EFFECT OF TEMPERATURE AND LIGHT ON SEED SOFTENING IN YELLOW SERRADELLA

C. K. Revell[1] and G. B. Taylor[2]

[1]Agriculture Western Australia, Northam, WA 6401
[2]CSIRO Centre for Mediterranean Agricultural Research, Wembley, WA 6014

Abstract

A field experiment involving pod burial treatments was conducted to describe the pattern of seed softening in the GEH72-1A accession of yellow serradella over the first summer/autumn in a mediterranean environment. Over 90% of hard seeds buried in December at a depth of 0.5 cm softened by the end of autumn, compared with less than 20% of seeds left at the soil surface. Seed softening was most active between 27 February and 12 March, when soil temperatures ranged from minima of 15-20^{o}C to maxima of 45-50^{o}C. Seeds taken from the soil surface on 5 March and treated in the laboratory with four gradual diurnal temperature cycles in the vicinity of 47/15^{o}C produced substantial seed softening, but only when the pods were treated in darkness. These results suggest that first year regeneration may be considerably enhanced by shallow tillage before the beginning of autumn.

Key words: Serradella, legume, hard seeds, seed softening, temperature, burial, light

Shallow burial of yellow serradella (*Ornithopus compressus* L.) pods, either after one summer at the soil surface (1) or early in the first summer (2), has been found to markedly accelerate seed softening. The precise timing at which seed softening occurred in these studies is unclear as measurements were made on only two occasions during the summer/autumn. Laboratory treatments based on a 2-stage temperature dependent process for the softening of seeds of subterranean clover (*Trifolium subterraneum* L.) (3) and burr medic (*Medicago polymorpha* L.) (4) have not been successful for yellow serradella (1, 2). The 2-stage process involves a period of high temperature preconditioning of the seed followed by a period of species-specific alternating temperatures which are responsible for the actual softening of the seed.

A field experiment and a series of laboratory experi-ments are reported in this paper. In the field experiment, the pattern of softening of hard seeds was determined for a single strain of yellow serradella whose pods were subjected to shallow burial, either early in summer or at the beginning of autumn after spending the summer at the soil surface. The objective of the laboratory experi-ments was to define the factors responsible for seed softening. Soil temperatures experienced at the time of most active seed softening in the field were used as a guide to the selection of appropriate laboratory treat-ments.

Materials and methods

Field Experiment

Pods of the GEH72-1A accession of yellow serradella were produced in ungrazed plots sown in June 1995 at Yelbeni in Western Australia (31^{o}15'S, 117^{o}45'E). Newly ripened pods were collected on 7 December and allocated at random into sample lots of 32 whole pods. The mean number of viable seeds in each lot was 182 (s.e. 3.6) with a mean weight per seed of 2.8 mg. The initial moisture content of the hard seeds was 3.8% (s.e. 0.1%).

The seed softening study was conducted from December 1995 to June 1996 at the Merredin Research Station (31^{o}29' S, 118^{o}16'E) on a gently sloping, loamy sand site. Pod lots were placed in fibreglass flywire envelopes (10 x 11 cm). Details of the treatment and sampling times are summarised in Table 1. A single pod lot was removed at each sampling time, except for the initial germination test and the soil surface treatments in March and June for which there were four replications. Each treatment was laid out as a single block, within which the sampling dates were randomised. Daily rainfall and soil temperatures at a depth of 0.5 cm were recorded at 30 minute intervals (Fig. 1). Germination tests were conducted by placing the pods on moist filter paper in petri dishes at 20°C for 14 days. Seedlings were counted and removed and the numbers of residual hard seeds determined. Seed softening was calculated from the decline in the numbers of hard seeds determined at each sampling time and adjusted for the number of pod segments recovered, using the procedure described by (1).

Table 1. Description of treatments (Tr) and time of sampling in the field experiment.

Tr	Description of treatments applied to pod lots	Number of pod lots
1	Initial germination test.	4
2	Soil surface from 28/12/95 and sampled 5/3/96.	4
3	Soil surface from 28/12/95 and sampled 11/6/96.	4
4	Buried at 0.5 cm on 28/12/95. Sampled weekly from 9/1/96 to 11/6/96.	23
5	Stored at 20°C until periods of one week in the field buried at 0.5 cm (commenced 28/12/95)	22 (1 each week until 11/6/96)
6	Soil surface from 28/12/95 to 5/3/96, then buried at 0.5 cm on 5/3/96. Sampled weekly from 12/3/96 to 11/6/96.	14

Laboratory experiments

A bulk sample of GEH72-1A pods from the same population as that used in the field experiment was preconditioned on the soil surface at Merredin from 28 December, 1995 to 5 March, 1996. After their recovery from the field the pods were broken into individual segments and allocated into lots of 50, before storing at 20°C prior to further treatment. At this stage 2.1% (s.e. 1.2) of the seeds were soft, nearly all of which were viable. Further treatments of the preconditioned seeds were conducted in a programmable temperature chamber, described by (4), in which the heat source was provided by incandescent light globes. Linear diurnal changes in temperature that were based on a time scale typical of field conditions were used to simplify the treatment comparisons. The level of photosynthetically active radiation produced by the chamber lights was 5.9 (s.e 1.4) mmol/m2/s, about 0.3% of full sunlight. Each seed lot was placed in a 10 ml glass bottle for subsequent treatment.

The first series of treatments are shown in Table 2 and examined various environmental influences for their effect on seed softening. All these treatments were subjected to diurnal temperature fluctuations involving a 6 hr rise from 15°C to 47°C, 1 hr at 47°C, a 6 hr fall from 47°C to 23°C and an 11 hr fall from 23°C to 15°C (abbreviated as 47/23/15°C). All treatments were subjected to four diurnal cycles of these temperatures, and some treatments were subjected to an additional four cycles. There was no replication of the treatments in this series owing to the limited supply of preconditioned seeds. The importance of sand cover was determined by covering the seeds with fine dry sand. The effect of light was determined by wrapping the bottle in a double layer of aluminium foil. As this procedure may also have affected the rate of temperature change experienced by the seeds, an additional treatment was included which involved blackening the light bulbs in the chamber with black paint and aluminium foil. Saturated or dry atmospheres were generated by the addition of water or silica gel to the bottles, respectively.

A second series of treatments was designed to investigate the sensitivity of seed softening in conditions of light and darkness in duplicate samples that were subjected to the range of diurnal temperature fluctuations shown in Table 3. The proportion of soft seeds in both series of treatments was determined by counting the seedlings and residual hard seeds after soaking the seeds for 14 days at 20°C.

Results

Field Experiment

In the initial germination test, 89.2% (s.e. 0.4) of the pod segments contained either hard or viable soft seeds, of which only 0.6% (s.e. 0.2) were soft. No rain fell between January and March. Rain in mid-April germinated most of the soft seeds which were subsequently killed.

Less than 20% of the seeds in pods on the soil surface softened during summer and autumn (Fig. 2). Most of this softening occurred after 5 March. In contrast about 90% of the seeds buried continuously at 0.5 cm softened by the end of autumn. The most rapid softening occurred between 27 February and 12 March. None of the samples buried for 1 week periods at 0.5 cm, after storage at 20^{o}C, showed any appreciable seed softening at any time. The burial of pods in March at 0.5 cm had a marked effect on seed softening with over 90% of the hard seeds softening after 5 to 7 weeks (Fig. 2).

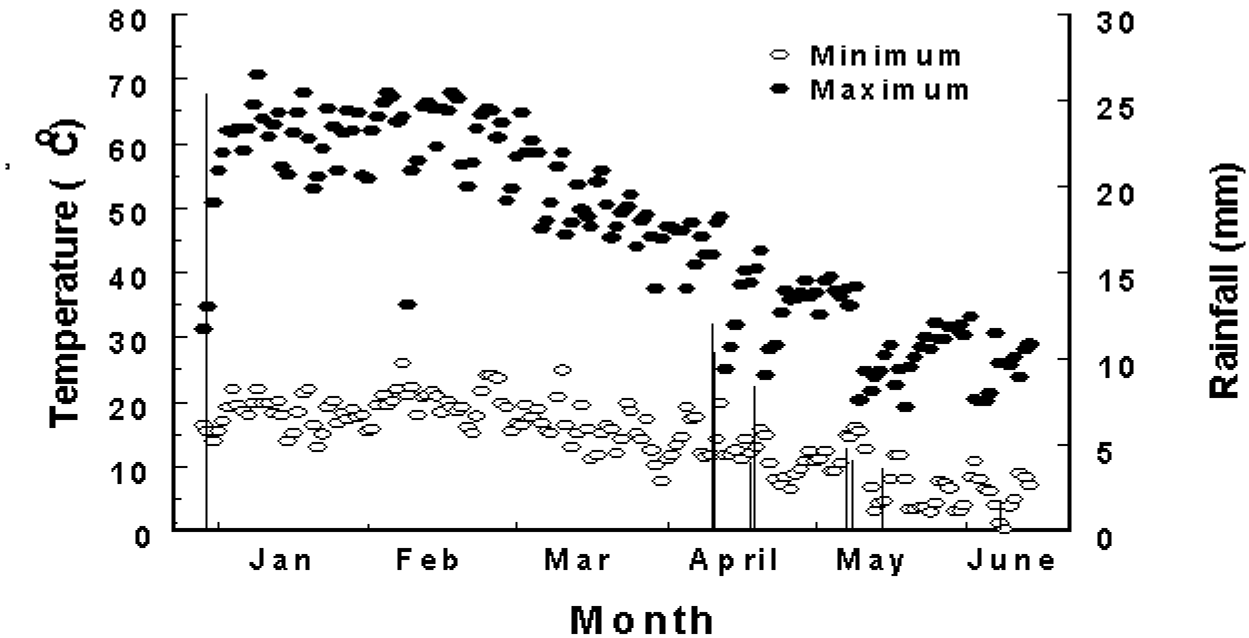

Figure 1: Daily rainfall (bars) and maximum and minimum temperatures recorded at a depth of 0.5cm at the seed softening site at Merredin durinf summer and autumn in 1996

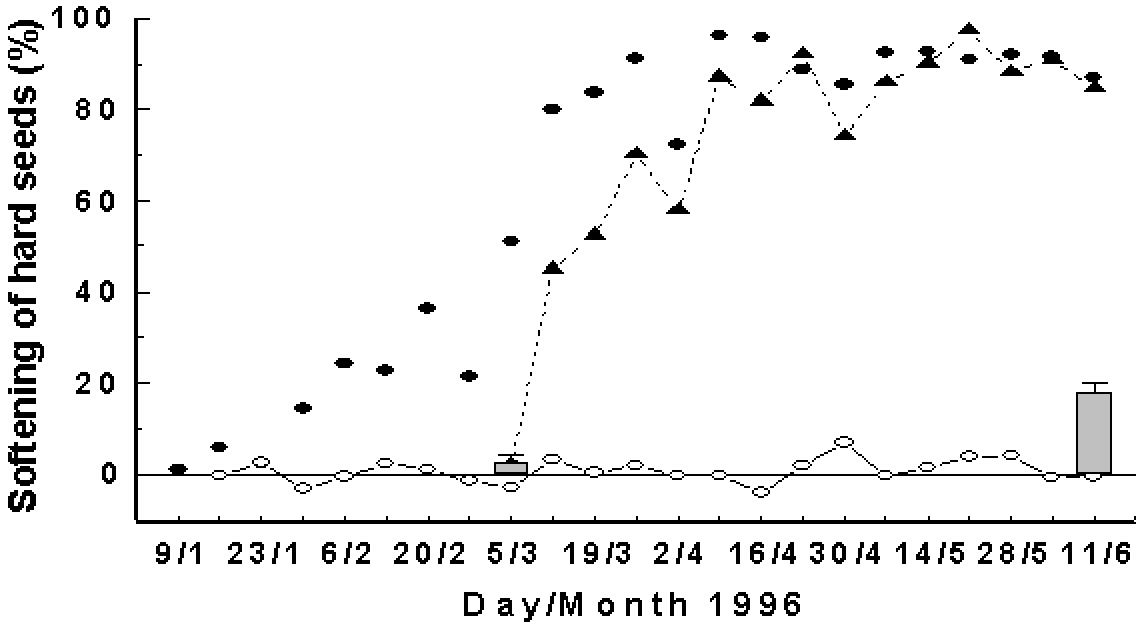

Figure 2: Softening of hard seeds (%) of GEH71-1a at Merredin in 1996 for pods buried continuously (solid circles) and for periods of one week (open circles) at 0.5 cm from the beginning of summer; and for pods buried continuously from 5/3/96 at 0.5 cm (solid triangles) following 10 weeks at the soil surface. Seed softening (%) in pods at the soil surface (columns) is shown in March and June (vertical bars indicate s.e. of the mean).

A high proportion of the daily maximum soil surface temperatures during the period of most active seed softening were in the range of 45 - 50°C, while the daily minimums were generally in the range of 15 - 20°C (Fig. 1).

Laboratory experiments

Some seed softening occurred in all of the first series of treatments involving temperature fluctuations of 47/23/15°C (Table 2). However, the proportion of seeds which softened was greatly increased by a sand covering and, in particular, by darkness, regardless of whether this was obtained by the aluminium foil wrapping or a darkened chamber. The greatest proportion of soft seeds was obtained after four temperature cycles. There was no effect of atmospheric moisture content on seed softening.

In the second series of treatments seed softening was increased by darkness under all temperature regimes, with most softening occurring in the 47/23/15°C treatment (Table 3).

Discussion

Results from the field experiment are consistent with those from an earlier study (2) which showed that con-tinuous shallow burial of pods during the first summer substantially increased the rate of softening of hard seeds when compared with that obtained in pods left on the soil surface. The lack of softening of seeds in pods that were buried for periods of only 1 week, particularly during March when continuously buried seeds were softening rapidly (Fig. 2), indicates that some preconditioning of the seeds at temperatures above 20°C is required before seed softening can occur.

Results from the laboratory experiments indicate that seed softening in yellow serradella is, indeed, a 2-stage process similar to that described for subterranean clover (3) and burr medic (4). However, the temperature requirements for the final stage of softening in yellow serradella are different from these other species (in the vicinity of 47/15°C cf. 60/15°C for subterranean clover and 35/10°C for burr medic). The relatively few diurnal temperature cycles required for the final stage of soften-ing (between 4 and 8) is consistent with the results obtained for burr medic (4), as is the absence of any effect of atmospheric moisture content during this stage of softening.

The requirement for darkness during the final stage of seed softening offers an explanation for the large softening response to burial. That some softening occurred under 'light' conditions in the chamber (Tables 2 and 3) may merely reflect the low intensity of this light treatment compared with that at the soil surface. The seeds appear to be particularly sensitive to light as the pod wall which surrounds them must intercept a high proportion of the incident light.

Table 2. Details of the laboratory treatments and their influence on seed softening.

a) Percentage of soft seed after four and eight cycles of 47/23/15°C alternating temperatures

Treatment	Soft seed (%)		
	First 4 cycles	Second 4 cycles	Total (8 cycles)
Illuminated chamber			
10ml bottle, no lid	27.1	16.7	43.8
10ml bottle, lid-sealed	17.6		
10ml bottle, no lid, shallow sand	35.4	27.1	62.5
10ml bottle, no lid, sand above and below seed	44.7	25.5	70.2
10ml bottle, no lid, DARK (wrapped in alfoil)	70.8	4.2	75.0
Darkened chamber			
10ml bottle, no lid	63.0		
10ml bottle, lid-sealed	48.9		
10ml bottle, lid-sealed, 4 drops water	62.2		
10ml bottle, lid-sealed, silica gel	60.9		

b) Percentage of soft seed after four cycles of varying alternating temperatures (s.e. of the mean in parentheses)

Alternating Temperature (°C)	Light	Dark
40/21/15	2.1 (2.1)	8.2 (0.1)
45/22/15	8.5 (1.8)	31.6 (9.2)
47/23/15	18.0 (3.7)	36.6 (7.4)
50/24/15	12.0 (1.1)	35.0 (4.6)
55/25/15	4.3 (0)	13.0 (2.6)

There was a substantial reduction in the proportion of seeds which were softened by the 47/23/15°C treatment in the second compared with the first series of laboratory treatments (Table 2). This reduction may be attributable to the longer period that these seeds were stored at 20°C before the treatments commenced. This may in turn suggest some reversal of the preconditioning process. Further work is required to determine whether the specific fluctuating temperatures and conditions of darkness necessary for seed softening in GEH72-1A are applicable to other varieties of yellow serradella. Until such time, the evaluation of seed softening patterns in new varieties should be conducted in the field and incorporate shallow burial treatments.

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