Establishing tagasaste from seed in Waikato hill country

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Abstract
Tagasaste (tree lucerne) can be difficult to establish from seed due to its hard seed coat and high levels of seedling mortality. Three experiments were performed. Experiment 1: Effect of gibberellic acid, potassium nitrate, seed nicking + soaking in warm water (38ºC for 24 hours), soaking seeds in boiling water and a cold-water control on germination. The highest germination percentage occurred from the warm and boiling water treatments. Experiment 2: Effect of slug bait, rhizobia and phosphorus fertiliser on establishment of tagasaste seed which had been hand sown into a herbicide-treated pasture. Of the 1600 hand-sown seeds, emergence was negligible, and no treatments varied significantly from the untreated control. Greater pasture suppression is required for successful establishment. Experiment 3: Examined removal of 20%, 40%, 60% or 80% of the height of a seedling on tagasaste branching. Branch number was similar for all treatments 83 days after trimming the main stem. Removing 20% or 40% of the seedling height produced three-fold greater edible dry matter than when 80% of the seedling height was removed ($P<0.001$). Although pruning treatments did not affect branch number, a less severe pruning regime resulted in the regrowth of the greatest amount of edible dry matter.

Keywords: browse, forage shrubs, tree lucerne, propagate

Introduction
Tagasaste (*Cytisus proliferus* var. *palmensis*) is a forage shrub originating from the Canary Islands that provides valuable drought feed when planted in low-rainfall hill country pastures. Farmers in various regions (*e.g.,* Waikato, Jon Sherlock, pers. comm.) are showing interest in its potential to enhance their farm system for multiple benefits, such as nitrogen (N) fixation, provision of fodder for bees, shelter for livestock, fodder for birds and aesthetics (Tozer et al*.,* 2021). The plant can be difficult to establish from seed due to its hard seed coat and being grazed readily by pest mammals and insects (Dann and Trimmer 2003; Wiley 2006).

Different techniques have been recommended to break dormancy in hard-seeded species. For example, sulphuric acid, gibberellic acid and potassium nitrate have been used to break dormancy in hard-seeded annual medic species (Balouchi and Sanavy 2006) and potassium nitrate in the hard-seeded legume, *Indigofera zollingeriana* (Simbolon 2019). At low concentrations, nitrates are absorbed by the seed and are incorporated into amino acids which are necessary for plant growth, and act as a plant signalling compound to stimulate germination (Duermeyer et al*.,* 2018). Gibberellic acid acts as a hormone to break dormancy and stimulate germination (Shu et al*.,* 2015). No published scientific research is available that compared the effect of these chemicals on tagasaste seed germination. Both of these compounds are commercially available and could provide a viable alternative for propagating tagasaste from seed - if more successful than the hot water treatment in breaking dormancy.

Fact sheets produced by government and rural professional sources in South Africa, Australia and New Zealand, are available which recommend soaking tagasaste seed in warm water to break its hard seed coat and stimulate seed germination (Dann and Trimmer 2003; Stace 2003; Esterhuizen and Esterhuizen 2012). Recommended temperatures and duration of soaking varies, and some suggest nicking the seed prior to soaking in warm water (pers. comm. cited in Townsend and Radcliffe 1987), although no published scientific research could be found to underpin these guidelines. Therefore, an experiment was designed to quantify the effect of gibberellic acid, potassium nitrate, and combinations of warm/hot water, with and without nicking of the seed, on tagasaste seed germination. Water temperatures were chosen such that they could be easily replicated by farmers or others wishing to propagating tagasaste (*i.e.,* hot tap water / boiling water).

Establishment of tagasaste from scarified seed in flat, arid environments, such as Western Australia, is well documented (Oldham et al*.,* 1991; Dann and Trimmer 2003). In Canterbury, tagasaste was successfully established from spring sowing in cultivated or herbicide-treated land, with 55% and 40% of viable seed, respectively establishing 12 weeks after sowing (Townsend and Radcliffe 1987). According to current published research, there are no data available on tagasaste establishment from seed in North Island hill

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country pastures, where the climate is different to the South Island, or how this is affected by critical factors, such as pests, nutrient enrichment or rhizobia.

Slug damage was observed on tagasaste seedlings in Canterbury, where rank growth of pasture created an ideal habitat for slugs (Townsend and Radcliffe 1990) and bait is recommended in Hawke's Bay Regional Council guidelines when establishing tagasaste (Anon 2002). Applying slug and snail bait may increase establishment success of tagasaste sown by seed, but this has not been investigated.

Application of phosphorus (P) can enhance tagasaste seedling growth. Moir et al. (2012) demonstrated a strong growth response of tagasaste seedlings to increasing P application in a controlled pot study, although the Olsen P required for 97% of maximum dry matter yield for tagasaste was low in comparison to many legumes. Phosphate fertiliser application is recommended in Australia when establishing tagasaste, although there is little information regarding base fertiliser recommendations (Dann and Trimmer 2003; Wiley 2006).

Rhizobia are critical for tagasaste as they enable it to fix atmospheric N necessary for shoot and root growth (Messaoud et al., 2015). To infect tagasaste with rhizobia, one recommendation has been to take soil from beneath an existing tagasaste stand and mix it into the soil where the new stand is to be established (AgFact 2003). While this could be achieved for small-scale plantings, using a commercially available rhizobium strain would be a more practical option for larger scale or aerial broadcasting. Tagasaste does not have highly specific rhizobia requirements (Wiley 2006) and rhizobia, which infect a range of legumes such as *Lotus corniculatus*, can be used (Gault et al., 1994). A strain of this rhizobia, EasyRhiz™ (Anon 2020), is commercially available. However, information is lacking on how it affects the growth of tagasaste seedlings.

Given the importance of soil P, rhizobia and slug bait and the lack of information regarding their effects, study was designed to investigate the impact of these factors on tagasaste seedling establishment in northern Waikato hill country pastures where herbicide had been applied. The hypothesis was that the addition of rhizobia and slug bait would increase seedling survival and growth up to one year after sowing while the addition of P fertiliser would increase the growth, but not the survival, of tagasaste seedlings.

Once established, seedlings can be managed to develop multiple stems from near ground level to protect the main stem from being ring-barked and killed by livestock. In Western Australia, pruning mature tagasaste trees has been recommended to encourage proliferation of finely branched new growth (Dann and Trimmer 2003) and pruning of one-year-old tagasaste plants at 50 cm above the ground level in Ethiopia enhanced biomass accumulation, which was attributed to increased lateral branching (Assefa et al., 2012). In Western Australia, a cutting height of 20-50 cm was recommended for a cut-and-carry system for established plantations of tagasaste (Dann and Trimmer 2003) and Townsend and Radcliffe (1987) encouraged branching by cutting the main stem from 1 m to 30 cm above the ground. However, there is no published research available on optimum pruning to promote multiple branching when establishing smaller seedlings in a New Zealand context. Therefore, a study was conducted to determine the effect of cutting height on stem branching and subsequent regrowth.

Materials and Methods

Experiment 1. Methods to maximise seed germination

Tagasaste seed was purchased from Proseed New Zealand, Amberley in November 2018. The seed had been sourced from wild populations in North Canterbury.

Three seed scarification treatments were compared in Stage 1:

- Treatment 1: Untreated control (cold tap water, ≈23°C)
- Treatment 2: 'GA_3': gibberellic acid applied at a rate of 1 g/L ProGibb® SG (a.i. gibberellic acid, 400 g/kg, Nufarm, New Zealand)
- Treatment 3: 'KNO_3': seeds soaked in a solution of potassium nitrate (KNO_3) (a.i. KNO_3, 60 g/L)
- Treatment 4: 'Nicking + warm water': a small incision was made with a scalpel at the distal end (nicking) of each seed prior to soaking in warm tap water maintained at 38ºC for 24 h.

To apply the treatments, a sheet of paper towel (22 cm x 22 cm, Countdown Essentials 2 ply Paper Towel) was folded in half, moistened with cold water (Treatment 1), the treatment solution (Treatments 2 and 3) or warm water (Treatment 4). Germination was tested using a standard assessment technique (Anon 2014). The towel was folded to create a midline, 50 seeds were placed on half of the towel and the unused portion folded over the seeds so they were enveloped between the layers. The seed-containing towel was again moistened with the treatment solution or warm or cold water to drip point, placed into a snap lock bag (17 cm x 18 cm), sealed, and placed in a controlled environment room (maintained at 25ºC with a daylength of 16 h) on 27 November 2018. There were four replicates of each of the four treatments, arranged in a Latin Square, randomised complete block design. The number of germinated seeds (with an emerged root or shoot) was counted 7 and 10 d after treatment application.

In August 2021, we further compared seed

scarification techniques (Stage 2). Two treatments were repeated: the control and the most successful treatment. The additional treatment (Treatment 5) involved freshly boiled water alone but without nicking:

- Treatment 1: Cold water control (as above)
- Treatment 4: Nicking + warm tap water (38ºC) for 24 h (as above)
- Treatment 5: Tagasaste seeds were placed in a container of freshly boiled water which was allowed to cool over 24 h.

The same process was followed as previously (Anon 2014) using the same source and number of seeds and the same controlled temperature room. Germination was assessed at 1, 6, 8, 10, 13, 17, 24 and 37 d after treatment application.

Experiment 2. Methods to increase establishment from seed

Site preparation

A trial area of 5 m^2 was selected on a perennial ryegrass-based pasture grazed sporadically by beef cattle, on a north-facing 12° slope, in northern Waikato (S37°, 32'55", E175°, 7'42").

The soil had the following pH and nutrient levels at the time of treatment application: pH=5.7, Olsen P=25 mg/L, potassium = 20 MAF (Ministry of Agriculture and Forestry) Quick Test units, calcium = 8 MAF Quick Test units, magnesium = 44 MAF Quick Test units and sodium = 7 MAF Quick Test units, organic matter content of 10.8% and total carbon of 6.3%, based on 30 bulked samples, collected to a depth of 0-75 mm and processed at Hill Laboratories, Hamilton.

The site was mown on 8 March 2019 to a height of approximately 5 cm.

On 2 April 2019, the site was fenced with cyclone fencing and rabbit-proof netting to exclude grazing livestock and pests, such as rabbits and goats.

Weedmaster TS450 (a.i. 540g/L glyphosate, Nufarm, New Zealand) was applied using a Solo 475 backpack at a rate of 1 l/ha to suppress vegetation eight days before the seed was sown. Herbicide was applied to suppress rather than remove resident vegetation to simulate likely management decisions appropriate for steeper hill county if aerially broadcasting tagasaste seed. Application rates were discussed with herbicide retailers and agronomists employed by herbicide companies, given the difficult requirement of needing to suppress competition without totally killing vegetation.

At the time of herbicide application, pasture height was approximately 10 cm and the pasture comprised 43% dead vegetation, 32% perennial ryegrass, 16% other grasses, 8% broadleaved weed species and 1% clover.

There were four treatments (slug bait, fertiliser, rhizobia and the control), with four replicates of each arranged in a Latin Square, randomised complete block design with blocks perpendicular to the hill side. Plots were 1 m^2 and each plot was separated by a 20 cm buffer.

Seed preparation and sowing

On 8-9 April, 1600 seeds (sourced from the same seed lot as Experiment 1) were nicked at the distal end and soaked in warm tap water (38ºC) for 24 h. The germination percentage of the seed was 69%, based on a test of a further 400 seeds treated in the same manner.

On 10 April volumetric soil moisture content averaged 12%, soil surface temperature averaged 21ºC and pasture cover averaged 1300 kg DM/ha. Soil moisture data (0-7.5 cm depth) were obtained using a calibrated time domain reflectometry (TDR) instrument with one reading per plot, temperature data were obtained using a thermometer with one reading per plot at ground level at 1000 h, and pasture cover was assessed using a rising plate meter (Jenquip, EC10, Feilding), with readings from 50 random positions across the site.

Using tweezers, each seed was sown approximately 5 mm below the ground surface in the centre of each of 100 (10 cm × 10 cm) cells in a 1 m^2 planting grid placed over each plot. Tweezers were used to bury the seed to simulate livestock trampling of the seed into the topsoil layer. One hundred seeds were sown in each plot, 400 in each treatment and a total of 1600 seeds across all treatments.

Treatment application

Treatments were applied on 10 April, comprising slug/snail bait application (10 kg/ha Slugout, a.i. 18 g/kg Metaldehyde, Nufarm, New Zealand), fertiliser application (500 kg/ha of DAP (di-ammonium phosphate), Ballance Agri Nutrients, New Zealand), rhizobia application (EasyRhiz™, soluble *Rhizobium leguminosarum* inoculant for lotus, Luisetti Seeds, Rangiora, New Zealand), and an untreated control (no application of slug bait, fertiliser or rhizobia). To apply the rhizobia, a 30 ml glass vial of freeze-dried EasyRhiz™ inoculant was mixed with 20 ml distilled water to create a slurry (Anon 2020). The seed was coated in the slurry and sown within 5 h of application. Lotus rhizobia was chosen as they can infect tagasaste (Gault et al., 1994). Slug bait was reapplied to the slug bait treatment on 26 April 2019. Emerged seedlings were counted 14, 16, 21, 60 and 91 days after sowing.

Experiment 3. Pruning management of tagasaste seedlings

Seedling preparation

Commercially grown tagasaste seedlings (ca. 10 months old) were sourced in July 2019. Seedlings were transplanted from root trainers into two litre pots (11

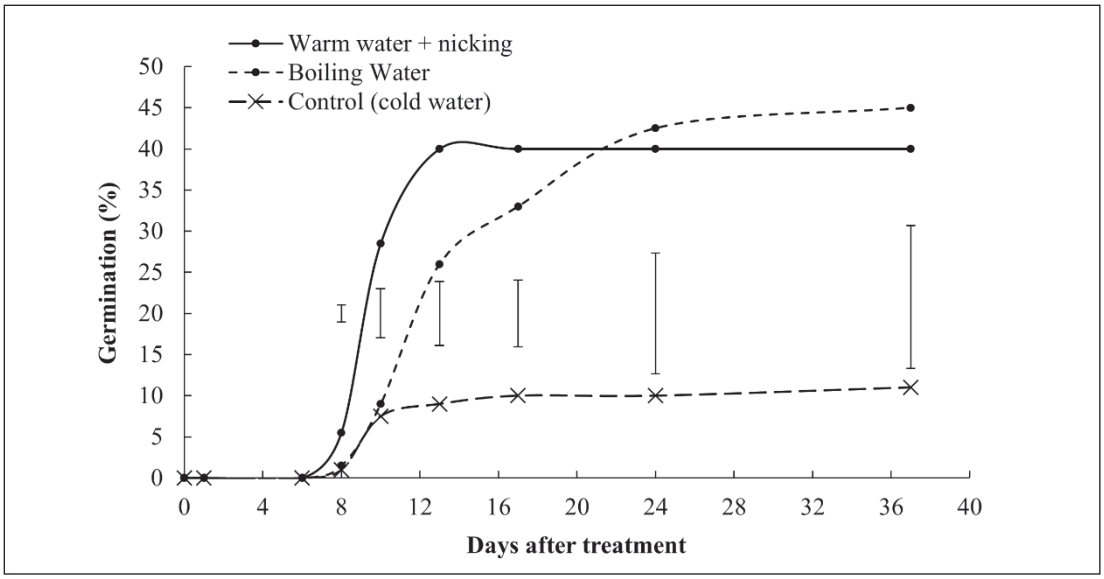

Figure 1 Germination of tagasaste seed placed in boiling water and left to cool for 24 h, nicked and placed in warm tap water (38ºC) and left to cool for 24 h, and cold tap water which was used as the control.

cm depth × 15.5 cm diameter) containing a potting mix comprising screened Dalton bark fines (65% v/v) and Daltons washed sand (35% v/v), enriched with 2 kg/m^3 of dolomite, fine gypsum and lime, 3 kg/m^3 Osmocote Exact 5/6 Standard Start, 0.50 kg/m^3 Osmoform NXT 22N and 0.75 kg/m^3 Permawet to ensure that macro- and micro-nutrients were supplied over six months (Daltons, Matamata, New Zealand). Plants were maintained on a concrete pad enclosed by rabbit-proof netting and watered to drip point three times weekly. Seedlings were stratified into ten blocks based on height, with each block containing four plants (40 plants in total), arranged in a randomised complete block design. All plants had a single main stem which averaged 43 cm in height and there was no difference in stem height between the treatments prior to imposing pruning treatments (P>0.05).

Treatment application

On 24 July, treatments were applied. The main stem was cut to a height of 36 cm, 27 cm, 18 cm or 9 cm, which equated to removal of ≈20%, 40%, 60% and 80% of the stem height, respectively.

Measurements

The total number of lateral branches was documented on d 0, 26, 41, 53, 68 and 83 after treatment application. Above-ground biomass above and below the cut point was harvested and edible portions (leaves and stem <5 mm diameter) separated, oven dried at 105ºC for 48 h and weighed.

Statistical analyses

Germination data from Experiment 1 were analysed for each separate date using ANOVA with block as the fixed effect and treatment as the random effect. A binomial analysis of tagasaste seedling emergence using ANOVA was undertaken on the number of seedlings present 49 d after sowing, and the number of seedlings that survived in Experiment 2. The number of branches on each measurement occasion and tagasaste edible DM were analysed using ANOVA for Experiment 3. All data were analysed in GenStat (GenStat 2021). No transformation of data was required to normalise the variance.

Results

Experiment 1. Methods to maximise seed germination

Stage 1: The highest germination percentage occurred from the nicking + warm water treatment (68% *vs.* <2% for all other treatments, P<0.001; Table 1). Establishment declined between d 7 and 10 because some of the seeds which had germinated on d 7 had died by d 10.

Stage 2: Approximately three weeks after seeds were treated, the boiling water and warm water treatments had a similar germination percentage (≈40% of seed treated), while only 10% of the seeds in the control (cold water) treatment had germinated (Figure 1). Seeds that had been nicked (nicking + warm water treatment) germinated more rapidly than seeds that were treated with boiling water. There was little new germination of seed after this time.

Experiment 2. Methods to increase establishment from seed

Establishment of tagasaste from sown seed was negligible. The first seedling was observed 21 d after sowing (Table 2). An average of 6 seedlings per treatment were present 49 d after sowing and a total of only two seedlings (emanating from a total of 1600 sown seeds) were present at the field site 91 d after sowing (Table 2).

The number of live seedlings present 49 d after sowing was similar for all treatments and averaged less than 2% of the total number of sown seeds ($P>0.05$; Table 2). Of the few seedlings that emerged, up to 66% died and there was no difference between treatments in seedling survival ($P>0.05$; Table 3).

Pasture height was approximately 15 cm 49 d after sowing and approximately 35 cm high 91 d after sowing. Slugs were observed 21 d after sowing in some of the plots to which slug bait had been applied.

Experiment 3. Pruning management of tagasaste seedlings

On four of the five measurement timepoints, the number of branches at the cut point was significantly higher in the 36 cm treatment than one or more of the other treatments ($P<0.05$; Figure 2). However, 83 d after treatment application, at the final measurement, there was no significant difference between treatments in the number of branches at the cut point, which averaged 2.7 per seedling ($P>0.05$).

Removing 20% or 40% of the height of the main stem produced three-fold greater total edible dry matter than when 80% of the main stem was removed (*i.e.*, cutting to 36 cm or 27 cm instead of to 9 cm above the growing media surface, $P<0.001$; Table 4). In contrast, pruning the seedlings severely (80%) compromised regrowth and had no benefits for increasing the branch number at the cutting point when compared to removal of the top 20% (Figure 2). The plants had a single main

Table 1 Effects of the cold-water treatment (Control), application of gibberellic acid (GA_3), potassium nitrate (KNO_3) and nicking the seed followed by soaking in warm tap water (38ºC) for 24 h, on the presence of germinated tagasaste seeds, 7 and 10 days after treatment. SED: Standard error of difference.

Days after treatment	Presence of live seeds (%)				SED	P value
	Control	GA_3	KNO_3	Nicking + warm water		
7	1	1	0	68	1.9	<0.001
10	2	2	0	56	3.0	<0.001

Table 2 Effect of fertiliser, rhizobia and slug bait on the total number of emerged tagasaste seedlings up to 91 d after hand-sowing tagasaste seeds (400 per treatment) on a north-facing hillside in Waikato. Control treatment: no fertiliser, rhizobia or slug bait.

Days after sowing	Number of live seedlings present			
	Control	+Fertiliser	+Rhizobia	+Slug bait
21	1	0	0	0
23	6	0	3	2
27	6	1	3	4
30	8	2	4	6
34	8	3	3	6
40	9	4	3	6
49	9	4	3	6
91	1	1	0	0

Table 3 Effect of fertiliser, rhizobia and slug bait on the percentage of live tagasaste seedlings present and seedling survival 49 days after hand-sowing tagasaste seeds (400 per treatment) on a north-facing hillside in Waikato. Control treatment: no fertiliser, rhizobia or slug bait. SED: Standard error of difference. 'ns': not significantly different ($P>0.05$).

Seedling presence and survival (%)	Control	+Fertiliser	+Rhizobia	+Slug bait	SED	P value
Seedling presence	2.3	1.0	0.8	1.5	1.2	ns
Seedling survival*	34	54	0	62	29	0.052

*Survival of the total number of emerged seedlings.

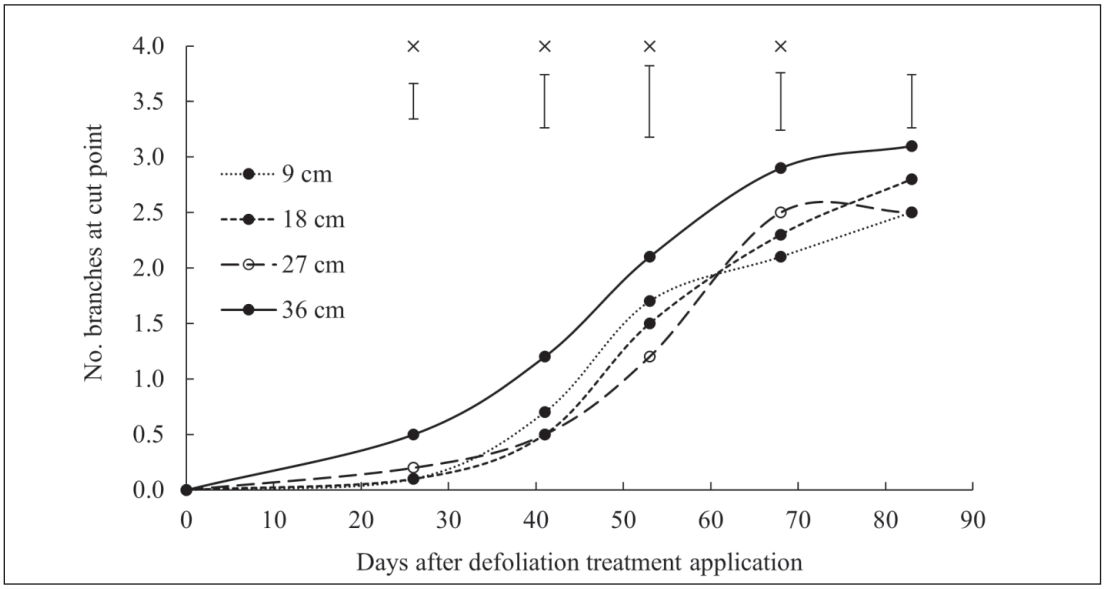

Figure 2 Effect of defoliating the main stem at 9 cm, 18 cm, 27 cm or 36 cm above ground level on the number of new branches at the cut point. Asterisks indicate the measurement times when there were significant differences between treatments (P<0.05).

stem throughout the study; no new branches grew from the plant base.

Discussion

A comparison of seed scarification techniques provided strong support for the current industry guidelines of soaking tagasaste seed in water that had just been boiled and allowed to cool (Dann and Trimmer 2003). While nicking the seed coat prior to soaking enabled faster germination, the overall success for both methods was similar. As nicking is highly labour intensive and did not enable greater germination, it is not recommended unless circumstances dictate that a speedier germination rate is required. The gibberellic acid and potassium nitrate methods, while successful on other hard-seeded species such as medics (Balouchi and Sanavy 2006) were insufficient to scarify and penetrate the hard seed coat of tagasaste.

Establishing tagasaste from scarified seed in the upper North Island hill country environment was not successful. Hence, because of the lack of seedlings it was not possible to determine if the addition of fertiliser, slug bait or rhizobia had any effect. A likely reason for the establishment failure was that the pasture was not sufficiently suppressed by herbicide application and that subsequent pasture regrowth outcompeted tagasaste seedlings. Townsend and Radcliffe (1987) found that tagasaste was successfully established from seed sown into cultivated land or resident pasture killed by herbicide application, while seed drilled into short pasture and broadcast seed failed to establish.

Pasture provides a habitat for invertebrate pests which may have predated the tagasaste seedlings. Visual observations showed that slugs were present at the site. Dead seedlings were not observed in this study, further lending support to the speculation that slugs or other invertebrate pests consumed the young seedlings which contributed to the lack of establishment; despite

Table 4 The effect of the percentage of the main stem removed on edible dry matter above and below the cut point, and total edible dry matter 83 d after treatment. The cutting height above the growing media surface is provided in parenthesis. SED: standard error of difference. Within rows, means followed by the same letters are not significantly different (P>0.05).

Edible dry matter (g)	Percentage removed					
	80% (9 cm)	60% (18 cm)	40% (27 cm)	20% (36 cm)	SED	P value
Below cutting point	1.3 $_a$	3.5 $_b$	4.5 $_b$	7.6 $_c$	0.77	<0.001
Regrowth above cut point	2.3 $_a$	5.4 $_b$	7.1 $_b$	3.3 $_a$	0.90	<0.001
Total edible dry matter	3.6 $_a$	8.9 $_b$	11.6 $_b$	10.9 $_b$	1.02	<0.001

slug bait application for one of the treatments. While an obvious conclusion was that resident pasture needs to be more strongly suppressed to remove competition, this may be difficult to achieve in North Island hill country for the sole purpose of establishing tagasaste. A less risky alternative for hill country may be to establish tagasaste using transplants, which is a commonly recommended establishment method (Aarssen 1989; Anon 2002; Dann and Trimmer 2003).

If establishing tagasaste transplants, the current results showed that removing a small proportion of the main stem (<40%) to a height of at least 27 cm above ground level resulted in optimal regrowth and edible dry matter production approximately three months after pruning. This was despite there being no effect of cutting height on the number of branches produced. The examples previously noted of increased branching in response to cutting were all from older stands (Assefa et al., 2012) and these benefits may not be directly transferable to younger seedlings, such as used in this study.

Conclusions

The hard seed coat of tagasaste can be easily broken by soaking seeds in boiling water that is then allowed to cool for 24 h. For successful establishment of tagasaste seedlings in North Island hill country pastures, competition from resident pasture must be strongly suppressed. It was not possible to conclude whether rhizobia, fertiliser or slug bait were beneficial for tagasaste growth or survival due to establishment failure. If trimming seedlings, it is recommended that less than 40% of the main stem be removed. Doing this is less likely to have a negative impact on branching and is likely to result in greater regrowth and edible dry matter accumulation than would occur from more severe pruning.

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