DEVELOPMENT AND FATE OF SEEDLINGS OF THREE TEMPERATE LEGUMES FOLLOWING OVERSOWING

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ABSTRACT

Oversown seeds of subterranean clover (*Trifolium subterraneum* L.), strawberry clover (*T. fragiferum* L.) and caucasian clover (T. *ambiguum* Bieb.) were monitored for 40 days to identify the stage of development at which the seed of these temperate pasture legumes failed at varying moisture levels. Intact sods of glyphosate-sprayed pasture were taken to a glasshouse, subjected to factorial combination of three soil surface moisture levels (low, medium, high) and three legume species. Overall, 70, 52 and 31% of seed of subterranean, strawberry and caucasian clovers,respectively, produced a visible radicle or seedling. The greatest loss of potential seedlings occured following imbibition and prior to germination. The order of decreasing germination, subterranean>strawberry>caucasian, reflected the order for decreasing seed size and seed vigour. Radicle death only occurred at low soil surface moisture and seedling death occurred mainly at high soil surface moisture.

KEYWORDS

Temperate legumes, soil moisture, accelerated aging test, radicle lengths, hard seed, ungerminated seed, oversowing

INTRODUCTION

In many areas of the world the nitrogen fertility and feed quality of pastures on non-arable land would be improved by the introduction of pasture legumes. When pasture seed is sown directly onto the soil surface the number of seeds that produce a seedling is low, usually less than 10 percent (Awan *et al*., 1993; Campbell, 1992). In New Zealand the percentage of viable oversown white clover (*Trifolium repens* L.) and subterranean clover (T. *subterraneum* L.) that produces an established plant when oversown on non-arable hill pastures ranges from five to 10 percent (Awan, 1995). The fate of oversown pasture seed has been shown to include insect predation, infection by pathogen, insufficient moisture for imbibition, failure of the radicle to penetrate the soil surface and death of seedlings from stress (Campbell, 1992). The objective of this research was to identify the stage at which the seed of three species of temperate pasture legumes failed to continue development to a seedling when oversown onto a range of soil surface moistures.

METHODS

A glasshouse experiment was conducted at Massey University, Palmerston North, New Zealand. Perennial ryegrass-white clover pasture was blanket sprayed with 12 l of Roundup (36% glyphosate) per ha and 20 d later intact sod pieces (420 x 300 x 50 mm) were transfered to the glasshouse. The gravimetric soil water content at sowing time was 30, 59 and 80% for the low, medium and high soil moisture treatments, respectively. A high moisture treatment was maintained by misting 200 ml water on the soil surface every 3 hr during daylight. The medium moisture treatment was maintained by misting 200 ml water daily at 09:00 hr, and the low moisture treatment was misted similarly, but was dried using oscillating fans (Awan *et al*., 1996). The legume species used were subterranean clover cv. 'Karridale', strawberry clover (T. *fragiferum* L.) cv. 'Grasslands Onwards' and caucasian clover (T. *ambiguum* Bieb.) cv. 'Monaro'. The experimental design was a randomised complete block with three moisture treatments and 3 legume species in a factorial combination in four blocks. Seed of these species were sown on the surface of the sod with one seed per 20 x 20 mm square in a grid (216 seeds) on 3-6 October, 1993. Subsequently, seed was pressed into the soil surface with a studded roller (1.28 Mg m^{-3}) which simulated sheep treading (Awan 1995). The sod (46 x 300 x 50 mm) was removed from each tray 2, 4, 6, 9, 12, 15, 20, 30 and 40 days after sowing (DAS). The number of ungerminated seeds, imbibed seeds, seeds with radicles and seedlings, dead or alive, on the soil surface and in the soil were counted and the radicle length measured. Standard seed germination (ISTA, 1985) and accelerated aging (AA) tests (Baskin, 1987) were performed on the treatment species. The data were analysed by ANOVA using general linear model procedure (SAS, 1989).

RESULTS AND DISCUSSION

The greatest number of lost seed occurred for strawberry clover, which had the smallest seed (Table 1). Overall, there were 100 seeds (47% of total) of caucasian clover that did not germinate even at high soil moisture, and there were 39 (18% of total) and 57 ungerminated seeds (26% of total) for subterranean and strawberry clovers, respectively. There was no radicle death for any legume species at high or medium moisture, but there was more seedling death (< 5%) at high moisture (Table 1). At low moisture the average radicle death was more than 10% for all the seed sown. Overall, a visible seedling or radicle was produced from 70, 52 and 31% of the seed of subterranean, strawberry and caucasian clover, respectively. Over all species and moisture levels less than 6% of seed produced a radicle or seedling and subsequently died. The standard laboratory seed germination test gave a lower percentage of ungerminated and hard seed compared to the AA test and oversown seed (Table 2). The oversown seed had 19, 35 and 53 percent of ungerminated and hard seed for subterranean, strawberry and caucasian clovers, respectively. Most of the ungerminated and hard seed was on the soil surface. Possibly, imbibed seeds of the smaller seeded caucasian and strawberry clovers were more susceptible to water loss than the larger seeded subterranean clover. Also, the AA test results suggested that the seed vigour of caucasian clover was lower, and contributed to the disparity between the standard germination tests and oversowing results. A similar disparity was reported by Chapman *et al*. (1985) for hill country oversowing of white clover cv. 'Grasslands Huia'. The number of viable seed is usually calculated from the standard seed germination test or the accelerated aging test, but these clearly do not reflect the germinability of oversown legume species. There was a significant interaction between the legume species and soil moisture treatments on radicle length on the soil surface ($P<0.05$). The radicle length was greater at low moisture (7.6±0.41 mm) than at medium and high moisture (4.1±0.41) in all species ($P<0.05$). The subterranean clover radicle length was longer (8.0 mm) at low moisture than for the other two species (7.0 mm). The radicle length at low moisture for strawberry (7.5 mm) and caucasian (6.4 mm) clovers increased relatively more, when compared to the other moisture treatments, than for subterranean clover ($P<0.05$). The difference in radicle length between the species at low moisture was not reflected in any difference in the proportion of radicle deaths. Of the seed that germinated, 17% of the subterranean and strawberry clover seed and 22% of the caucasian clover seed failed to develop into a seedling due to radicle death (Table 1). Although radicle death from desiccation was a major cause of seedling loss following oversowing at low moisture, it appeared to affect all three species similarly. Therefore, the proportion of ungerminated seed was the major determinant of the number of oversown seeds that produced a seedling after 40 d. Presumably, the germination process was arrested in these seeds before a radicle becomes visible. Greater losses of potential seedlings were observed from the pre-appearance phase than the post-appearance phase for Huia white clover by Chapman *et al*. (1995). In contrast, Barker and Zhang (1988) found seedling losses for white clover 'Grasslands Tahora' were split equally between pre- and post- appearance phases.

In conclusion, the greatest loss of potential seedlings from the three legume species resulted from seed that failed to produce a visible radicle. Subterranean clover produced the most seedlings from oversown seed, caucasian clover the least and strawberry clover was intermediate. These differences between the three species were due to either differences in seed size or seed vigour.

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Table 1
Fate of 216 seed of three clover species oversown onto intact pasture sods from 0-40 d. Figures in parentheses are the percentage of total number of seed that was accounted for.

Species	Imbibed but ungerminated seed Moisture	Ungerminated and hard seed (0-4 DAS)	Radicles (6-40 DAS)	Radicles died	Seedling alive	Visible died	Total (% or seed seedlings	Seed not found sown)	(unaccounted)
Subterranean clover	Low	9	49	27	37	0	95	216	0
		(3)	(23)	(13)	(17)		(44)	(100)	
	Medium	9	36	0	24	2	141	211	5
		(4)	(17)		(11)	(1)	(67)	(98)	
	High	11	32	0	26	10	104	182	34
		(7)	(17)		(14)	(5)	(57)	(84)	
Strawberry clover	Low	13	59	14	19	0	49	153	
		(8)	(39)	(9)	(12)		(32)	(71)	63
	Medium	11	58	0	11	5	82	167	49
		(7)	(30)		(7)	(3)	(49)	(77)	
	High	12	54	0	10	11	90	177	39
		(7)	(30)		(6)	(6)	(51)	(82)	
Caucasian clover	Low	24	94	17	24	2	33	192	24
		(13)	(48)	(9)	(12)	(1)	(17)	(89)	
	Medium	18	106	0	9	3	55	190	26
	(8)	(56)		(5)	(2)	(29)	(88)		
	High	19	101	0	11	10	47	188	28
		(10)	(54)		(6)	(5)	(25)	(87)	
SEM		1.2	3.6	2.1	2.5	1.4	3.8		
Significance									
Species	**	***	**	***	ns	***			
Moisture	***	*	***	***	***	***			
Species x moisture	*	ns	*	ns	ns	***			

Table 2
Percentage (average) of hard and ungerminated seed of three clover species present in standard seed germination test, accelerated aging test and oversown on soil surface and then trampled.

Species	Standard seed germination test	Accelerated aging test	Seed oversown on soil surface
Subterranean clover cv. Karridale	6	10	19
Strawberry clover cv. G. Onward	15	19	35
Caucasian clover cv. Monaro	27	49	53
SEM	6.08	11.08	9.82