

The development of Pallaton raphanobrassica for New Zealand farming systems

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Abstract

Raphanobrassica (*Raphanus x Brassica* L., cv. 'Pallaton') is an allotetraploid, produced from kale (*Brassica oleracea* L.) x radish (*Raphanus raphanistrum* subsp. *sativus* L.). It is a multi-graze forage, selected for high cumulative yield, water efficiency and resistance to clubroot (*Plasmodiophora brassicae*). This paper describes the breeding, agronomy and nutritive value of 'Pallaton', which can be grazed *in situ* between 42 and 70 days after sowing (DAS) with no specific crop maturity requirement. Cumulative yield of three consecutive harvests, analysed by meta-analysis of eighteen trials from 2013 to 2019 on three sites, showed 'Pallaton' had a greater yield of 16,254 kg DM/ha than forage rape (*B. napus* spp. *biennis*, cv. 'Titan') and leafy turnip (*B. rapa*; syn. *B. campestris* cv. 'Pasja II') at 12,639 and 10,965 kg DM/ha, respectively. A further trial, comparing nutritive values, demonstrated 'Pallaton' has a similar profile to 'Titan'. 'Pallaton' may be rotationally grazed for up to twelve months when initially grazed by livestock at 350 - 400 mm plant height between 42 and 70 DAS. Livestock consuming 'Pallaton' are susceptible to the same potential animal health concerns as other *Brassica* species, but represents a forage option for sheep, beef, deer and dairy farming, particularly those in challenging environments.

Keywords: Forage brassica, yield, nutritive value

Introduction

Forage brassica are fodder crops grown as part of rotation systems to produce additional summer, autumn or winter feed, where pasture growth is limited. Brassica crops, such as forage rape (*Brassica napus* ssp. *biennis*), leafy turnip (*B. rapa*; syn. *B. campestris*), bulb turnip (*B. rapa* ssp. *rapifera* or *napobrassica*; syn. *B. campestris*), swede (*B. napus* ssp. *napobrassica* or *rapifera*), and kale (*B. oleracea* ssp. *Acephala*), account for an estimated 300,000 ha (Dumbleton, *et al.*, 2012) and 200,000 ha (D. Thomas, personal communication, 2020) of land use in New Zealand and Australia, respectively. These crops can provide high-quality feed options (de Ruyter *et al.*, 2007; Westwood and Mulcock, 2012) and superior dry matter (DM) yields (Judson and Edwards, 2008) to traditional pasture swards, which are valuable during periods of low soil moisture (Fletcher

et al., 2010) or limiting temperatures (Andreucci *et al.*, 2012; 2016; Dumbleton, *et al.*, 2012). Demand for flat cultivatable land for dairy production and horticulture has resulted in sheep, beef and deer enterprises being pushed to finish stock in more marginal land areas. This change requires forage crop options that are suitable for growing in land with lower soil moisture availability and low fertility. Raphanobrassica (cv. 'Pallaton') is a multi-graze forage brassica species developed from a kale x radish hybrid. The breeding, agronomic performance and nutritive value of 'Pallaton' are described in this paper in comparison with other forage brassica options commonly grown on-farm.

Breeding

The need to expand forage brassica options for grazing animals in agronomically challenging conditions was a key driver for the formation of 'Forage Innovations Ltd.', a joint venture between Plant and Food Research and PGG Wrightson Seeds. The aim was to utilise intergeneric hybridisations between kale and radish. The expectation was that kale would provide high DM yields, quality and palatability traits, while the radish contributes increased drought, insect and disease tolerance traits. 'Pallaton' is an allotetraploid, which combines the genomes of more than one species. Naturally occurring allotetraploids most likely originate through either 2n gametes or chromosome doubling of chance F1 hybrids between two distinct species of the same genus, or from different genera (Baker *et al.*, 2017). Early interspecific hybrids between *Brassica* and *Raphanus* species for human consumption were largely unsuccessful, until work by Russian geneticist Karpechenko (1928) successfully crossed *B. oleracea* (2n=18, Figure 1). and *R. sativus* (2n=18). The hybrid formed from these two species is a diploid (2n=18), containing one set of kale chromosomes (CC genome, n=9) and one set of radish chromosomes (RR genome, n=9). However, these F1 progeny are sterile, forming gametes that contain one complete set of *Brassica* chromosomes and one complete set of *Raphanus* chromosomes.

Intergeneric hybrids are commonly cross-incompatible, which results in the premature breakdown of endosperm and early abortion of embryos (Nishi *et al.*, 1970). To restore fertility to the gamete, plants

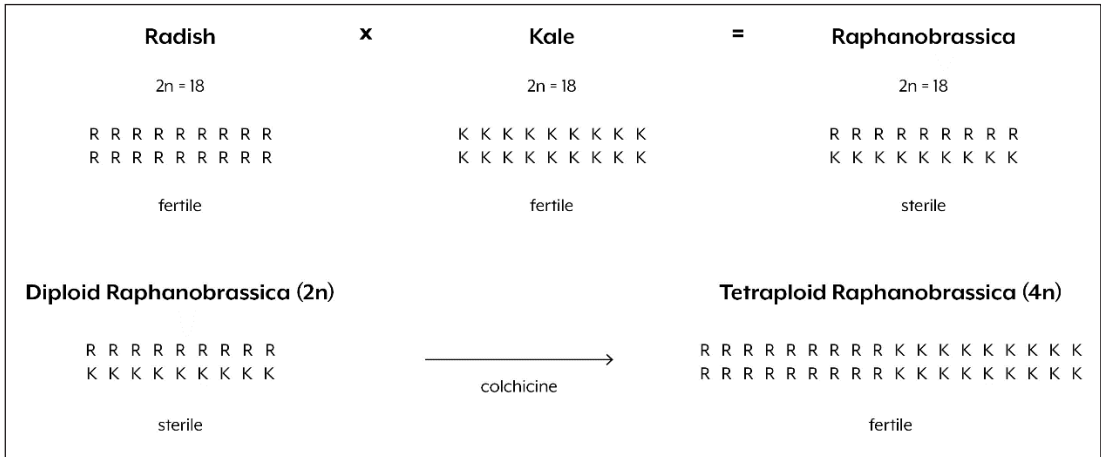


Figure 1 Development of interspecific fertile Raphanobrassica hybrids between Brassica and Raphanus by chromosomal doubling

are treated with colchicine, which induces polyploidy (Figure 1). This enables the pairing of chromosomes during meiosis. The colchicine treatment combined with different *in vitro* embryo rescue techniques (Rodrigues-Otubo *et al.*, 2000) can produce a plant that contains two sets of *B. oleracea* chromosomes and two sets of *R. sativus* chromosomes (4n=36), resulting in fertility in the F2 progeny.

‘Pallaton’ was selected from a phenotypically variable test population and viable and fertile, intergeneric single plant progeny then entered a traditional field plant breeding programme from 2003 – 2014. Over the twelve-year period, plants were selected for high cumulative forage yield; plant persistence under multiple grazing; reduction of the presence of early flowering; high water use efficiency (Chakwizira *et al.*, 2021 *in press*); and glasshouse selection for clubroot resistance. The final candidate then underwent extensive regional trials that compared yield to existing forage rape and leafy turnip cultivars.

Clubroot resistance

Clubroot, caused by the *Plasmodiophora brassicae* fungus, is one of the most serious diseases of brassicas. Consequently, there has been a considerable effort in New Zealand and internationally to develop resistant cultivars. The genetics of resistance to clubroot is complex, due to there being multiple factors involved. These include the diverse sources of resistance among the plant genotypes, and the presence of the large and evolving population of clubroot pathotypes. Each individual raphanobrassica single plant progeny was screened for clubroot resistance using a glasshouse screening assay against the most pathogenic Southland field isolate (SLD1), which has heavily infected the clubroot-resistant swede variety ‘Aparima Gold’.

Individual plants were inoculated with a known amount of SLD1, grown for four to six weeks (dependent on growth rate) and scored from 0 (no disease symptoms) to 3 (heavily infected). Matilda (*B. rapa*) was used as the susceptible control and achieved an infection score of 3, compared to the parent plants of ‘Pallaton’ which had an average infection score of 1.

Agronomic performance

Eighteen plot trials were carried out between 2013 and 2019 to assess the yield and regrowth potential of ‘Pallaton’ raphanobrassica with control cultivars across several brassica species (Table 1). ‘Pallaton’ was entered in all eighteen trials, early maturing forage rape (*cv.* ‘Titan’) in sixteen of the eighteen and leafy turnip (*cv.* ‘Pasja II’) in four of the eighteen. Trials were located across three sites, with sixteen occurring at an irrigated site at Kimihia Research Centre in Lincoln, Canterbury, one on a non-irrigated farm near Oxford, North Canterbury, and one at a non-irrigated site at Ruakura Research Centre in Hamilton, Waikato.

All trials had a complete randomised block design, with sixteen trials having four replicates, one with three replicates (trial 4), and one with six replicates (trial 18). All were spring sown in mid to late October. Plot sizes differed at sites: 1.2 x 6 m at Kimihia Research Centre and 1.5 x 6 m at both Oxford and Ruakura Research Centre. All plots were drilled in 150 mm drill rows at a depth of <10 mm using an Oyjord cone seeder with a sowing rate of 4 kg/ha for ‘Titan’ and ‘Pasja II’ and 8 kg/ha of ‘Pallaton’.

All trials (except six, seventeen and eighteen) were harvested 100 mm above soil level, with Wintersteiger Cibus F forage plot harvester. Total plot fresh weight (kg) was measured by the plot harvester. Subsamples of approximately 400 grams per plot were collected

and weighed before and after herbage was dried in a purpose-built oven at 90°C for 48 hours, then used to calculate DM percentage.

Trial eighteen was assessed by cutting a 1 m² quadrat per plot to 100 mm above soil level, with the remainder grazed with sheep to a residual of 100 mm. Trials six and seventeen, were measured as for trial eighteen, but remaining biomass was removed by mowing with a ride on mower to the 100 mm residual.

There were three harvesting events in each trial, defined by optimum crop maturity of control cultivars for harvest one. Regrowth cuts, harvest two and three were determined by either or both variables: growth and time. Harvest one occurred from 53 – 112 DAS, harvest two events from 30 – 84 days after harvest one and harvest three events from 33 – 87 days after the second harvest.

The data from all trials were analysed using a combined restricted maximum likelihood (REML) meta-analysis, with cultivar and cultivar x cut length (days between harvests) as fixed effects, fitting trials and replicates as blocking terms and allowing each trial to have its own residue variance. This was fitted with Genstat 21 (VSN International, 2020) using $\alpha=0.05$.

Yield from Harvest 1, 2 and 3, total cumulative yield, yield by day effect 1, 2, 3 and total yield by day effect were analysed with mean days reported for each

growth period (Table 2). Yield by day effect quantified the differences between cultivars, relative to ‘Pallaton’ thus always having a value of zero when days to harvest differ from mean days.

The first trial was conducted in 2013, whereby the yield of ‘Pallaton’ was compared with conventional forage brassica cultivars with a range of different maturity types. These included, early and late maturing forage rape (recommended grazing 70 – 120 DAS), through to longer maturing kale cultivars (150 – 200 DAS). Throughout these first trials, ‘Pallaton’ had relatively high yields early in crop life, comparable with forage rape and kale. A reduction in leaf senescence, typically seen with other forage brassica species also allowed increased grazing flexibility of ‘Pallaton’ up to 100 DAS. However, crop utilisation, regrowth potential and feed quality were reduced with delaying grazing (150 – 200 DAS). Subsequently, trial four and trial sixteen respectively compared ‘Pallaton’ to a range of early maturing leafy turnip and forage rape cultivars, with trials often including harvesting several regrowth events. Through this process, the place of ‘Pallaton’ in farming enterprises was defined. Current recommendations are that the first graze of ‘Pallaton’ should occur at 42 – 70 DAS when plant height is between 350 – 400 mm and continued repeat grazings can occur as plant height regrows to the maximum of 400mm.

Table 1 Overview of eighteen trials in combined trial analysis of raphanobrassica (cv. ‘Pallaton’), forage rape (cv. ‘Titan’) and leafy turnip (cv. ‘Pasja II’).

Trial No.	Year	Location	Replicates	Sowing date (dd/mm/yy)	Harvest* 1 (days)	Harvest 2 (days)	Harvest 3 (days)
1	2013	Kimihia	4	24/10/13	82	66	60
2	2014	Kimihia	4	23/10/14	89	64	57
3	2015	Kimihia	4	30/10/15	53	30	33
4	2015	Kimihia	3	30/10/15	98	70	87
5	2016	Kimihia	4	13/10/16	110	70	83
6	2016	Oxford	4	18/10/16	87	75	61
7	2016	Kimihia	4	13/10/16	98	82	83
8	2016	Kimihia	4	13/10/16	112	69	82
9	2017	Kimihia	4	24/10/17	55	35	37
10	2017	Kimihia	4	25/10/17	84	63	58
11	2017	Kimihia	4	24/10/17	91	63	52
12	2018	Kimihia	4	19/10/18	56	39	37
13	2018	Kimihia	4	19/10/18	82	57	60
14	2018	Kimihia	4	19/10/18	96	54	66
15	2019	Kimihia	4	21/10/19	94	40	65
16	2019	Kimihia	4	09/10/19	65	40	44
17	2019	Ruakura	4	17/10/19	62	84	71
18	2019	Kimihia	6	29/10/19	69	50	59

* Harvest 1 = days to first harvest; Harvest 2 and Harvest 3 = days from previous harvest.

'Pallaton' and 'Titan' were not different ($P > 0.05$) at Harvest 1, and produced 9,010 and 9,073 kg DM/ha, respectively (Table 2). However, both yielded more ($P < 0.001$) than 'Pasja II' at 6,745 kg DM/ha. At the second harvest (Harvest 2) 'Pallaton' yielded 4,839 kg DM/ha, significantly more than 'Titan' and 'Pasja II' at 2,735 kg DM/ha and 3,154 kg DM/ha respectively ($P < 0.001$). At the third harvest (Harvest 3), 'Pallaton' yielded 2,007 kg DM/ha, which was again more ($P < 0.001$) than 'Titan', 1137 kg DM/ha, and 'Pasja II', 1,063 kg DM/ha.

Total cumulative yield across harvests, showed 'Pallaton' produced 16,254 kg DM/ha, which was more ($P < 0.01$) than 'Titan' (12,639 kg DM/ha), and 'Pasja II' (10,965 kg DM/ha). The initial yield of 'Titan' in these trials was comparable to the expected crop yields of early maturing forage rapes (70 – 90 DAS) at 8 – 10.5 t DM/ha (de Ruiter *et al.*, 2009). Although, there was difficulty comparing studies, due to crop yields being highly influenced by environmental conditions, crop management and husbandry (Keogh *et al.*, 2011).

Yield by day effect compared expected yield differences between 'Pallaton', 'Titan' and 'Pasja II' according to the period of growth when days to harvest differ from reported mean days. For example, at Harvest 2 (Table 2), if harvesting was at 60 days after the previous harvest, 'Titan' produced 2,104 kg DM/ha ($P < 0.05$) less than 'Pallaton'. The yield difference outside mean harvesting days can be calculated using the following equation, where DAS can be used interchangeably with days after the previous harvest.

$$\text{Yield difference}_{x\text{DAS}} = \text{Yield difference}_{\text{means DAS}} + \text{Yield by day effect} \times \text{DAS difference}$$

For example, if 'Pallaton' and 'Titan' crops were then harvested at 'Harvest 2', 75 days after previous harvest instead of 60 days, the expected difference in

yield between these two species was 2884 kg DM/ha in favour of 'Pallaton', *i.e.*:

$$\text{Yield difference}_{75\text{DAS}} = -2104 + (-52) \times (75 - 60)$$

$$\text{Yield difference}_{75\text{DAS}} = -2884 \text{ kg DM/ha}$$

Another example, if 'Pallaton' and 'Pasja II' crops were then harvested at 'Harvest 3', 50 days after the previous harvest instead of 63 days, the expected difference in yield between these two species is 1139 kg DM/ha in favour of 'Pallaton', *i.e.*:

$$\text{Yield difference}_{50\text{DAS}} = -944 + (15) \times (50 - 63)$$

$$\text{Yield difference}_{50\text{DAS}} = -1139 \text{ kg DM/ha}$$

Nutritive value

A randomised complete block trial with four replicates was established at an irrigated site at Kimihia Research Centre, Lincoln, Canterbury to compare the nutritive value of 'Pallaton' and 'Titan'. Four 0.5 ha replicates of each brassica were sown on 1 November 2017 following an application of 250 kg/ha of diammonium phosphate. At 69 DAS, three randomly selected 1 m² quadrat samples per plot were cut to 3 cm above soil level and manually separated into leaf and petiole, and stem. Approximately 300 g of plant material from each plot of each brassica species was chilled and transported to a commercial laboratory (R J Hill Laboratories Ltd, Hamilton, New Zealand) for wet chemistry analysis of DM, metabolisable energy (MJME), digestibility (DOMD), crude protein (CP), acid detergent fibre (ADF), neutral detergent fibre (NDF), soluble sugars (SS), lignin and ash (Table 3). Two whole plants per plot from two replicates of each brassica species were freeze-dried and transported to Plant and Food Research, Lincoln, Canterbury for analysis of glucosinolate concentrations. Data were analysed using analysis of variance (ANOVA) implemented in GENSTAT Version

Table 2 Yield (kg DM/ha) at three harvesting events (Harvest 1-3) for raphanobrassica (cv. 'Pallaton'), forage rape (cv. 'Titan') and leafy turnip (cv. 'Pasja II') combined over eighteen trials (2013 – 2019) at three sites in New Zealand.

Cultivar	Harvest 1 kg DM/ha	Yield by day effect 1	Harvest 2 kg DM/ha	Yield by day effect 2	Harvest 3 kg DM/ha	Yield by day effect 3	Total yield kg DM/ha	Total yield by day effect*
'Pallaton'	9010 ^a	0	4839 ^a	0	2007 ^a	0	16254 ^a	0
'Titan'	9073 ^a	-18	2735 ^b	-52	1137 ^b	-3	12639 ^b	-18
'Pasja II'	6745 ^b	-50	3154 ^b	-54	1063 ^b	15	10965 ^b	-36
Mean days		86		60		63		209
P-value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Wald/df	366.58	97.33	1156.93	208.22	1821.48	107.65	785.41	113.05
LSD _{5%}	867		615		251		1412	

* Yield by day effect was the difference between two entries by each extra day of growth relative to 'Pallaton'. Subscript letters in common represent means that are not significantly different ($P > 0.05$) using Duncan's new multiple range test.

19 with significance declared at $P < 0.05$.

'Pallaton' and 'Titan' plants were harvested at 69 DAS, a relatively long sowing to grazing interval for 'Pallaton' so nutritive value data may not fully representative of plant nutritive value for 'Pallaton'. A more suitable test would be to compare 'Pallaton' against an earlier maturing forage brassica, leafy turnip (grazed 42 – 70 DAS).

'Titan' had a higher DM ($P < 0.001$) than 'Pallaton' at 9.6% DM and 8.1% DM, respectively (Table 3), both DM values were relatively low, compared to previous studies. Higher values for forage rapes have been recorded, for example 13.8% (Keim *et al.*, 2020), 14.3% (Westwood and Mulcock, 2012) and 14.5% (Islam *et al.*, 2012), reflecting the impacts of variable environmental conditions, crop management practices (Keogh *et al.*, 2011) and cultivar selection (Westwood and Mulcock, 2012).

Leaf proportion was high ($P < 0.05$) for 'Pallaton' compared with 'Titan' at 91.5% and 83.3%, respectively. Conversely whole plant ME was lower ($P = 0.033$) for 'Pallaton' (12.2 MJME/kgDM) than 'Titan' (12.4 MJME/kgDM). The MJME content was high for both brassica species, compared to typical MJME content of summer pastures, particularly under dry conditions.

Soluble sugar content of 'Titan' and 'Pallaton' differed significantly ($P < 0.001$) at 18.2% and 16.0%, respectively. Levels of SS in 'Titan' and 'Pallaton' were higher than those reported for forage rape of 9.8% non-structural carbohydrate (Keim *et al.*, 2020) but relatively low compared to values for rape of between 25.1% and 29.8% water soluble carbohydrate for seven cultivars of forage rape (Westwood and Mulcock, 2012). Soluble sugar content of forage brassicas can be highly variable, for example water soluble carbohydrate content of 'Goliath' rape ranged from 9.0% - 18.4% (Islam *et al.*, 2012), with variation attributed to sowing date, DAS and first versus subsequent harvests of forage

regrowth. The higher SS content of 'Titan' may reflect the early maturity of 'Titan' (70 – 90 DAS) compared to 'Pallaton', which does not mature. Future studies could consider sequential harvests of 'Titan' and 'Pallaton' to investigate time by SS and other plant quality attribute, interactions as plants approach or pass maturity.

Ash content was significantly ($P < 0.001$) higher for 'Pallaton' than 'Titan' (Table 3). Ash content provides an approximation of mineral content of feed as well as an indicator of possible soil contamination. Other investigations of mineral content of 'Pallaton' (Foley, pers.comm.) revealed a similar profile of minerals to other Brassica species; so, the variance in ash content remained unexplained.

Crude protein content of 'Titan' and 'Pallaton' was high for both species at 21.3 and 22.1% CP, respectively, and did not differ significantly ($P = 0.28$) between species. Sowing date, grazing and management practices including N fertiliser application (Islam *et al.*, 2012; Keogh *et al.*, 2011) brassica species and cultivar (Westwood and Mulcock 2012) can all influence the CP content of forage *Brassica* species. Neutral detergent fibre and ADF content did not differ significantly ($P > 0.05$) between 'Titan' and 'Pallaton'; values were similar to those previously reported for forage rape (Islam *et al.*, 2012; Keim *et al.*, 2020; Westwood and Mulcock, 2012).

'Pallaton' contained low concentrations of the glucosinolates epiprogoitrin ($P < 0.001$) and progoitrin ($P < 0.001$), with concentrations of 0.14 and 3.32 $\mu\text{mol/g DM}$, respectively. In contrast, 'Titan' contained 0.30 $\mu\text{mol/g DM}$ epiprogoitrin and 8.40 $\mu\text{mol/g DM}$ progoitrin. Nitrile and epithionitrile derivatives of progoitrin have been implicated as potential hepatotoxins in a cattle-specific condition known as 'Brassica Associated Liver Disease' (BALD) (Collett *et al.*, 2020). The concentration at which progoitrin may induce BALD in cattle is unknown and the risk

Table 3 Proportion of leaf and stem and whole plant nutritive value ($n=48$) for raphanobrassica (cv. 'Pallaton') and forage rape (cv. 'Titan') as percentage dry matter (DM).

Cultivar	Leaf %	Stem %	DM %	MJ ME	DOMD	CP	ADF	NDF	SS	Lignin	Ash
'Pallaton'	91.5	8.7	8.1	12.2	76.1	22.1	19.3	22.2	16.0	4.6	15.7
'Titan'	83.3	16.6	9.6	12.4	77.4	21.3	18.2	21.9	18.2	4.8	13.6
P-value	<0.001	<0.001	<0.001	0.039	0.033	0.280	0.078	0.800	<0.001	0.582	<0.001
S.E.M.	<0.01	<0.01	0.14	0.07	0.42	0.56	0.41	0.70	0.43	0.14	0.28
LSD _{5%}	0.02	0.03	0.40	0.19	1.18	1.58	1.15	1.97	1.21	0.39	0.80

DM% = dry matter percentage;

MJME = megajoules of metabolisable energy;

DOMD = digestibility;

CP = crude protein;

ADF = acid detergent fibre;

NDF = neutral detergent fibre; SS = soluble sugars.

is influenced by multiple plant and animal factors, including brassica species, onset of the development of plant reproductive structures, use of N and S fertiliser, animal age, concurrent presence of rumen acidosis, and animal physiological state, specifically late gestation in cattle (Dalley *et al.*, 2015). Progoitrin concentrations of 30 $\mu\text{mol/g}$ DM or more in swedes may induce BALD under some but not all conditions. An investigation of an outbreak of BALD in dairy cattle consuming swedes during 2014 (Dalley *et al.*, 2015) revealed a mean progoitrin concentration for all parts of swede plants of 24.8 $\mu\text{mol/g}$ DM, with reproductive parts of swede plants containing more than 35 $\mu\text{mol/g}$ DM.

With a whole plant progoitrin content of less than the 10% reported for reproductive sections of swedes, the risk of BALD in cattle consuming 'Pallaton' should be considered relatively low. However, animal health could still be compromised if high-risk factors are present, such as inappropriately high use of N and S fertilisers; a high proportion of flowering plant parts; and/or concurrent presence of rumen acidosis in cattle, particularly during the latter stages of gestation.

Conclusions

Raphanobrassica (*cv.* 'Pallaton'), a hybrid of kale x radish, represents a new forage brassica option for New Zealand and Australian farming systems. 'Pallaton' was evaluated for yield and regrowth in eighteen trials over six years against two other brassica species. It had a greater cumulative yield, particularly in regrowth harvest events, than the forage rape 'Titan' or leafy turnip 'Pasja II', which suggested superior regrowth. Based on single-site data, the feed profile of 'Pallaton' was largely comparable with 'Titan' and similar to other published values, hence it represents a viable feed option for sheep, beef, deer and dairy enterprises.

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REFERENCES

Andreucci, M. P., Black, A., & Moot, D. J. (2012). Cardinal temperatures and thermal time requirements for germination of forage brassicas. *Agronomy New Zealand*, 42, 181-191.

Andreucci, M. P., Moot, D. J., Black, A. D., & Sedcole, R. (2016). A comparison of cardinal temperatures estimated by linear and nonlinear models for

germination and bulb growth of forage brassicas. *European Journal of Agronomy*, 81, 52-63. <https://doi.org/10.1016/j.eja.2016.08.010>

Baker, R. L., Yarkhunova, Y., Vidal, K., Ewers, B. E., & Weinig, C. (2017). Polyploidy and the relationship between leaf structure and function: implications for correlated evolution of anatomy, morphology, and physiology in Brassica. *BMC Plant Biology*, 17(3). <https://doi.org/10.1186/s12870-016-0957-3>

Chakwizira, E., Dumbleton, A., Maley, S. (2021). Water use efficiency of raphanobrassica and forage rape. *Agronomy Society of New Zealand*, in press.

Collett, M. G., Matthews, Z. M., & Parton, K. H. (2020). Hepatotoxicity of two progoitrin-derived nitriles in New Zealand white rabbits. *Toxins*, 12(11), 695-708. <https://doi.org/10.3390/toxins12110695>

Dalley, D., Verkerk, G., Kyte, R., McBeth, C., Petch, S., Kuhn-Sherlock, B., . . . Morley, C. (2015). *Swede associated toxicity in dairy cattle during winter 2014*. Hamilton, New Zealand: DairyNZ.

de Ruiter, J. M., Dalley, D. M., Hughes, T. P., Fraser, T. J., & Dewhurst, R. J. (2007). Types of supplements: Their nutritive value and use. In P. V. Rattray, I. M. Brookes, & A. M. Nicol, *Pasture and supplements for grazing animals* (pp. 97-115). New Zealand Society of Animal Production Occasional Publication No. 14. <https://agris.fao.org/agris-search/search.do?recordID=US201300122197>

de Ruiter, J., Wilson, D., Maley, S., Fletcher, A., Fraser, T., Scott, W., . . . Nichol, W. (2009). *Management practices for forage brassicas*. Christchurch, New Zealand: Forage Brassica Development Group.

Dumbleton, A., Gowens, S., Conner, A., Christie, M., Kenny, P., Mulcock, H., & Charteris, B. (2012). Cleancrop(TM) Brassica System: The development of herbicide. *Proceedings of the New Zealand Grassland Association*, 74, 25-30.

Fletcher, A. L., Sinton, S. M., Gillespie, R. N., Maley, S., Sim, R. E., de Ruiter, J. M., & Meenken, E. D. (2010). Drought response and water use efficiency of forage brassica. *Agronomy New Zealand*, 105-117. <https://www.cabdirect.org/cabdirect/abstract/20143056229>

Griffiths, A. J., Gelbart, W. M., & Miller, J. H. (1999). *Modern genetic analysis: Changes in chromosome number*. New York: Freeman. <https://www.ncbi.nlm.nih.gov/books/NBK21229/>

Harberd, D. J., & McArthur, E. D. (1980). Meiotic analysis of some species and genus hybrids in the Brassicaceae. In S. Tsunoda, K. Hinata, & C. (. Gomez-Campo, *Brassica Crops and Wild Allies, Biology and Breeding* (pp. 65-87). Tokyo: The Japan Science Society Press. <https://www.cabdirect.org/cabdirect/abstract/19801689305>

Islam, M. R., Garcia, S. C., & Horadagoda, A. (2012). Effects of residual nitrogen, nitrogen

- fertilizer, sowing date and harvest time on yield and nutritive value of forage rape. *Animal Feed Science and Technology*, 177(1-2), 52-64. <https://doi.org/10.1016/j.anifeedsci.2012.08.002>
- Judson, H. G., & Edwards, G. R. (2008). Survey of management practice of dairy cows grazing kale in Canterbury. *Proceedings of the New Zealand Grassland Association*, 70, 249-254.
- Karpechenko, G. D. (1928). Polyploid hybrids of *Raphanus sativus* X *Brassica oleracea* L. *Bulletin of Applied Botany*, 17, 305-408. <https://doi.org/10.1007/BF01740955>
- Keim, J. P., Gandarillas, M., Benavides, D., Cabanilla, J., Pulido, R. G., Balocchi, O. A., & Bertrand, A. (2020). Nutrient concentrations and profile of non-structural carbohydrates vary among different Brassica forages. *Animal Production Science*, 60(12), 1503-1513. <https://doi.org/10.1071/AN19472>
- Keogh, B., McGrath, T., & Grant, J. (2011). The effect of sowing date and nitrogen on the dry-matter yield and nitrogen content of forage rape (*Brassica napus* L.) and stubble turnips (*Brassica rapa* L.) in Ireland. *Grass and Forage Science*, 67(1), 2-12. <https://doi.org/10.1515/2161-962X.1011>
- Nishi, S., Kawada, J., & Toda, M. (1970). Studies on the embryos culture in vegetable crops. III. On the conditions affecting to embryo culture of interspecific hybrids between cabbage and Chinese cabbage. (pp. 73-88). Japan, Ser. A9: Bulletin of Horticultural Research. <https://www.cabdirect.org/cabdirect/abstract/19710306414>
- Rodrigues-Otubo, B. M., de Penteadó, M. I., & do Valle, C. B. (2000). Embryo rescue of interspecific hybrids of *Brachiaria* spp. *Plant Cell, Tissue and Organ Culture*, 61, 175-182. <https://doi.org/10.1023/A:1006478407814>
- VSN International. (2020). *GENSTAT for Windows 21st Edition*. Retrieved from VSN International, Hemel, Hempstead, United Kingdom: genstat.co.uk
- Westwood, C. T., & Mulcock, H. (2012). Nutritive evaluation of five species of forage brassica. *Proceedings of the New Zealand Grassland Association*, 74, 31-38.