Stoloniferous red clover cv. Rubitas is a valuable companion to PRG and phalaris

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Abstract
In established pasture swards, legumes contribute and transfer nutrients to non-legume species, but are thought to contribute and transfer little during the establishment phase. The nutrient contribution of stoloniferous red clover *Trifolium pratense* cv. Rubitas during the pasture establishment phase may have been underestimated. To test this hypothesis a pot study was sown in April 2014. Perennial ryegrass *Lolium perenne* (PRG) cv. Reward was sown alone at 25kg/ha or in combination at two sowing rates, 12 and 20kg/ha with Rubitas at 5kg/ha. Phalaris (*Phalaris aquatica*) cv. Advanced AT was sown alone at 5kg/ha or with 3kg/ha Rubitas. Rubitas was also sown alone at 6kg/ha. After establishing for 75 days, plants were exposed to defoliation intervals of 10, 20 and 40 days defoliated 8, 4 and 2 times, respectively. These defoliation interval treatments were combined with three residual heights of 25, 50 and 100 mm. Material harvested was hand separated into species, weighed and dried. Results for all treatments and means showed a significant ($P<0.0001$) effect of species, defoliation interval, species by defoliation interval and residual height by defoliation interval. For treatments that included Rubitas, there was a significant ($P<0.0001$) increase in DW yield of the companion grass when compared to the PRG or phalaris sown alone. The inclusion of Rubitas increased the combined DW yield of PRG and phalaris by 72% and 179% respectively compared to PRG or phalaris sown alone. Rubitas nutrient contribution and transfer may affect regrowth recovery after defoliation. Further work will seek to quantify Rubitas establishment mechanisms and enhance management for economic and environmental gains.

Key words
Companion sowings, pasture establishment, defoliation intervals

Introduction
Legumes including members of the *Trifolium* genus have a symbiotic relationship with nitrogen (N) fixing bacteria when they are fully established actively nodulating plants (Boller and Nösberger, 1987; Høgh-Jensen and Schjørring, 1997). The method that the roots of the legumes use to establish contact with symbiotic bacteria is not clearly defined. Previous research in forest ecosystems suggest an exudate is generated from leguminous trees to grasses, as an attractant to N fixing bacteria in the soil (Sierra et al., 2007). If this is occurring in pasture mixes of clover and grass (Lesuffleur et al., 2013), grasses may also be seeking out this medium to aid in rapid root establishment. Perennial ryegrass (PRG) and white clover *Trifolium repens* pastures are commonly used to support intensive grazing animal production (Cunningham et al., 1994). Clovers are often observed to be the less dominant component of a grass/clover composite sward under intensive grazing management systems, particularly for those fertilized with increasing rates of synthetic N fertiliser.

Mature decaying clover roots, shoots and leaves release N back into the soil to be taken up by plant roots (Evans, 1977) and recycled again (Dahlin and Stenberg, 2010; Unkovich, 2012). During establishment the N contribution is less defined. The productivity of clover is influenced by establishment strategies that enhance legume content and pereniality as longer established swards hold larger nutrient reserves in the soil (Peoples et al., 2013). It is claimed that clovers do not contribute to the N pool until decaying plant parts became part of the soil nutrient pool (Laidlaw et al., 1996; Ledgard and Steele, 1992). The presence of a clover companion during establishment is not expected to improve the DM yield of the grass.

Following sowing, grazing management during establishment is critical to achieving a productive and persistent pasture. Defoliation interval and defoliation residual heights have been intensively studied in established PRG/white clover pastures (Chapman et al., 1996; Rawnsley et al., 2014) but little work has been done with stoloniferous red clover and grasses during the establishment phase.This experiment aimed to investigate the effects of varying defoliation interval and residual height treatments on dry weight (DW) yields during the establishment of differing mixed clover and grass swards.

Methods

Six pasture treatments were sown in 200 mm pots as monocultures of stoloniferous red clover cv. Rubitas 6 kg/ha, Reward PRG 25 kg/ha, Advanced AT phalaris 5 kg/ha and combinations of Rubitas 5 kg/ha & Reward 20 kg/ha, Rubitas 5 kg/ha & Reward 12 kg/ha, Rubitas 5 kg/ha & Advanced AT 3 kg/ha. Pots were initially located outside and species treatments sown in April 2014. In May 35 days after sowing (DAS) four replicates of each treatment were placed in a glass house maintained at 20^{o}C arranged as a completely randomised design. Rubitas stoloniferous red clover was scarified and inoculated with Rhizobium Group B. Fertiliser Starter blend + Mg 1000kg/ha 30 DAS, trace elements Basix Reset™ 15L/ha 31 DAS and Potassium 50kg/ha 66 DAS were applied. Plants were thinned to represent plant density for each sowing rate. At 65 DAS all treatments received a co-variant cut to 50 mm. The first defoliation treatments commenced 75 DAS. Treatments were defoliated for 80 days at 10, 20, and 40 days to residual heights of 25 mm, 50 mm, and 100 mm and re- randomised (CRD) after defoliation. All material was cut, collected, weighed, hand separated into species composition and dried at 60 ^{o}C for 48 hours. Cumulative dry matter (DM) yields were analysed using proc mixed in SAS 9.3 assuming a completely randomised design. After examining quantile plots of residuals a log transformation was selected. Effects were considered significant at P<0.05.

Results

Fixed effects for all treatments and means showed significant effects ($F_{5, 157}$=63.15, P<.0001) of species and species by defoliation interval ($F_{10, 157}$=5.95, P<.0001). A three way interaction of species by defoliation interval by residual height was also significant (P<0.0002). The effect of residual height or species by residual height was not significant (P>0.05). Comparing species effects (Table 1) the DM results were consistently higher (P<0.0001) when Rubitas was sown as a companion with PRG. PRG sown with Rubitas at 20kg/ha produced 66% more DM, PRG at 12kg/ha 77% more than PRG sown alone. Phalaris sown with Rubitas produced 179% more DM than phalaris sown alone. There was no significant (P>0.05) difference in PRG DW yield (97.31 kg/ha) at the higher and lower sowing rates of 20 or 12 kg/ha when in combination with Rubitas. The yield increase for the Reward PRG only component at the 20 kg sowing rate was 27% and at 12 kg sowing rate was 32%. The Advanced AT phalaris only component yield increase was 72%.

Table 1.The cumulative mean dry matter yield (kg DM/ha) of each species treatment. Values presented are the back transformed means. Values in parenthesis are the transformed means± the standard errors % clover and grass shown for monoculture and combination treatments

species	Yield kg/DM /ha	% clover	% grass
Reward PRG	853.17 (6.66±0.182)	0	100
Reward20/Rubitas	1414.27 (7.14±0.182)	20	80
Reward12/Rubitas	1511.58 (7.15±0.182)	24	76
Advanced AT phalaris	361.94 (5.81±0.190)	0	100
Advanced AT/Rubitas	1008.72 (6.63±0.182)	43	57
Rubitas	869.51 (6.43±0.202)	100	0

There was a significant (P<0.0001) species by defoliation interval effect on DM yield (Table 2). A defoliation interval of 40 days resulted in a 71% and 60% decline in cumulative DM yield in comparison to defoliation intervals of 20 and 10 days, respectively. A defoliation interval of 20 days resulted in a significantly higher (37% increase) cumulative DM yield in comparison to a defoliation interval of 10 days.

Table 2.The cumulative dry matter yield (kg/DM/ha) for each species treatment by each defoliation interval treatment. Values presented are the back transformed means. Values in parenthesis are the transformed means± the standard errors

species	defoliation interval		
	10 days	20 days	40 days
Reward PRG	864.09	1218.14	477.29
Reward20/Rubitas	1466.12	2011.04	765.63
Reward12/Rubitas	1713.22	2180.52	641.01
Advanced AT phalaris	313.83	527.56	244.43
Advanced AT/Rubitas	1239.34	1494.43	292.37
Rubitas	902.03	1498.30	208.19
average	1083.11	1488.33	438.15
SE	(6.80±0.182)	(7.19±0.182)	(5.91±0.196)

Discussion

The legume of choice in high production irrigated systems is white clover. White clover has a tap root not unlike red clover initially. The tap root is replaced with adventitious roots at around two years after sowing white clover (Black et al., 2009). Storage of nutrients is somewhat restricted after this occurs in white clover. Rubitas may improve yield as a legume companion with grasses.This plant unlike other red clovers has a distinct advantage as it is stoloniferous. This attribute makes it more suited to repeated defoliation (Seresinhe et al., 1994; Van Minnebruggen et al., 2014) giving it some similarity in management to white clover. The inclusion of Rubitas as an alternative legume to white clover may suit irrigated pastures sown in autumn or spring.

In this experiment both PRG and Phalaris responded to autumn establishment under irrigation when sown with Rubitas as a legume companion. Defoliation interval was shown to be important but residual height had no effect on DM yield. Rate of sowing in PRG had no effect on DM yield. Reward PRG sown as a monoculture was similar in DM to Rubitas sown alone suggesting a combination of the two species would produce a similar DM yield. However, in this trial the yield was shown to be greater in the companion sowing than in the monoculture combined yields. In the Advanced AT / Rubitas mixed sward the above theory of similar DM yield would hold, as the individual DM of phalaris and Rubitas was similar to when they were sown together. Advanced AT is slow to establish and the lower DM yield was possibly due to this morphological factor.

Defoliation interval results across all treatments support the current thinking that more frequent defoliation can decrease DM production. When the defoliation frequency is too great, as shown in the 10 day defoliation interval results, some depression in yield would be expected. When the defoliation frequency was extended to 40 days a larger depression in yield occurred. The 20 day interval was found to be optimal for defoliation and regrowth even with three residual heights imposed. The species selected and the defoliation intervals imposed favoured a response to 20 days based on PRG optimum interval for regrowth. The increase in DM produced at establishment with PRG cannot be explained by inclusion of Rubitas. The slower establishing phalaris combination showed a greater increase in DM but this can be attributed to the presence of Rubitas.

Conclusion

Rubitas red clover has the potential to contribute at establishment, as well as the longer term life of the pasture in combination with PRG or phalaris. The mechanism by which DM yield is affected and enhanced by the inclusion of Rubitas needs explanation. Measurement of components in the grass/clover composite sward and growth indices would add greatly to the knowledge of establishment interaction. Future field experiments should include white clover combinations as well as Rubitas when testing establishment effects on DM.

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