Development of Embryo Dormancy and Abscisic Acid Concentration During Seed Maturation in Beech

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Nuts of beech (*Fagus sylvatica*) were harvested from a single tree at nine different developmental stages during the period 3 June to 28 September 1993. Seeds were tested in a standard germination tests at 5 and 15C. Seeds incubated at 15C were unable to germinate at any stage of development. Seeds harvested after 16 August had reached a point of maturity where they were able to germinate at 5C. To test the effect of covering structures on dormancy, seeds harvested 16 August and later were germinated as whole seeds, seeds without pericarp, and seeds without pericarp and testa (naked embryos). Again only seeds incubated at 5C germinated after at least 3 months at 5C meaning that the seeds possess true embryo dormancy, requiring cold treatment for dormancy breakage.

At all stages of development ABA was extracted and quantified by a radioimmunoassay. The development in ABA concentration showed a pattern well known from other orthodox seeds, with a sharp rise in concentration at the time where the seeds reached maximum fresh weight. The ABA content decreased during the maturation phase—upon full maturity we found a low concentration of ABA. Concurrently with a high level of ABA an increase in protein accumulation is found, especially in the heat-stable fraction. These heat stable proteins belong to a group of proteins called late embryogenesis abundant (LEA) proteins. The function of these proteins are yet unknown, but their appearance in practically all species with orthodox seeds has been correlated to development of desiccation tolerance and dormancy.

INTRODUCTION

Mature seeds of beech (*Fagus sylvatica*) are dormant and require a cold treatment to break dormancy before the seeds are able to germinate. The cold treatment is traditionally performed at 5C on fully imbibed seeds. Because the seed lot is heterogeneous, some seeds will have their dormancy broken before others and start to germinate. If the treatment is interrupted some seeds will still be dormant and unable to germinate in a seed bed. The result is a partial loss of a seed lot either caused by dormancy, or caused by damage to germinated seeds during sowing.

This study is part of a project with the main aims to develop efficient methods to break dormancy without germination, and to get a better understanding of dormancy mechanisms in tree seeds on a molecular level.

MATERIALS AND METHODS

Plant Material. Beech nuts were collected from a single tree in Lundtofte, every 2 weeks from the beginning of June to the end of September.

Germination. Seeds were germinated in 3 or 4 replicates of 25 seeds on top of filter paper in germination boxes. The filter paper was supplied with deionized water from a wick, ensuring constant moisture content during the germination period. The beech nuts were germinated in the dark at constant temperature at 5 or 15C

ABA Extraction and Purification. Seeds for ABA extractions were removed from the capsules at the day of harvest and frozen in liquid nitrogen. Lyophilized seeds were ground in a pistil mortar (minimum 10 seeds) and duplicate extractions were made for each sample. One hundred milligrams dry matter was extracted in 4 ml of 0.02 M sodium-phosphate buffer (pH 7.3). To stop enzymatic degradation of conjugates, the samples were immediately heated in boiling water for 5 min (Loveys and Dijk, 1988). The extraction continued over night at 5C. Solid matter was spun down and 1 ml of extract was taken out. Three drops of 1M H_2SO_4 were added and ABA was extracted in 3 × 1 ml of water-saturated ethyl acetate. Ethyl acetate and buffer were thoroughly mixed and separated by centrifugation to ensure an effective extraction. The organic phase was passed through a Sep Wac. silica cartridge (Waters Associated, 3 ml, 500 mg sorbent). The elute plus further 3 ml wash (water-saturated ethyl acetate) was pooled and reduced to dryness under a stream of air. ABA was redissolved in 1 ml of water.

ABA Measurements. The concentration of ABA was measured according to Quarrie et al. (1988).

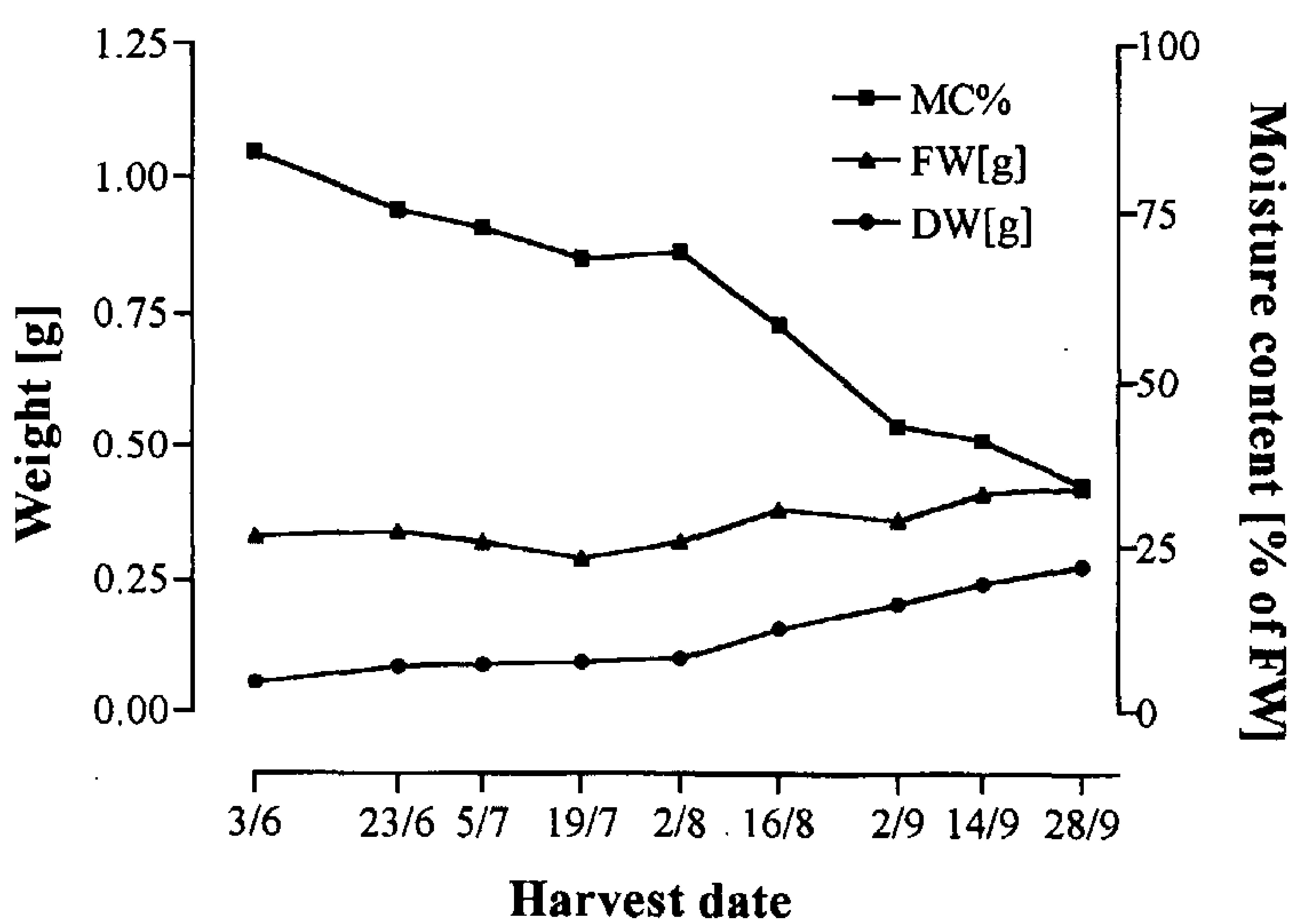

Figure 1. Fresh weight, dry weight and moisture content during seed development in *Fagus sylvatica*. Each point is an average of two measurements.

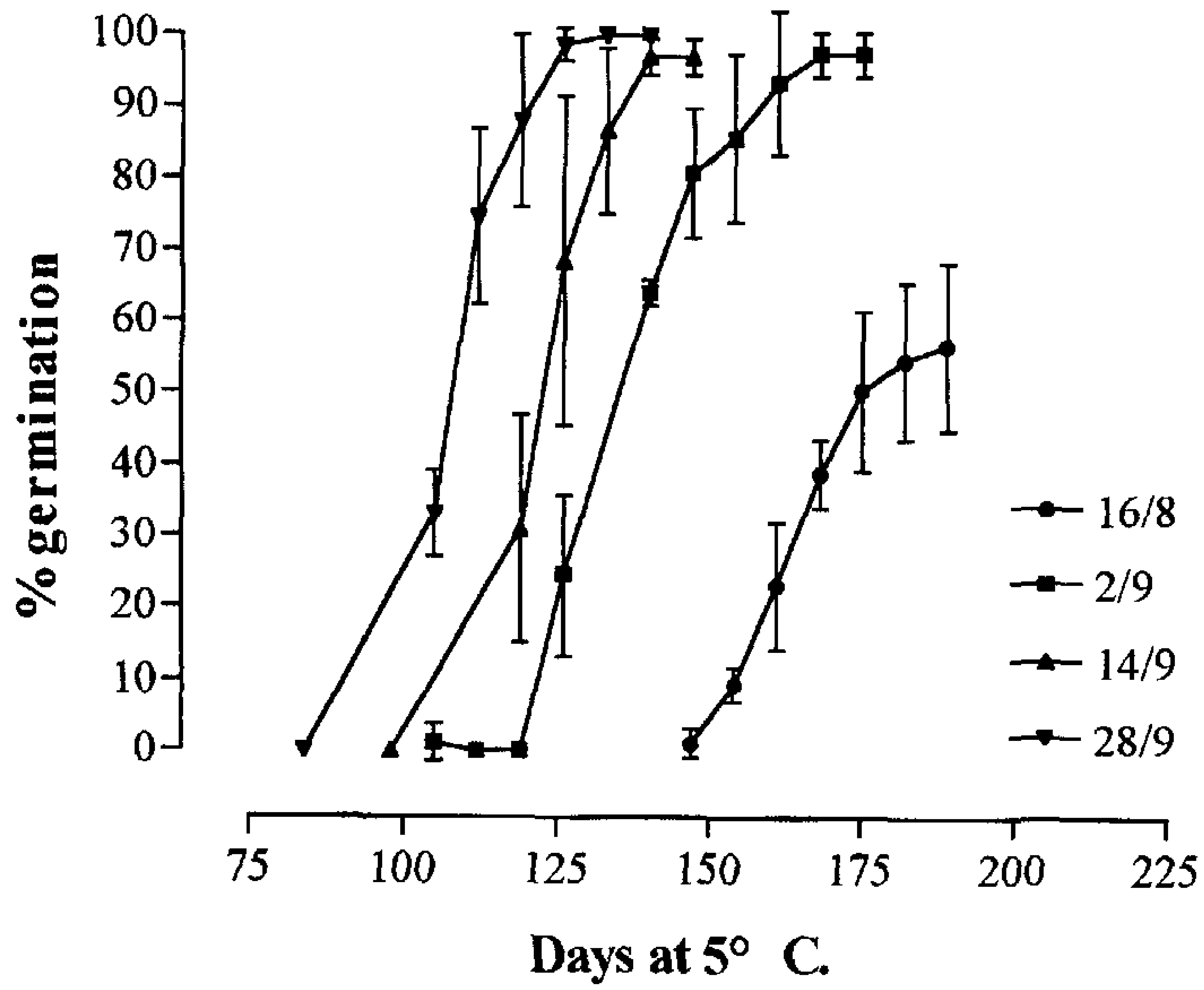

Figure 2. Accumulated germination for seeds harvested at different developmental stages. Harvest dates according to legends. Each point is average of 3 or 4 replicates ± S.D.

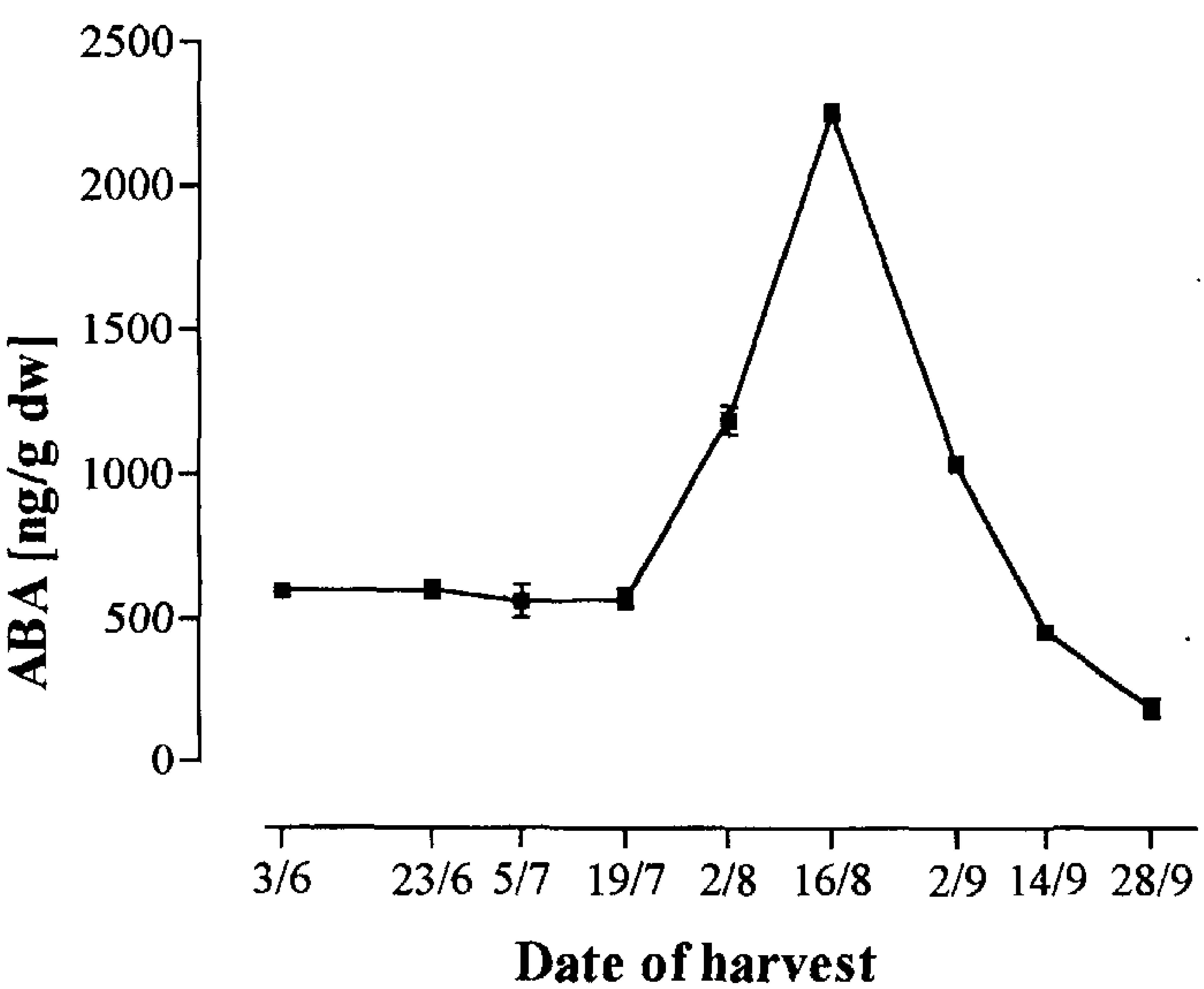

Figure 3. Abscisic acid concentration on dry weight basis at different developmental stages. Each point is an average of two extractions ± S.D.

RESULTS

From 3 June to 16 August the seed fresh weight increased which correlated to a visible increase in size of the embryo. The size increased fast during a short period from 5 July to 2 August, when the embryo filled the pericarp. The pericarp only changed slightly in size during the period examined. The moisture content showed a rather constant drop from 84% at 3 June to 34% at 28 September when the seeds were shed (Fig. 1).

No seeds were able to germinate at 15C, but seeds harvested 16 August and later were able germinate at 5C. From 16 August to 2 September the number of germinable seeds increased, but seeds harvested 2 September and later germinated to nearly 100%. The speed of germination increased during the maturation phase (Fig. 2)—meaning the later the harvest the lesser the dormancy.

The changes in ABA concentration showed a pattern well known to other species including both woody and herbaceous species. The concentration on a dry-weight basis is constant until 19 July, after which a sharp rise in concentration occurs with the highest concentration reached at 16 August, the same date the seeds reached a point of maturity where they were able to germinate. During seed maturation the ABA concentration declines, and at full maturity the ABA concentration is even lower than the one found in very young seeds (Fig. 3).

Proteins accumulated in the seeds after they filled the pericarp and we found an increasing protein concentration during the period 2 August to 28 September. The accumulation of heat-stable proteins started 16 August which coincided with the time of highest ABA concentration. Especially in the acidic part of the gel, a group of heat-stable proteins accumulate during the maturation phase of seed formation.

DISCUSSION

In this study we are unable to conclude, whether the higher ABA level at 16 August is correlated to development of dormancy, as the seeds are already deeply dormant, as they reach the point of maturity, where they are able to germinate. We are therefore unable to distinguish between changes connected to dormancy and changes correlated to maturation of the seeds. One role of ABA in seeds could be to prevent precocious germination while the seeds are still on the mother plant, as ABA is known to be an efficient inhibitor of germination. The correlation between the high ABA concentration and the ability to germinate after cold treatment on 16 August, supports this theory. The fact that the ABA concentration drops upon full maturity implies that ABA alone does not control dormancy, as the fully mature seeds are dormant and require about 3 months at 5C to break dormancy.

Heat-stable proteins have been reported to accumulate late in seed development in several species. Blackman et al. (1991) found a correlation between accumulation of heat-stable proteins and the development of desiccation tolerance in soybean (*Glycine max*). Several LEA proteins are heat stable, e.g., dehydrins (Close et al., 1993), and ABA inducible (Skriver and Mundy, 1990). The correlation between high ABA content in the seeds and accumulation of heat-stable proteins supports the theory that ABA is inducing transcription of LEA genes.

The function of *LEA* proteins is unknown but their hydrophilic nature implies that they are involved in protection against desiccation damage. Further studies will examine the development of desiccation tolerance during seed maturation in correlation to changes in ABA concentration in the seeds, and the use of antibodies against dehydrins will show if the accumulation of dehydrins is correlated to development of desiccation tolerance.

LITERATURE CITED

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