IMPLICATIONS OF BIOMECHANICAL CHARACTERS OF SUBTERRANEAN CLOVER ON FEEDING BY REDLEGGED EARTH MITE AND INTAKE BY SHEEP

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ABSTRACT
The biomechanical strength of cultivars of subterranean clover (*Trifolium subterraneum*) that were either resistant or susceptible to feeding by redlegged earth mite was measured as the force required to penetrate their leaves with a small, tubular punch. Cotyledons and trifoliate leaves of cultivars resistant to redlegged earth mite had a higher resistance to penetration than cultivars susceptible to redlegged earth mite. Cotyledons needed a higher force to penetrate than trifoliate leaves. The possible implications for intake by ruminants of differences between the cultivars in biomechanical characters are also considered.

KEYWORDS
Biomechanical characters, force to penetrate, strength, *Trifolium subterraneum*, redlegged earth mite

INTRODUCTION
Biomechanical characters of plant material influence both its resistance to feeding by invertebrates and to intake by ruminants. Hochuli (1996) provided a critical appraisal of the role of biomechanical properties of plants as a defense mechanism against insect herbivores and concluded that, despite some terminological confusion, correlative evidence supports the notion that biomechanical properties are important inhibitors of insect herbivory,and that several direct studies suggest that biomechanical properties are associated with reduced fitness and feeding performance of insect herbivores. For example, Jiang and Ridsdill-Smith (1996a,b) reported a strong, negative correlation between the force required to penetrate the surface of cotyledons of subterranean clover (*Trifolium subterraneum*) with a small, tubular punch and a feeding damage score caused by redlegged earth mite (RLEM) (*Halotydeus destructor*) (Acari: Penthaleidae) over two weeks (r^2 = 0.752).

There are several reports of negative correlations between biomechanical properties of plant material and intake by ruminants. In an early study, Chenost (1966) defined a 'fibrosity index' as the electrical energy required to pulverise a fixed amount of dried forage, and a strong negative correlation was established with voluntary dry matter intake by sheep (r^2 = 0.808). Later studies have established similar relationships for other biomechanical properties and plant materials of differing stages of maturity. For example, Mackinnon et al. (1988) reported a negative correlation between the shear strength of leaves of perennial ryegrass (*Lolium perenne*) in the vegetative stage of development and voluntary feed intake, and Baker *et al.* (1993) observed a negative correlation between the energy required to both compress and shear mature, dry subterranean clover with the voluntary dry matter intake by sheep.

Ridsdill-Smith *et al.* (1994) showed that for six cultivars of subterranean clover a feeding damage score caused by RLEM, and the number of RLEM present after 5 weeks was not correlated with eating rate by sheep. In this paper we consider the importance of biomechanical strength of subterranean clover on feeding by RLEM, and whether the differences that were observed by Ridsdill-Smith et al. (1994) between the cultivars of subterranean clover may have been due in part to differences in their biomechanical properties.

MATERIALS AND METHODS
Nine cultivars of subterranean clover were selected which were known to be either resistant or susceptible to feeding by RLEM. The esistance of a cultivar was determined by scoring the feeding damage which occurred when it was exposed to an equal number of mites; those with low feeding damage score were considered to be resistant. Seeds were sown in a 4:1 sand:loam mixture in pots (130 mm top diameter, 100 mm in height) as described by Ridsdill-Smith and Gillespie (1993), and maintained in a glasshouse. Of the 9 cultivars sown, five were resistant and two were susceptible to feeding by RLEM. Cotyledons were harvested 12 days after seeding, and trifoliate leaves were harvested 40 days after seeding. In both cases their biomechanical strengths were measured immediately after harvest using a Hounsfield Testing Instrument. Individual cotyledons or leaves were pierced through the top surface with a small (1.6 mm leaves of trifoliates.

RESULTS AND DISCUSSION
The force required to penetrate both cotyledons and trifoliate leaves of cultivars of subterranean clover resistant to RLEM was higher than that for cultivars susceptible to RLEM (Table 1). On average, was a significant, positive correlation between the resistance ofcotyledons to penetration and that of trifoliate leaves (r^2 = 0.574, $p<0.02$).

The force required to penetrate the upper surface of the cotyledons was higher than that required to penetrate the surface of trifoliate leaves. Cotyledons have a higher water content than trifoliate leaves, which may confer higher resistance to penetration through increased turgor pressure. Cotyledons and trifoliate leaves also differ significantly in their anatomical and chemical characters (Esau, 1977), and a number of these characters have been reported to contribute significantly to their biomechanical characters. For example, leaf cellulose concentration influences leaf tensile strength (Wilson, 1965) and sclerenchyma concentration influences leaf shear strength (John *et al*., 1989).

When feeding of RLEM and abundance of mites on different cultivars of subterranean clover was compared with rate of intake by sheep of those cultivars, there were significant differences between the cultivars in the amount of feeding damage by mites and the eating rate by sheep (Ridsdill-Smith *et al*., 1994). The number of mites produced was negatively correlated with the concentration of phytoestrogen Biochanin A, but only 68% of the variation in the number of mites could be explained. Eating rate by sheep was best explained by a linear regression equation including sward height and the concentration of soluble sugars, but this relationship explained only 29% of the variation in eating rate (Ridsdill-Smith *et al*., 1994). The biomechanical characters of the plant material may contribute a significant proportion of the remaining variation. We have established that the resistance to penetration appears to influence feeding damage score by RLEM, and relationships between biomechanical characters and feeding by sheep have been established previously (Mackinnon *et al*., 1988; Baker *et al*., 1993). Further, Dynes *et al.* (1993) reported a 14% lower rate of intake by sheep eating subterranean clover cv. Dalkeith than when they ate cv. Trikkala when plants were in their vegetative stage of development, and this may have been a reflection of a 44% higher energy required to shear cv. Dalkeith than cv. Trikkala (Henry et al., 1996).

Biomechanical properties appear to have an important role in determining both feeding by RLEM and intake by sheep. Further work is in progress to determine which plant characteristics influence their biomechanical properties and their role in determining feeding by redlegged earth mite and eating rate and total voluntary intake by sheep.

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Table 1
Force to penetrate (N) cotyledons and trifoliate leaves of cultivars of subterranean clover that are susceptible or resistant to redlegged earth mite (RLEM). Cultivars were tested 12 days after seeding for cotyledons and 40 days after seeding for trifoliate leaves.

	Force to penetrate (N) (± s.e.)	
	Cotyledons (12 days after seeding)	Trifoliate leaves (40 days after seeding)
Cultivars susceptible to RLEM:		
Goulburn	1.095 ± 0.029	0.632 ± 0.028
Trikkala	1.033 ± 0.022	0.490 ± 0.013
Seaton Park	1.013 ± 0.015	0.674 ± 0.034
Denmark	1.124 ± 0.031	0.679 ± 0.028
Mean	***1.066 ± 0.030***	***0.619 ± 0.051***
Cultivars resistant to RLEM:		
YL020	1.360 ± 0.051	0.717 ± 0.054
YL013	1.216 ± 0.029	0.743 ± 0.034
SM020	1.341 ± 0.040	0.767 ± 0.038
SM018	1.347 ± 0.051	0.942 ± 0.035
SM015	1.091 ± 0.032	0.651 ± 0.050
Mean	***1.271 ± 0.058***	***0.764 ± 0.054***