percentage of hard seeds for each site from day 0 to 220 is presented in Fig. 22. Seed lots from Three Springs and Cunderdin had a higher percentage of initial hard seed (95%) than Dandaragan (88%), but seed softening was greater and both sites finished with a lower percentage of hard seed in June (50 to 57% cf. 70%).

Figure 22. Percentage of hard seed from 0 to 220 days for Cunderdin, Dandaragan and Three Springs.



For the second Dandaragan sampling, the hard seed break down from December 2019 to June 2020 is presented in Fig. 23. The percentage of hard seed was highly significant for the main effect of days (0 to 175 days). Days 0 and 45 were statistically the same, day 90 was significantly lower and days 135 and 175 were statistically the same but were significantly lower than day 90. The hard seed level remained high in the first two months at 79%, but seeds placed on the soil for 90 days started to soften reaching 64.8% of hard seed by the 14 February 2020, before the unseasonal rainfall at the end of February 2020. At day 135, in mid-April 2020, the hard seed level dropped to 39% and was not significantly different by day 175 on the 2 June 2020 with 34.2% of hard seeds remaining.

Figure 23. Percentage of hard seed for the main effect of days from 0 to 175 at Dandaragan.



While the extent of seed softening varied between sites, a general pattern emerged with slow initial softening, accelerating during early autumn (March-April), and then slowing into late autumn/winter. Such a pattern would offer some protection against false breaks of season.

4.6 Herbicide tolerance

4.6.1 Experiment 1 (2017) Post-emergent herbicides on a 2-year-old tedera seed crop

Visual phytotoxic symptoms (yellowing, chlorosis and/or necrosis) and biomass reduction (%) of two-year old tedera 1 month after 15 post-emergent herbicides were applied is presented in Table 33.

Table 33. Visual phytotoxic symptoms (yellowing, chlorosis and/or necrosis) and biomass reduction (%) of two-year old tedera 1 month after application of 15 post-emergent herbicides.

Herbicide	Rate g a.i./ha	Biomass reduction (%)	Yellowing (%)	Chlorosis (%)	Necrosis (%)
		14 July 2017			
Control		0a[A]	0a	0a	0a
Bentazone	1440	2a	5ab	10b	0a
Cyanazine	1080	5a	32c	28d	27c
Flumetsulam	32	0a	10ab	0a	0a
Diflufenican	100	3ab	0a	15bc	0a
Bromoxynil	400	3ab	12ab	20cd	15b
Butroxydim	45	2a	5ab	0a	0a
Imazamox + Imazapyr	24.75+11.25	0a	60d	0a	0a
Bromoxynil + Diflufenican	250+25	3ab	0a	48e	5a
Propyzamide	1000	2a	0a	0a	0a
Linuron	500	5ab	15b	22cd	12b
Imazamox	35	2a	10ab	0a	0a
Clethodim	120	0a	12ab	0a	0a
Saflufenacil	23.8	10b	33c	7ab	25c
Saflufenacil + Paraquat	23.8+375	58c	0a	0a	58d
Imazethapyr	98	3ab	17b	0a	0a
l.s.d. (5%)		7	14	9	9

[A]Figures in the columns that share a common letter are not significantly different ($p<0.05$).

One month after fifteen herbicides were applied to 2-year-old tedera plants (14 July 2017), thirteen caused no significant biomass reduction, with the exceptions being saflufenacil and saflufenacil + paraquat. Flumetsulam, imazamox, butroxydim, propyzamide and clethodim did not produce significant visual symptoms. Imazamoz + Imazapyr produced the most yellowing, bromoxynil + diflufenican produced the most chlorosis and saflufenacil + paraquat produced the highest necrosis score as expected, since paraquat is a desiccant herbicide.

4.6.2 Experiment 2 (2018). Post-emergent herbicides on a 1-month-old tedera stand

There was a significant effect on tedera biomass by the application of the broad-leaf selective herbicides on the visual biomass reduction estimates conducted on the 13 August 2018 and 14 September 2018 (Table 34).

Table 34. Response of 1-month-old tedera seedlings to post-emergent broadleaf selective herbicides applied on 5 August 2018 (assessed at 8 days and ~6 weeks post application) at Dandaragan.

Broad-leaved herbicide	Rate g a.i./ha	Biomass reduction on tedera (%) 13 August 2018	Biomass reduction on tedera (%) 14 September 2018
Control		3 ab[A]	0 a
Flumetsulam + Diuron	20+50	0 a	0 a
Flumetsulam	20	0 a	0 a
Diflufenican	100	10 b	0 a
Prometryn	400	3 ab	3 ab
Imazamox	35	10 b	5 ab
Imazethapyr	98	18 b	8 ab
Oxyfluorfen	120	79 c	12 b
Pyraflufen-ethyl	8	interaction[B]	interaction
l.s.d.		8	10

[A]Figures in the columns that share a common letter are not significantly different ($p<0.05$).
[B]Interaction with grass herbicide. 82% (13 August 2018) or 60% (14 September 2018) if combined with haloxyfop or butroxydim; otherwise, 0%.

The broad-leaf selective herbicides that produced no biomass reduction to tedera were flumetsulam + diuron and flumetsulam while diflufenical produced some initial biomass reduction but plants fully recovered by two months. Herbicides that produced minor biomass reduction but not significantly different to the control were imazamox, prometryn and imazethapyr. Oxyfluorfen caused a severe biomass reduction, but plants had significantly recovered 10 weeks after application.

There was a significant interaction between the broadleaf selective herbicide Pyraflufen-ethyl and two grass selective herbicides. Pyraflufen ethyl was highly damaging for tedera when combined with haloxyfop or butroxydim. On the 13 August 18, pyraflufen-ethyl+butroxydim and pyraflufen-ethyl+haloxyfop had a tedera biomass reduction of 83.3 and 80.0% respectively, while pyraflufen-ethyl+propyzamide and pyraflufen-ethyl+control had 0.0% biomass reduction. On the 14 September 18, both, pyraflufen-ethyl+butroxydim and pyraflufen-ethyl+haloxyfop had a tedera biomass reduction of 60.0%, while, again, both pyraflufen-ethyl+propyzamide and pyraflufen-ethyl+control had 0.0% biomass reduction. Except when combined with Pyraflufen-ethyl, grass-selective herbicides caused no significant biomass reduction when combined with any other broadleaf selective herbicide.

4.6.3 Experiment 3 (2018). Post-emergent herbicides on a 1-year-old tedera stand

The broad-leaf selective herbicides significantly affected the biomass of tedera and broad-leaf weeds, mainly capeweed (*Arctotheca calendula* (L.) Levyns) (Table 35). The broad-leaf selective herbicides that had least reduction on tedera biomass were flumetsulam + diuron, flumetsulam, prometryn and diflufenican. The best control of capeweed was achieved with flumetsulam + diuron, imazethapyr, prometryn and imazamox. Saflufenacil desiccated the whole plot initially but tedera plants showed good recovery with passage of time.

Table 35. Response of 1-year old tedera stand and cape weed plants to post-emergent broadleaf herbicides applied on 28 June 2018 at Dandaragan, assessed ~6 weeks and 11 weeks after application.

Broad-leaved herbicide	Rate g a.i./ha	Biomass reduction on tedera (%) 13 August 2018	Biomass reduction on tedera (%) 14 September 2018	Cape weed control (%) 13 August 2018	Cape weed control (%) 14 September 2018
Control		0 a[A]	0 a	0 a	0 a
Flumetsulam + Diuron	20+50	2 a	3 ab	95 e	91 e
Imazamox	35	3 ab	15 cd	32 bc	77 de
Diflufenican	100	3 ab	5 abc	15 ab	38 bc
Prometryn	400	5 abc	3 ab	54 cd	78 de
Flumetsulam	20	6 abc	3 ab	19 ab	60 cd
Imazethapyr	98	13 bcd	13 bcd	64 d	85 de
Oxyfluorfen	120	13 cd	17 de	13 ab	33 b
Pyraflufen-ethyl	8	17 d	8 abcd	12 ab	13 ab
Saflufenacil	23.8	70 e	27 e	100 e	92 e
l.s.d.		9	10	23	26

[A]Figures in the columns that share a common letter are not significantly different ($p<0.05$).

The grass selective herbicides tested caused no significant reduction to the biomass of tedera, but significantly reduced the grass weeds, mainly annual ryegrass (*Lolium rigidum* Gaud.) (Table 36). All grass selective herbicides controlled more than 80% of the grasses with no significant differences between treatments on 14 September 2018.

Table 36. Response of post-emergent grass selective herbicides on a 1-year-old tedera stand and grass weeds, assessed ~6 weeks and 11 weeks after application.

Grass herbicide	Rate g a.i./ha	Biomass reduction on tedera (%) 13 August 18	Grass control (%) 13 August 18	Grass control (%) 14 September 18
Control		13	19 a[A]	0 a
Haloxyfop	104	12	49 ab	80 b
Butroxydim	45	14	85 bc	88 b
Propyzamide	1000	13	96 c	93 b
l.s.d.		n.s.	38	17

[A]Figures in the columns that share a common letter are not significantly different ($p<0.05$).

4.6.4 *Experiment 4 (2020). Pre-emergent and post-emergent herbicides on 1-month-old seedlings*

Seedlings in the pre-emergent treatments were counted 1 month after sowing on 29 April 2020 and about 1 month after application for the post-emergent treatments on 28 May 2020 (Table 37). The pre-emergent application of terbuthylazine at both doses, and post-emergent application at the lower does, significantly reduced the plant population, being highly damaging for tedera.

Table 37. Number of tedera seedlings/m² one month after herbicide application.

Herbicide	Rate g a.i./ha	Seedlings/m² (Pre)	Seedlings/m² (Post)
Propyzamide	2000	26 a[A]	21 a
Control		23 ab	20 a
Prosulfocarb + S-metolachlor	4000+600	20 ab	15 ab
Propyzamide followed by Flumetsulam+Diuron	1000+20+90[B]	20 ab	20 a
Prosulfocarb + S-metolachlor	2000+300	20 ab	20 a
Propyzamide	1000	17 b	21 a
Terbuthylazine	900	6 c	8 b
Terbuthylazine	1800	2 c	20 a
l.s.d.		8	8

[A]Figures in the columns that share a common letter are not significantly different ($p<0.05$).
[B]Rate was doubled for post emergent herbicide.

Comparison of the un-sprayed control and herbicide treatments using visual biomass reduction assessments taken on 28 May 2020 and the biomass cuts taken on 25 August 2020 are presented in Table 38. There was no significant effect of time of application or interaction of herbicide by time of application.

Table 38. Visual assessment of tedera biomass reduction taken on the 28 May 2020 and biomass cuts taken on the 25 August 2020, 2 and 5 months after herbicide treatment application at Northam

Herbicide	Rate g a.i./ha	Visual biomass reduction (%)	Biomass (kg/ha)
Untreated Control		0 a[A]	6791 a
Propyzamide followed by Flumetsulam+Diuron	1000+20+90[B]	3 ab	6768 a
Propyzamide	2000	5 ab	6560 ab
Prosulfocarb+S-Metolachlor	2000+300	14 b	5824 bc
Prosulfocarb+S-Metolachlor	4000+600	9 ab	5719 bc
Propyzamide	1000	6 ab	5541 c
Terbuthylazine	900	57 c	2851 d
Terbuthylazine	1800	72 d	1097 e
l.s.d (0.05)		11	897

[A]Figures in the columns that share a common letter are not significantly different ($p<0.05$).
[B]Rate was doubled for post emergent herbicide.

Application of propyzamide alone or followed by flumetsulam + diuron had no significant negative effect on plant population and crop biomass (visual) as compared to untreated control and these were the only two treatments whose biomass was not significantly lower than the control. Prosulfocarb+S-metolachlor at both rates and propyzamide at the lower rate resulted in moderate biomass reduction, while terbuthylazine at both rates was highly damaging to tedera.

4.6.5 *Experiment 5 (2020). Post-emergent herbicides on 5-month-old plants*

Three weeks after spraying on the 21 September 2020, the effect of 11 herbicides on flowering tedera was visually assessed (Table 39). There was a highly significant effect of most herbicides on the flowering of tedera and only the two diflufenican treatments and the flumetsulam + diuron treatment were not significantly different from the un-sprayed control. Despite high variability in the biomass results, the effect of herbicide was significant and four treatments at their highest rate were significantly less productive than the un-sprayed control: MCPA ester + diflufenican, MCPA ester + bromoxynil + diflufenican, bromoxynil + diflufenican and MCPA ester + bromoxynil.

Table 41. Effect of herbicides on tedera flowering three weeks after application (21 September 2020) and biomass production on the 18 December 2020.

Herbicide Active ingredient	Rate g a.i./ha	Flowering reduction (%) 21 Sept 2020	Biomass (kg/ha) 18 Dec 2020
Un-sprayed control		0 a[A]	5830 a
MCPA ester + Bromoxynil	250+250	90 d	5524 a
Diflufenican	100	5 a	5384 a
Diflufenican	300	5 a	4943 ab
Flumetsulam +Diuron	40+180	10 ab	4430 abc
Bromoxynil + Diflufenican	250+25	30 bc	4339 abc
MCPA ester + Diflufenican	250+25	95 d	4268 abc
MCPA ester + Bromoxynil + Diflufenican	250+250+25	82.5 d	3914 abc
MCPA ester + Diflufenican	750+75	97.5 d	3423 bc
MCPA ester + Bromoxynil + Diflufenican	750+750+75	100 d	3140 bc
Bromoxynil + Diflufenican	750+75	47.5 c	2957 c
MCPA ester + Bromoxynil	750+750	100 d	2851 c
l.s.d.		21	1948

[A]Figures in the columns that share a common letter are not significantly different ($p<0.05$).

Echoing the effect on flowering, diflufenican at both rates and flumetsulam + diuron were the only treatments that had no significant negative effect on tedera biomass. Either two-way mixes of MCPA ester, bromoxynil and diflufenican or their three-way mixes reduced tedera biomass and significantly reduced the number of flowers.

4.6.6 Experiment 6 (2020). Post-emergent herbicides on 1-month-old seedlings

Eight weeks after spraying 99 pots with 33 herbicide treatments, the experiment was harvested. Pots with two or less plants remaining were removed from the dataset prior to analysis. The herbicide treatment pyraflufen (label and double label rate) and bromoxynil at the highest rate killed almost all plants in all three replicates, therefore these treatments were not included in the statistical analysis, as they had no data.

The herbicide treatment effect on shoot dry weights and plant height were highly significant with a grand mean of 0.9 g/plant and 8.9 cm respectively. Treatments including bromoxynil, mesotrione, fluroxypyr, and imazamox + imazapyr significantly reduced the shoot dry weight in comparison with the un-sprayed control. Regarding plant height, five treatments (imazamox + imazapyr, bromoxynil + diflufenican, fluroxypyr, mesotrione and flumioxazin) were significantly shorter than the control. The six treatments that had diuron either alone or in mixture with other herbicides were the tallest plants (Table 40).

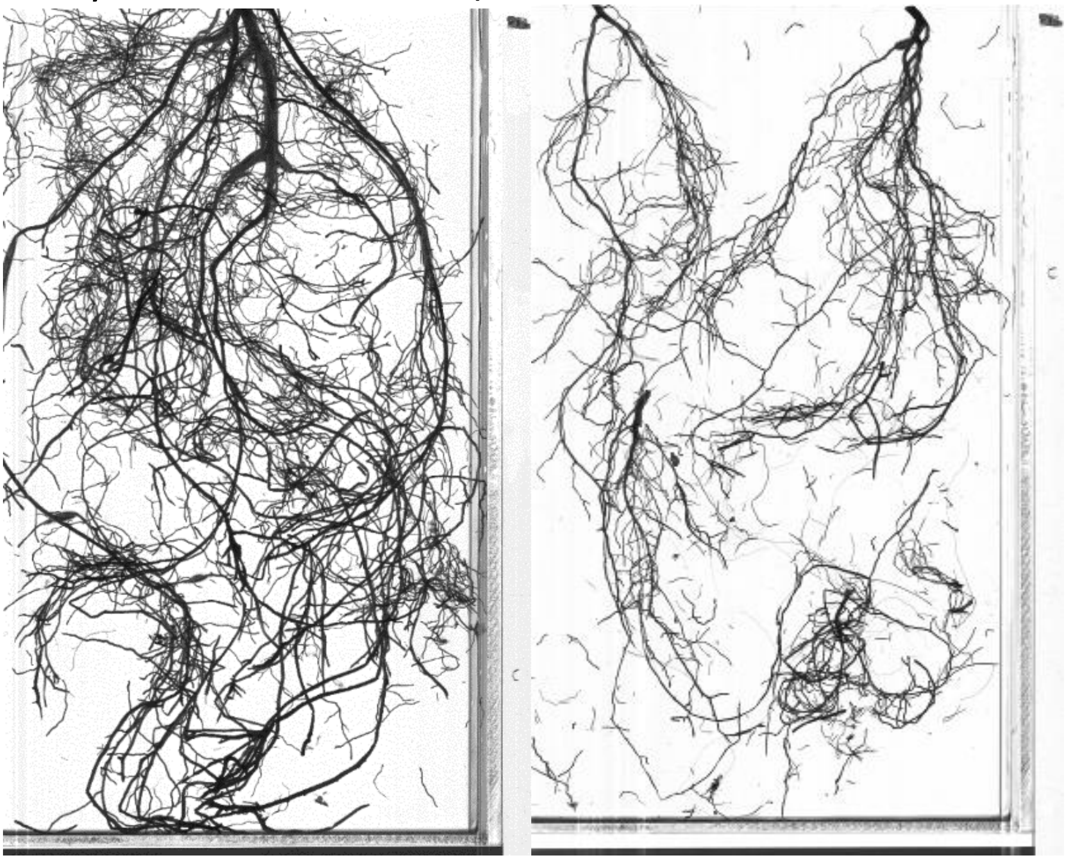
The herbicide treatment effects on root dry weight, average root diameter and total root volume (root scanning image, Fig. 24) were highly significant (Table 40). The mean root dry weight was 0.25 g/plant and the treatments significantly lower than the un-sprayed control were flumioxazin, bromoxynil + diflufenican, and mesotrione at both rates and bromoxynil at the highest rate. The grand mean for the average diameter was 0.54 mm. The four diflufenican treatments, fomesafen and fluroxypyr at their highest rate and carbetamide had thicker roots, while bromoxynil + diflufenican at both rates and mesotrione and diuron at their highest rate had thinner roots than the un-sprayed control. The total root volume grand mean was 1.95 cm^3 and only both rates of bromoxynil + diflufenican and mesotrione had lower volume than the un-sprayed control.

Table 40. Shoot dry weight, plant height, root dry weight, root average diameter and total root volume for tedera herbicide tolerance pot experiment at Northam in 2020

Herbicides	Rate g a.i./ha	Shoot Dry Weight (g/plant)	Plant height (cm/plant)	Root Dry Weight (g/plant)	Root average Diameter/Plant (mm/plant)	Total root volume (cm^3/plant)
Flumetsulam + Diuron	20+90	1.52	13.17 *A	0.41	0.56	2.66
Carbetamide	4140	1.51	11.87	0.32	0.55	2.42
Diflufenican + Pyraflufen	100+8	1.45	9.36	0.36	0.52	2.07
Diflufenican	400	1.39	9.83	0.40	0.60 *	3.08
Fomesafen	360	1.34	10.74	0.45	0.62 *	3.21
Carbetamide	2070	1.30	12.08	0.41	0.59 *	2.84
Fomesafen	180	1.22	8.39	0.32	0.58	2.03
Flumetsulam + Diuron	40+180	1.19	12.79 *	0.30	0.56	1.93
Diflufenican	200	1.17	10.20	0.33	0.62 *	2.25
Un-sprayed control		1.11	9.46	0.35	0.51	2.05
Diflufenican + Flumetsulam + Diuron	50+20+90	1.09	13.73 *	0.30	0.52	1.74
Diflufenican + Pyraflufen	200+160	1.06	9.50	0.24	0.48	1.69
Diflufenican + Flumetsulam + Diuron	100+40+180	1.01	12.49	0.27	0.49	1.87
Diuron	450	0.96	12.77 *	0.28	0.48	1.64
Diflufenican	50	0.96	6.92	0.25	0.60 *	2.10
Diflufenican	100	0.90	7.46	0.23	0.61 *	2.05
Bromoxynil	250	0.79	7.73	0.19	0.50	1.64
Diuron	900	0.75	12.58	0.20 *	0.44 *	1.26
Fluroxypyr	50	0.75	7.06	0.40	0.58	3.12
Flumioxazin	180	0.71	7.34	0.16 *	0.49	1.18
Imazamox+ Imazapyr	10+4.5	0.70	6.88	0.20	0.56	1.40
Flumioxazin	90	0.59	6.10 *	0.13 *	0.55	0.97
Imazamox+ Imazapyr	20+9	0.54 *	3.62 *	0.23	0.56	1.75
Bromoxynil	500	0.54 *	6.64	0.11 *	0.46	0.88 *
Bromoxynil + Diflufenican	500+50	0.42	5.75	0.06 *	0.39 *	0.47 *
Fluroxypyr	100	0.42 *	2.87 *	0.25	0.64 *	1.99
Bromoxynil + Diflufenican	250+25	0.33 *	3.38 *	0.05 *	0.44 *	0.52 *
Mesotrione	192	0.22 *	5.50 *	0.07 *	0.43 *	0.59 *
Mesotrione	96	0.20 *	7.19	0.05 *	0.49	0.48 *

[A]Results with a * are significantly different to the un-sprayed control ($p<0.05$).

Figure 24. (Left) Scanned root image of un-sprayed tedera control; (Right) roots of tedera sprayed with bromoxynil + diflufenican at 250+25 a.i./ha.



4.6.7 Experiment 7 (2021). Post-emergent herbicides on a 3-year-old tedera stand

Results are presented in Table 41 for visual biomass reduction in comparison with control on the 22 July 2021 (4 weeks after application) and 24 August 2021 (eight weeks after application) and biomass yield taken on 31 August 2021 (nine weeks after application) for a 3-year-old tedera crop after being sprayed with 22 herbicide treatments.

Table 41. Visual biomass reduction in comparison with control on the 22 July 2021 and 24 August 2021 and biomass yield on 31 August 2021 for a 3-year-old tedera crop after being sprayed with 22 herbicide treatments.

Herbicide	Rate a.i./ha	Biomass reduction (%) 22 July 2021	Biomass reduction (%) 24 August 2021	Biomass (kg/ha) 31 August 2021
Carbetamide	2070	0 a[A]	0 a	1406 a
Diflufenican + Flumetsulam + Diuron	200+40+180	0 a	0 a	1348 ab
Fomesafen	180	3 ab	10 ab	1308 ab
Un-sprayed Control		0 a	0 a	1262 ab
Flumetsulam + Diuron + Picolinafen	20+90+37.5	13 bcd	10 ab	1258 ab
Flumetsulam + Picolinafen	20+37.5	17 cde	3 ab	1212 abc
Fomesafen	360	5 ab	10 ab	1174 abc
Diflufenican + Flumetsulam + Diuron	100+20+90	3 ab	10 ab	1173 abc
Diflufenican + Flumetsulam + Diuron	400+80+360	3 ab	7 ab	1144 abc
Diflufenican	100	5 ab	3 ab	1134 abcd
Flumetsulam + Diflufenican	20+100	8 abc	7 ab	1087 abcde
MCPB+MCPA+Flumetsulam	600+40+20	25 efg	37 c	1080 abcde
2,4-DB	500	23 def	50 de	986 abcdef
2,4-DB + Flumetsulam	1000+20	37 h	60 efg	966 abcdef
Flumetsulam + Diuron	40+180	3 ab	0 a	922 abcdefg
Picolinafen	37.5	13 bcd	10 ab	859 bcdefg
MCPB + MCPA + Flumetsulam	1200+80+40	30 fgh	53 e	753 cdefg
MCPB + MCPA + Flumetsulam	2400+160+80	35 gh	57 ef	639 defgh
2,4-DB	1000	32 fgh	60 efg	620 efgh
2,4-DB	2000	38 h	67 fg	520 fgh
Flumetsulam + Diuron + Picolinafen	40+180+75	13 bcd	13 b	441 gh
Saflufenacil + Paraquat	23.8+375	50 i	40 cd	434 gh
Glyphosate	450	40 hi	70 g	231 h
l.s.d.		11	11	496

[A]Figures in the columns that share a common letter are not significantly different ($p<0.05$).

There was a highly significant main herbicide effect for the tolerance of tedera for the two visual assessments of biomass reduction in comparison with the control and the biomass cut. In the July observations, application of carbetamide, fomesafen, flumetsulam, diuron and diflufenican either alone or in mixture with other herbicides resulted in less than 5% reduction in biomass. Two months after spraying (August), tedera visual biomass was similar to the un-sprayed control for these five herbicide treatments along with picolinafen at the lower rate alone or in mixtures, except for the higher dose of flumetsulam+diuron+picolinafen.

At the end of August (nine weeks after application), the biomass was on par with untreated control for the all the above-mentioned treatments with the addition of the lower spraying rates of MCPB+MCPA+flumetsulam and 2,4-DB and 2,4-DB+flumetsulam. Long-lasting damage to the 3-year-old-tedera stand was caused by the high application rates of 2,4-DB (1000 and 2000) and MCPB+MCPA+flumetsulam, flumetsulam+diuron+picolinafen, saflufenacil+paraquat and glyphosate.

4.6.8 Experiment 8 (2021). Pre-emergent and post-emergent herbicides on 1-month-old seedlings

Plant counts taken on the 14 December 2021 had a grand mean of 54.6 plants/m^2 and no significant differences among the herbicide treatments. The effect of the herbicide treatments on biomass was highly significant and results are presented in Table 42.

Table 42. Biomass (kg/ha) of the herbicide treatments taken on the 14 December 2021 on 2-month-old plants.

Herbicide	Rate	Timing	Biomass (kg/ha)
Fomesafen	360	IBS[A]	1397 a[C]
Flumetsulam+Diuron	20+90	Post-emergent	1320 ab
Fomesafen	360	Post-emergent	1304 ab
Fomesafen	600	PSPE[B]	1296 ab
Diflufenican+Flumetsulam+Diuron	100+20+90	Post-emergent	1292 ab
Fomesafen+Diuron+Flumetsulam	240+450+40	IBS	1194 abc
Fomesafen	180	Post-emergent	1147 abc
Fomesafen+Diuron	240+450	IBS	1090 abc
Fomesafen	720	IBS	1023 abcd
Un-sprayed control			1021 abcde
Fomesafen	300	PSPE	998 abcdef
Fomesafen+Diuron	240+90	Post-emergent	938 abcdef
Fomesafen+Clopyralid	240+30	Post-emergent	930 abcdef
Clopyralid	90	PSPE	902 abcdef
Aclonifen+Diflufenican+Pyroxasulfone	400+66+100	IBS	883 abcdef
Flumetsulam+Diuron	40+450	IBS	816 bcdef
Clopyralid	45	Post-emergent	749 cdef
Imazamox+Imazapyr	24.8+11.2	Post-emergent	549 d
Imazamox+Imazapyr	12.4+5.6	Post-emergent	538 d
Aclonifen+Diflufenican+Pyroxasulfone	400+66+100	Post-emergent	510 d
l.s.d.			538

[A]IBS – Incorporated by Sowing
[B]PSPE – Post-Sowing Pre-Emergent
[C]Figures in the columns that share a common letter are not significantly different (p<0.05).

All herbicide treatments with fomesafen, flumetsulam, diuron and diflufenican were well tolerated by Lanza® tedera when sprayed pre-emergent (IBS or PSPE) or post-emergent. Tedera sprayed with imazamox+imazapyr at both rates, and aclonifen+diflufenican+pyroxasulfone post-emergent were the least productive, although not significantly different to the un-sprayed control due to large variability in the experiment.

4.6.9 Summary results from 2017 to 2021 of Lanza® tedera tolerance to pre-and post-emergent herbicides

A total of nine pre-emergent and 44 post-emergent herbicide treatments were evaluated in eight herbicide tolerance experiments from 2017 to 2021. Experiments 4 and 8 evaluated pre-emergent herbicides, experiments 2, 4, 5, 6 and 8 evaluated post-emergent herbicides in one-month-old seedlings and experiments 1, 3 and 7 evaluated post-emergent herbicides in tedera plants 1-year-old or older. Some common weeds in WA such as annual ryegrass, capeweed and wild radish are controlled by specific herbicides, however, full list of weeds controlled by each herbicide, can be obtained from Moore and Moore (2021) or their respective commercial labels in the country of interest.

The herbicides evaluated that can control grasses when applied pre-emergent (IBS) were propyzamide, prosulfocarb+S-metolachlor and aclonifen+diflufenican+pyroxasulfone. Propyzamide at the highest dose (2000 a.i. g/ha) in experiments 4 caused no significant negative effect on tedera plant population and crop biomass, however when applied at 1000 a.i. g/ha, there was a significant reduction in biomass. Same lower rate of experiment 4 was applied across all experiment 8 without causing biomass reduction to tedera. Prosulfocarb+S-metolachlor (experiment 4) caused no significant reduction in tedera plant numbers, but there was a significant reduction in biomass in comparison with the un-sprayed control. Aclonifen+diflufenican+pyroxasulfone (experiment 8) caused 14% reduction in Lanza® biomass in comparison with the un-sprayed control, but it was not statistically significant. This herbicide being a ready-mix product of three herbicides (e.g. Mateno® Complete) is a promising option from a grass weed control and herbicide resistance management point of view; however, it will require further evaluation before being recommend for use in tedera as pre-emergent IBS. The three post-emergent grass selective herbicides butroxydim (experiments 1 to 3), clethodim (experiment 1) and haloxyfop (experiments 2 and 3) caused no significant damage to Lanza®. Propyzamide was also evaluated as post emergent (experiments 1 to 4) and caused no damage to Lanza®. Carbetamide is a pre-emergent grass selective herbicide that was only evaluated as post-emergent in experiments 6 and 7. Results were outstanding with no damage to tedera, and it can be recommended for pre and post emergent applications. Prosulfocarb+S-metolachlor was also sprayed post-emergent (experiment4) and had similar results to the pre-emergent application, there was no significant reduction in plant numbers, but there was a reduction in biomass in comparison with un-sprayed control. All the above-mentioned herbicides except aclonifen+diflufenican+pyroxasulfone, are registered in grain legumes for control of a range of grass weeds including annual ryegrass in Australia. Aclonifen+diflufenican+pyroxasulfone is registered in wheat and barley for control of a range of grass weeds in Australia. Use of carbetamide and propyzamide post-emergent could help manage herbicides Group 1 and 2 resistant annual ryegrass populations in tedera-phase of crop sequence in Australia. Resistance to herbicides Group 1 and 2 in annual ryegrass is quite widespread in Australia (Saini et al., 2014, Broster et al., 2019a, Broster et al., 2019b).

The broadleaf pre-emergent herbicides (Table 43) that had no significant reduction in Lanza® biomass in comparison with un-sprayed fomesafen to control radish pre-emergent and the double mix of fomesafen+diuron and the triple mix of fomesafen+diuron+flumetsulam to control capeweed and radish (pre and post-emergent). Flumetsulam+diuron or clopyralid to control post emergent capeweed, aclonifen+diflufenican+pyroxasulfone to suppress post emergent capeweed were also statistically similar to unsprayed control, but they caused more than 10% biomass reduction, therefore more research is required to recommend this herbcides at the dose applied.

Table 43. Tedera tolerance to pre-emergent herbicides to control pre and post-emergent broadleaf weeds. Herbicides/doses (a.i. g/ha) highlighted in green had biomass statistically similar to un-sprayed control (less than 10% biomass reduction), highlighted in yellow were also statistically the same as un-sprayed control (more than 10% biomass reduction) and highlighted in red had significantly less biomass than the un-sprayed control.Herbicide	Exp4	Exp8
Aclonifen+Diflufenican+Pyroxasulfone (IBS)		400+66+100
Fomesafen (IBS)		360; 720
Fomesafen (PSPE)		300; 600
Fomesafen+Diuron (IBS)		240+450
Fomesafen+Diuron+ Flumetsulam (IBS)		240+450+40
Flumetsulam+Diuron (IBS)		40+450
Clopyralid (PSPE)		90
Terbuthylazine (IBS)	900;1800	

The evaluation of tedera tolerance to post-emergent herbicides was conducted on 1-month-old seedlings to maximize the weed control when weeds were still small and most susceptible. Tedera seedlings tolerance from five experiments are presented in Table 44.

Table 44. Tolerance of 1 month old seedlings of Lanza® tedera to post-emergent herbicides to control broadleaf weeds. Herbicides/doses (a.i. g/ha) highlighted in green had biomass statistically similar to un-sprayed control (less than 10% biomass reduction), highlighted in yellow were also statistically the same as un-sprayed control (more than 10% biomass reduction) and highlighted in red had significantly less biomass than the un-sprayed control.

Herbicide	Exp2	Exp4	Exp5		Exp6[1]				Exp8
Aclonifen+Diflufenican+ Pyroxasulfone									400+66+ 100
Bromoxynil					250	500	1000		
Bromoxynil+Diflufenican			250+25	750+75	250+25	500+50			
Diflufenican	100		100	300	50	100	200	400	
Diflufenican+Pyraflufen					100+8	200+16			
Diflufenican+Flumetsulam+Diuron					50+20+90	100+40+180			100+20+90
Diuron					450	900			
Flumetsulam+Diuron	20+50	20+90; 40+180	40+180		20+90	40+180			20+90
Flumetsulam	20								
Flumioxazin					90	180			
Fluroxypyr					50	100			
Fomesafen					180	360			180; 360
Fomesafen+Diuron									240+90

Fomesafen+Clopyralid								240+30
Imazamox+Imazapyr					10+4.5	20+9		12.4+5.6; 24.8+11.2
Imazamox	35							
Imazethapyr	98							
Clopyralid								45
MCPA+Bromoxynil			250+250	750+750				
MCPA+Diflufenican			250+25	750+75				
MCPA+Bromoxynil+ Diflufenican			250+250+25	750+750+75				
Mesotrione					96	192		
Oxyfluorfen	120							
Prometryn	400							
Prosulfocarb + S-Metolachlor		2000+300; 4000+600						
Pyraflufen-ethyl	8				16	32		
Terbuthylazine		900;1800						

[1]Colour category assigned based on shoot and root biomass reduction (%)

From the 27 herbicide treatment combinations evaluated with either one or multiple doses and up to five experiments, the most consistently well tolerated herbicide by tedera seedlings was fomesfen up to double the label rate (for other crops). Fomesafen is a herbicide widely utilized to control weeds in soybean crops (Oliveira et al., 2017). Tedera and soybean are genetically close relatives (Nelson et al., 2020, Pazos-Navarro et al., 2011), therefore the genetic mechanisms of tolerance in soybean might apply to tedera. Fomesafen is not registered for use in clovers and medics and in fact, there are papers reporting damage in white clover (*Trifolium repens* L.) (Schuster et al., 2015) and lucerne (*Medicago sativa* L.) (Hijano et al., 2013). Further studies are required in WA, but it might be possible to control some of the clovers and medics in tedera stands with fomesafen. Flumetsulam and diuron were tolerated well either alone or in mixes with other herbicides. Diflufenican was well tolerated up to four-times the label rate, but some early damage occurred in experiments 2, 5 and 6. Kelly (2008) reported flumetsulam and diflufenican as safe herbicides well tolerated by tedera seedlings. Gray (2011) and Seednet at Keith (unpublished) reported 14% and 10% biomass reduction for flumetsulam and 30% and 40% biomass reduction for diflufenican, that were more damaging than results than in our experiments. Different combinations of flumetsulam, fomesafen, diuron and diflufenican can provide good control of capeweed and radish. Gray (2011) also reported a 55% biomass reduction for imazamox (12.3 a.i. g/ha) and a 30% biomass reduction for imazethapyr (35 g a.i./ha) that agrees with our yellow classification. Prometryn and fomesafen+clopyralid at label rates were also well tolerated but needs further evaluation as they were only evaluated in one experiment. MCPA and Bromoxynil were tolerated at label rates, but they caused damage at higher rates. Kelly (2008) reported tedera seedling to be susceptible to MCPA (375a.i. g/ha) and moderately susceptible to bromoxynil (300 a.i. g/ha), while Gray (2011) reported 80% reduction in biomass for bromoxynil sprayed at 56 a.i. g/ha. None of these two herbicides should be recommended on Lanza® tedera as these recorded low crop safety margins.

Adult tedera tolerance from three experiments is presented in Table 45.

Table 45. Tolerance of adult Lanza® tedera (one year old or older) to post-emergent herbicides to control broadleaf weeds. Herbicides/doses (a.i. g/ha) highlighted in green had biomass statistically

similar to un-sprayed control (less than 10% biomass reduction), highlighted in yellow were also statistically the same as un-sprayed control (more than 10% biomass reduction) and highlighted in red had significantly less biomass than the un-sprayed control.

Herbicide	Exp1[1]	Exp3	Exp7		
2,4-DB			500	1000	2000
2,4-DB+Flumetsulam			1000+20		
Bentazone	1440				
Bromoxynil	400				
Bromoxynil+Diflufenican	250+25				
Cyanazine	1080				
Diflufenican	100	100	100		
Diflufenican+Flumetsulam+Diuron			100+20+90	200+40+180	400+80+360
Flumetsulam+Diuron		20+50	40+180		
Flumetsulam	32	20			
Flumetsulam+Diflufenican			20+100		
Flumetsulam+Picolinafen			20+37.5		
Flumetsulam+Diuron+ Picolinafen			20+90+37.5	40+180+75	
Fomesafen			180	360	
Glyphosate			450		
Imazamox+Imazapyr	24.75+11.25				
Imazamox	35	35			
Imazethapyr	98	98			
Linuron	500				
MCPB+MCPA+ Flumetsulam			600+40+20	1200+80+40	2400+160+80
Oxyfluorfen		120			
Picolinafen			37.5		
Prometryn		400			
Pyraflufen-ethyl		8			
Saflufenacil	23.8	23.8			
Saflufenacil+Paraquat	23.8+375		23.8+375		

[1]Colour category assigned based on biomass reduction (%), yellowing (%), chlorosis (%) and/or necrosis (%).

From the 26 herbicide treatment combinations evaluated with either one or multiple doses and up to three experiments, the most consistently well tolerated herbicides by adult plants of Lanza® tedera were very similar to the herbicides tolerated by the seedlings. Diflufenican and flumetsulam up to four-times the label rate, and fomesafen up to double the label rate and mixes with diuron. Different combinations of two or three of these herbicides can provide good control of capeweed and radish. Kelly (2008) also reported flumetsulam and diflufenican as safe herbicides on mature tedera plants while MCPA and glyphosate caused damage. Moore (2014) also supported the safe use of flumetsulam (10-times the label rate) and reported that adult tedera plants can recover with less than 10% biomass reduction four weeks after spraying with 10-times the label rates of imazamox and imazethapyr. Prometryn at label rate was also well tolerated but needs further evaluation as it was only evaluated in experiment 3. Saflufenacil+paraquat desiccated the tedera stand, however tedera as a perennial species was able to recover from being desiccated and grew back very well. This management practise can be very useful in winter when heavy weed infestations of several annual species could be present, and this is an effective way of controlling a diverse range of weeds. Tedera seed crops also had tolerance to being desiccated annually with diquat sprayed at 600 g/ha before harvesting seed in late spring.

These results identified well tolerated herbicides by tedera but should not be taken as recommendations as off-label use of herbicides on tedera cannot be advised.

4.7 Demonstration sites sown in WA in 2017 and 2018

4.7.1 Dandaragan, Yallalie Downs - Establishment with a grass species companion – Panic grass

In 2017, at Dandaragan, Yallalie Downs Farm (Table 1), a split plot experiment was sown with the main plot of with or without 20 kg/ha of P fertilizer and three sub plots as pure panic grass, pure tedera or alternate rows of tedera and panic grass with two replicates. Tedera was sown at 10 kg/ha and panic grass at 5 kg/ha in rows 44 cm apart.

This demonstration site established well and was grazed with cattle by the farmer as part of the normal grazing of the panic grass paddock. Photographs of winter growth in August 2018 and after grazing in December 2018 are presented in Figs. 25 and 26.

Figure 25. Tedera plot before defoliation - 22 August 2018

Figure 26. Tedera and panic grass plot after defoliation - 11 December 2018

Total rainfall for 2019 was only 187 mm, however tedera plants survived being grazed by cattle several times (Fig. 27).

Figure 27. Tedera at Yallalie Downs in August 2019.

This site was under cattle grazing as part of the paddock until 2021.

4.7.2 Moora

The 5 ha demonstration site in Moora (Table 3) planted on 21 June 18 established well across the whole site, except in small sections located low in the landscape that were waterlogged and with a thick infestation of toad rush (*Juncus bufonius* L.). A photo of the site on the 19 November 2018 is presented in Fig. 28.

Figure 28. Tedera demonstration site at Moora - 19 November 2018.

A photo of the site one year later, on the 14 November 2019, is presented in Fig. 29. During 2019, this site had been grazed with sheep during winter/spring, and the rainfall was about half of normal annual rainfall (percentile 0%). This site was grazed by sheep for two years and then returned to cropping as it was in prime cropping country.

Figure 29. Tedera demonstration site at Moora - 14 November 2019

4.7.3 Mingenew

The tedera demonstration paddock at Mingenew (Table 3) had two soil types. One was very sandy and after sowing in June 2018, strong windstorms caused severe wind damage to the tedera seedlings. The top half of the paddock was a better yellow sand that did not blow as much in the windstorm and tedera established well (Fig. 30). This site has been grazed until 2020 with a good stand of tedera in the areas that established well.

Figure 30. Tedera demonstration site at Mingenew - 11 September 18.

4.7.4 Perenjori

The site at Perenjori (Table 3) was sown in mid- August 2018, followed by one of the driest years on record. The seedlings survived the dry seasons very well as shown in Fig. 31 (top), taken on the 26 June 2019. They were severely defoliated in October 2019 (Fig. 31 – bottom left) and then showed good recovery even without any rain (Fig. 31 – bottom right) by the 13 November 2019.

Figure 31. Perenjori - 26 June 2019 at Perenjori (top), severe defoliation in October 2019 (bottom left) and regrowth on 13 November 2019 (bottom right).

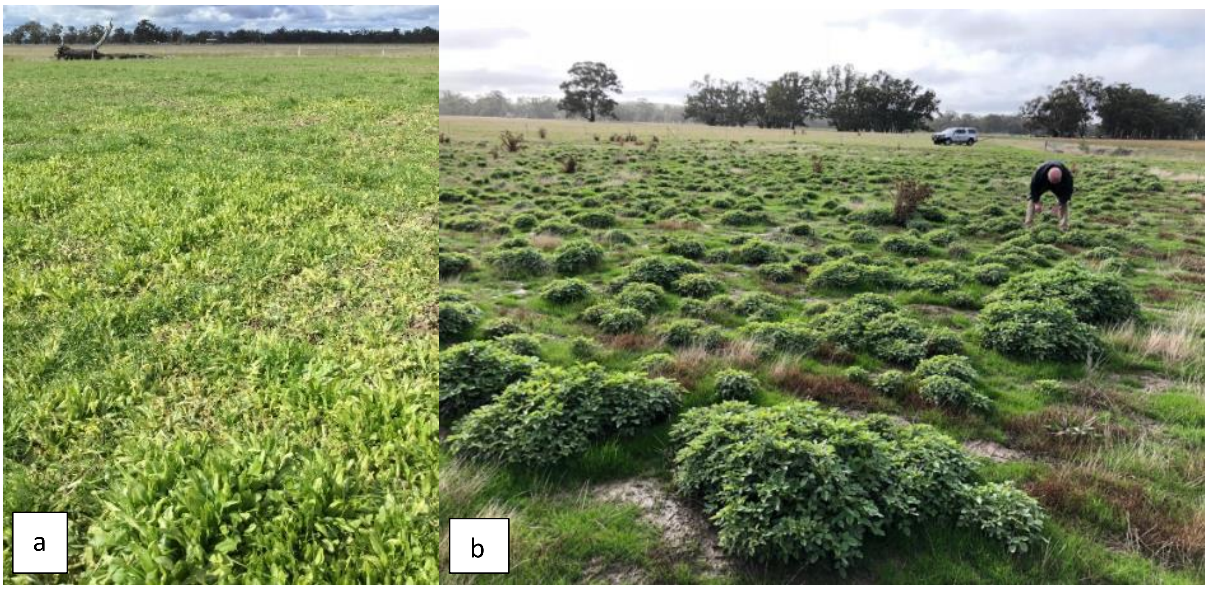
4.8 Demonstration sites in Eastern Australia

? Indication of hectares established/monocultures/strip or inter row sowing

4.8.1 Stawell

In May 2017, a demonstration site was established at Stawell (Table 3). Tedera was completely covered by weeds at establishment (Fig. 32a) due to the lack of information regarding herbicide tolerance at that time. Continuous grazing was employed as a management tool to keep weeds under control and to favour tedera and the site was able to have a remarkable recovery by the following year (Fig. 32b).

Figure 32. (a) weeds covering the young tedera seedlings after sowing in August 2017; (b) tedera recovered in June 2019.



This site is still ongoing in Fig. 33 in December 2021 with minimal specific management

Figure 33. Tedera site at Stawell in December 2021.

4.8.2 Hart Grower Group

The tedera at the SA Hart Field Day site (Table 3) was sown in a pasture herbicide tolerance demonstration at the main field day together with annual crops in autumn 2018. Tedera survived the summer and autumn very well and it was covered with plastic to avoid being sprayed during the knock-down of the paddock before sowing wheat in 2019 (Fig. 34). Wheat was harvested in December 2019 and a tedera sward remained after harvesting (Fig. 34 – bottom left and right).

Figure 34. Tedera in autumn 2019 (top left) and covered before knock down spray before cropping (top right). Tedera after wheat crop was harvested in 2019 (bottom left and right).

4.8.3 Boat Harbour, Tasmania

A demonstration site in Boat Harbour, Tasmania was established in 2019. A photo of the site in May 2021 is presented in Fig. 35. The site is still used under grazing in March 2022.

Figure 35. Two-year old tedera stand in Boat Harbour, Tasmania.

4.8.4 Other sites in eastern Australia

Locations of several other demonstration sites in eastern Australia are reported in Table 4. The tedera SA site at Wudinna was under-sown in a wheat paddock. Initial establishment was successful. Stock was kept out to preserve plant numbers for on-going observation. The tedera site in Victoria at the Joel site was established in autumn and retained for a couple of years. Due to severe drought in the eastern States in 2019, the SA site of Cummins and the NSW sites of Womboota and Forbes were not sown.

5. Conclusion

Tedera is a Mediterranean forage legume that can provide out-of-season green feed to fill feed-gaps. This project confirmed the results of previous research project B.PBE.0027 "Sheep production from tedera" that demonstrated tedera can be grazed at strategic times to fill feed gaps with excellent animal production results and no ill-effects to grazing animals. A reliable agronomy package has been developed to establish tedera in the right place, at the right time, in the right way and to manage it to maximize its potential value. Tedera needs to grow without competition as a pure crop for the first year for a successful establishment. Therefore, tedera's tolerance to pre-emergent and post-emergent herbicides has been a key research area. By implementing all the recommendations and guidelines in the agronomy package, tedera stands can be successfully established and managed to maximize high quality green biomass production at strategic times of the year to fill feed gaps.

5.1 Key findings

Establishment techniques
Key elements of a tedera agronomic establishment package have emerged from this study which involve early planting (close to the start of the growing season) and shallow sowing (2 cm depth) at 15 kg/ha of sowing rate and narrow row spacing (22 cm apart). Very similar practices are in common use for cereal establishment when grown in the same regions negating the need for any specialized equipment for tedera establishment (Real, 2022).

Defoliation management
The best overall management is to defoliate frequently when tedera is under stress and allow it to grow and accumulate biomass when there are good growing conditions.

Fertilization response to P, K, N and S
The optimum level in the soil and plant tissue for each nutrient to produce more than 90% of maximum biomass is reported. For the first time we have information for either soil content and/or plant tissue concentration of each of these nutrients if they are at deficient, adequate, or toxic levels.

Hard seed break down pattern
While the extent of seed softening varied between sites, a general pattern emerged with slow initial softening, accelerating during early autumn (March-April), and then slowing into late autumn/winter. Such a pattern would offer some protection against false breaks of season.

Herbicide tolerance
To control grass weeds such as annual ryegrass, propyzamide and carbetamide can be safely used as pre or post-emergent options in tedera. Post-emergent application of butroxydim, clethodim and haloxyfop can be recommended to control Group 1 herbicide susceptible annual ryegrass and other grass weeds in tedera.

The broadleaf pre-emergent herbicides that can be recommend in tedera were clopyralid to control post emergent capeweed, fomesafen to control pre-emergent radish and the double mix of fomesafen+diuron, flumetsulam+diuron and the triple mix of fomesafen+diuron+flumetsulam to control pre-emergent capeweed, pre and post-emergent radish and other broadleaf weeds.

The most consistently well tolerated post-emergent herbicides by seedlings and adult plants of Lanza® tedera were diflufenican, diuron, flumetsulam, fomesafen, and their two- or three-way mixes that will provide good control of pre- and post-emergent capeweed and radish.

Desiccants such as paraquat or diquat were also well tolerated by tedera adult plants. Tedera plants showed good quick recovery after desiccation with these herbicides.

Demonstration sites

Demonstration sites were successfully established around Australia, with 3 in WA, 8 in Vic, 19 in SA, 18 in NSW and 1 in Tasmania. Demonstration sites in eastern due to limited seed supply at the time, sites ranged in size from less than 500 m2 to over 1 ha.

Summer and autumn grazing trial

The grazing experiment at Kojonup during 2017 again demonstrated that tedera can be grazed to reduce or eliminate expensive hand-feeding in summer-autumn by using the simplest and least expensive grazing management, continuous grazing. Either continuous or rotational grazing of tedera increased animal live weight and condition and eliminated hand-feeding for 84 days during February to May. At equivalent stocking rates (5 DSE/ha), sheep grazing tedera could gain 5 to 6 kg/head more than sheep grazing a lucerne paddock. MIDAS modelling has previously indicated that shifting a Central Wheatbelt farm with 35 % annual pasture to a mix of 9 % annual and 26 % tedera pasture could increase mixed farm income by more than 30%, and that tedera may be suited to 6.3 M ha in WA alone. These benefits come from tedera's out-of-season feed-base reducing supplementary feeding and allowing higher value ewe/prime lamb systems to replace wether/wool systems. These results are consistent with three summer and autumn grazing experiments previously conducted at Dandaragan and Kojonup (Real et al., 2018).

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5.2 Benefits to industry

In Mediterranean-like climates with a winter/spring growing season two key feed-gaps were identified as the shoulders of the growing season. One shoulder arises from February/March after the stubbles lose their quality to May/June before the annual species are ready to be grazed. The second shoulder can be from the end of the season about mid-October when annuals start to senesce to mid-December when first stubbles become available. Tedera can grow all year round, but its production can be best utilized at these two strategic times, being a perfect fit for the needs of the red meat industry. Previous trials demonstrated that tedera grazing could maintain or increase animal live weight and condition, while reducing or eliminating expensive hand-feeding for around 3 months between December and May, using either continuous or rotational grazing. At stocking rates of 10 DSE/ha and from early Feb to late April, sheep grazing tedera could gain 5 to 10 kg/head more than sheep fed grain at a maintenance level. MIDAS modelling has previously indicated that shifting a Central Wheatbelt farm with 35 % annual pasture to a mix of 9 % annual and 26 % tedera pasture could increase mixed farm income by more than 30%, and that tedera may be suited to 6.3 M ha in WA alone. These benefits come from tedera's out-of-season feed-base reducing supplementary feeding and allowing higher value ewe/prime lamb systems to replace wether/wool systems.

This report again demonstrated that tedera has lower nutrient input requirements, phosphorus in particular, than lucerne, a comparable perennial legume. This nutrient efficiency will save farmers money by reducing inputs and increases the adaptation of perennial legume pastures in poorer soils.

This project developed the agronomy package for sowing tedera in the right soil, at the right time, following the right guidelines to maximize establishment success and maximize its biomass production of the best quality to be used at strategic times, complementing other feedbase options available to growers.

6. Future research and recommendations

All experiments and demonstration sites in this project were established with T15-1218⁽ᵖ⁾ (Lanza®). This was the only tedera variety protected under the Plant Breeder's Rights (PBR) Act 1994 (Real, 2016), and commercially available since 2019. In 2021, PBR in Australia accepted a second cultivar of tedera with improved cold tolerance, also bred by DPIRD and co-owned with MLA. Seed increase of the new cultivar commenced in 2022. The expectation is that most of the agronomy package developed with Lanza® will be applicable to the new cultivar. However, regional adaptation and herbicide tolerance are two key areas for future research for the new cultivar once seed becomes commercially available.

Tedera needs to establish in a weed-free situation to develop a strong and deep root system to survive the first dry season. Herbicides are a very important tool to achieve this objective if it cannot be sown into a weed-free paddock. Several herbicides were identified that are well tolerated by tedera and that can be used pre- and/or post-emergent to control grasses and broadleaf weeds. When it became available in 2019, tedera was a completely new commercial species to worldwide agriculture, and therefore, there is no herbicide specifically registered for tedera use. It will be essential to continue the research work to seek tedera-specific use registration for the key herbicides reported in this project.

Tedera is a species that can be used strategically to fill feed-gaps and this project confirmed the value of that strategic use. Projects such as the "Summer / Autumn liveweight gain from Tedera" project hosted at the Producer Demonstration Site (PDS) of the Moora Miling Pasture Improvement Group (MMPIG) are key to support and to show the benefits of tedera at paddock scale using animal production data generated by farmers. This is a very powerful research model to drive adoption, and more PDS sites with Lanza® and the new cultivar in WA and eastern Australia will be very important to showcase the benefits of tedera to accelerate its wide-spread adoption. We suggest a network of demonstration sites should be established to provide growers and agronomists with confidence in the agronomy package of tedera and to demonstrate and measure the animal production/economic benefits of including tedera in the production system.

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