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Variability in short and long-term seed dormancy in clover plants from a single population

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Summary - Plants of the same species of clover (*Trifolium* spp.) from the same site of collection may differ in dormancy strategies. Variation within a population was found in the level of hardseededness both at maturity and after 6 months on the soil surface, and in the half-life of hard seed breakdown. This variation may allow for a particular dormancy pattern to be easily selected by agronomists without the need for extensive germplasm collection.

Key-words: hardseededness, dormancy, intra-population variation, *Trifolium*

Résumé - *Les plantes de la même espèce de trèfle, et venant du même endroit, peuvent avoir des stratégies dormantes différentes. On a trouvé une variabilité au sein de la méme population concernant la dureté de graine aussi bien à maturité qu' après avoir passé six mois au sol et aussi concernant la demi-vie de la décomposition de la graine dure. Cette variabilité peut permettre aux agronomes de selectionner les graines à structure dormante particulière sans nécessiter la collection de gènes.*

Mots-clès*: la dureté de graine, le caractère dormant, la variabilité de l'intrapopulation,* Trifolium

Introduction

Annual pasture legumes rely on hardseededness (dormancy due to a water-impermeable seed coat) to prevent germination after out of season rainfall (short-term dormancy) or to spread germination between seasons (long-term dormancy). The ecological importance of hardseededness as a hedge against unfavourable conditions is well documented (Phillipi and Serger, 1989, Taylor and Ewing, 1992).

Hardseededness is typically measured by pooling the seed of a number of plants collected at a single site. This method is used to compare hard seed breakdown between sites of collection (Ehrman and Cocks, 1996; Norman *et al.,* 1998; Loi *et al.*, 1999). Pooling of seed results in the hard seed measurements being an average of the plants tested, thus there is no indication of variation within populations for this trait.

Dormancy and maturity are partially substitutable reproductive strategies (Venable and Brown, 1998; Ehrman and Cocks, 1996). Variation in maturity within populations is well documented (Cocks, 1992) and it is therefore reasonable to think that there may be complementary variation in hardseededness. The aim of this experiment was to measure variation in rate, pattern and level of hard seed breakdown among plants from within the same population.

Materials and Methods

Origins of accessions and production of seed

Seeds of *Trifolium tomentosum* L., *T. stellatum* L. and *T. campestre* Gmel. were collected along several transects in a single paddock (3 ha) in Tel Hadya, Syria (35°55'N, 36°55'E, 340 mm rainfall) in 1997. The seeds were sown as single spaced plants at the Shenton Park Research Station (31.56° S,115.47°E) near Perth, Western Australia, on the 5 May 1998. Plants were irrigated and fertilised as necessary and were harvested in November 1998 after drying off. Four plants of each species were randomly chosen for hard-seed testing.

Hard seed breakdown

All the seed was removed from each plant by gently rubbing the inflorescences between soft corrugated rubber mats. The seed from each plant was thoroughly mixed before counting into 40 lots of 100 seeds. Initial hardseededness was tested immediately using 4 lots from each plant and the remaining 36 lots were sewn into fine mesh cotton bags. On the 8th of December 1998 (start of summer) the bags were laid on the soil surface in full sun on a site cleared of vegetation.

During the summer and autumn two samples were removed from the field for hard seed testing every 7 to 14 days (intensity of testing was increased during the period of maximum hardseed breakdown). In total there were 14 sampling dates, the last being the 16th of June 1999. On this final date 8 samples were removed.

The level of hardseededness was determined by moistening the sample with deionised water for 2 weeks at 15°C in a petri dish, then counting the unimbibed seeds that remained. The proportion of unimbibed (hard) seeds was calculated. The hard seeds in the initial samples were scarified after this test then allowed to imbibe, to confirm 100% viability and absence of embryo dormancy.

Statistical analysis

Logistic curves were fitted to the data to determine the rate and half-life of hard seed breakdown. For each curve the parameter values and their standard errors were calculated, and an estimate of goodness of fit was obtained. The significance of the variation in the values of each parameter among plants of the same species was determined using an F-test.

Results and Discussion

The fitted logistic curves (Fig. 1) vary considerably, not only between the three species but also between individual plants of each species, and this is confirmed by the statistical analysis (Table 1). There is within species variation for short and long-term seed dormancy in the plants collected from the same site. The level of hardseededness after 6 months on the soil surface varied significantly between the 4 plants tested of each of the 3 species, as did the half-life of hardseededness. The initial level of hardseededness was variable in *T. stellatum* and *T. tomentosum*, but this variability was not correlated with date of maturity.

The variation among plants in the parameters of the logistic curves suggests that individual populations contain genetic variation with regard to dormancy traits. This indicates either that plants in the same microenvironment are able to persist using different short and long-term dormancy patterns, or else that there are distinct niches within the environment at a given site. Ehrman and Cocks (1996) associated increasing aridity with declining variation in reproductive strategies. The site of collection for this study is relatively arid (compared to other places where these species are found) yet individual plants appear to have differing dormancy strategies.

Alternatively, since the site was sloping there is a good chance that micro-environments vary: Cocks (1992) was able to correlate flowering time in subterranean clover to position on slope.

Table 1: Pattern in breakdown of hardseededness among individual plants of three *Trifolium* species during 6 months of field exposure[1].

Species	Plant	Initial hardseed (%)	6 months later (%)	Half life (days)	R^2 (%)
T. stellatum	A	90 (2.5)	48 (2.6)	133 (8.8)	75.5
	B	97 (1.8)	75 (1.8)	110 (8.8)	73.7
	C	92 (3.0)	52 (2.8)	91 (6.9)	80.3
	D	80 (1.7)	61 (2.5)	88 (6.6)	69.2
	F	**	**	**	
T. tomentosum	A	97 (1.3)	67 (1.8)	136 (6.0)	79.5
	B	98 (1.0)	69 (3.2)	145 (7.0)	88.3
	C	98 (1.6)	67 (4.5)	105 (6.4)	81.3
	D	99 (0.5)	88 (1.6)	146 (9.8)	79.2
	F	*	**	**	
T. campestre	A	97 (1.6)	58 (4.1)	145 (6.8)	83.5
	B	97 (0.8)	86 (2.5)	112 (7.7)	70.8
	C	100 (1.4)	65 (4.3)	133 (7.9)	90.6
	D	98 (0.9)	72 (2.3)	121 (4.0)	89.9
	F	n.s.	**	**	

[1]Standard errors are in parentheses. R^2 represents the fit of the curve to the raw data. Asterisks represent the significance of the variation in the curve parameters among plants: * P<0.05; **P<0.001; n.s. is not significant.

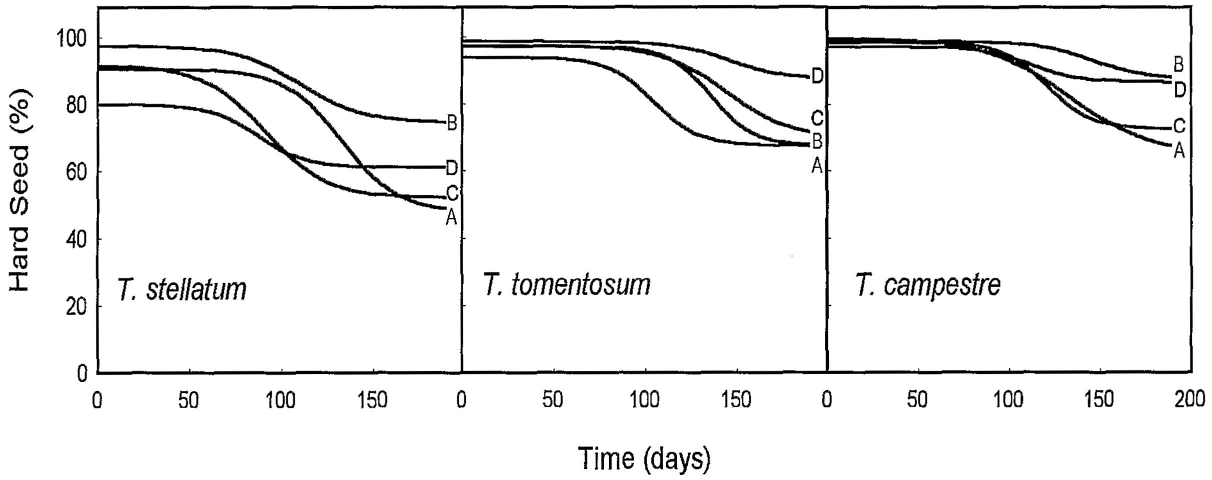

Figure 1: Fitted logistic curves for breakdown of hardseededness in 4 plants of each of 3 clover species collected at the same site in Syria.

Conclusions

Plants of the same species from a single site of collection demonstrated variation in dormancy characteristics. The agronomic implications of this study are that a range of dormancy strategies can perhaps be found without extensive germplasm collection. This applies both to long-term dormancy strategy (represented by the level of hardseededness at the end of the testing period) and short-term strategy (represented by the half life of hardseededness). Previously it has been postulated that in order to find different hardseed breakdown patterns or dormancy levels for inbreeding species it is necessary to collect from a range of different environments.

Variation in hardseededness within populations could be necessary for the populations to persist in environments that vary in both time and space. In selecting new cultivars plant breeders need to remember site variation and the genetic variation that will increase the likelihood that their selections will be adapted to this site variation. This is especially important for inbreeding populations that are unlikely to rapidly generate new diversity.

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