possible that a different temperature regime or a longer acclimation period would cause asparagus seedlings to become artificially acclimated to temperatures below -6.5°C.

Literature Cited


Relationship Between Viability, Endosperm Integrity, and Imibed Lettuce Seed Density and Leakage

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Abstract. Imibed nonviable lettuce (Lactuca sativa L.) seeds have been shown to have lower density than imibed control seeds. The purpose of this study was to investigate density differences associated with seed death. The relationship between endosperm integrity and the volume, density, and leakage of imibed control and heat-killed lettuce seeds was studied. After an 8-hr soak, heat-killed seeds imbibed 23% more water than control seeds. The percentage of heat-killed seeds with density of 1.08 g cm−3 was 2%, compared to 90% for the control. Mean electrical conductivity of the steep water was similar for heat-killed and control seeds. Seeds were punctured to rupture the endosperm layer surrounding the embryo. Puncturing the heat-killed seeds decreased total water uptake, as measured by decreased swelling, and increased density compared to intact heat-killed seeds. Leachate from punctured heat-killed seed had a 41% higher mean conductivity than that from punctured control seed. These data suggest that the undamaged endosperm restricted leakage of electrolytes from the embryo to the soak water. We speculate that the endosperm caused osmotically active solutes to accumulate in the extra-embryonic fluid of heat-killed seeds. This accumulation of solutes decreases the water potential inside the embryonic pouch, resulting in a greater uptake of water from the environment. The additional water uptake by heat-killed seeds would increase seed swelling and decrease seed density relative to control seeds.

Table 1. Volume quotients and electrical conductivities of control or heat-killed seeds that were punctured or left intact.

<table>
<thead>
<tr>
<th>Seed condition</th>
<th>Volume quotient (V/Vo)</th>
<th>Conductivity (µA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.05 ± 0.22</td>
<td>11 ± 1</td>
</tr>
<tr>
<td>Heat-killed</td>
<td>3.97 ± 0.25</td>
<td>10 ± 1</td>
</tr>
<tr>
<td>Punctured</td>
<td>2.47 ± 0.03</td>
<td>17 ± 1</td>
</tr>
<tr>
<td>Punctured heat-killed</td>
<td>2.35 ± 0.04</td>
<td>24 ± 1</td>
</tr>
</tbody>
</table>

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Fig. 1. Weight and volume quotient changes during various periods for control (---) and heat-killed (- -) lettuce seeds (means ± SE).

Fig. 2. Density class distributions of lettuce seeds in response to puncturing and heat-killing (means ± SE).

The purpose of this study was to investigate density differences associated with seed death and to propose a physiological mechanism to explain the density differences. The integrity of the endosperm was studied in relation to volume, weight, density, and leakage associated with viability of imbibed seed.

‘Montello’ lettuce seed (Harris-Moran, Rochester, N.Y.) were used in all studies and 96% germinated; these seeds are referred to as control seeds. Seeds referred to as heat-killed, were obtained by autoclaving samples from the same lot at 121°C for 20 min.

Weight and volume of seeds were recorded after soaking control or heat-killed samples 1, 2, 4, 6, 8, 24, or 48 hr in distilled (d) water at 25°C using specific gravity bottles (4, 7). There were four replications of 2-g samples for each treatment. The weight quotient (Wt/Wt₀) and the volume quotient (V/V₀) were calculated by dividing the weight or volume of the seeds after soaking for a specific time by the initial weight (Wt₀) or volume (Vt₀) of the seeds. Four seed treatments were used in the remainder of the experiments to study relationships between endosperm integrity and seed viability: control, heat-killed, punctured control, and punctured heat-killed. Punctured seeds were produced by inserting a pin with a 0.6-mm diameter completely through the widest part of the dry seed. Volume quotients were determined after an 8-hr soak, again using specific gravity bottles.

Density class distributions were obtained by imbibing seeds in aerated water for 8 hr at 25°C in 40 × 5-cm glass columns. There were three replications of 200 seeds each. After imbibition, seeds were immediately separated into density classes of 0.02 g·cm⁻³ increments using a float–sink procedure with various aqueous solutions of Maltrin 500 (Grain Processing Corp., Muscatine, Iowa) (5, 12). Electrolyte leakage measurements were conducted on 50 seeds of each treatment by submerging single seeds in 1 ml of deionized-distilled water for 8 hr. Electrolyte leakage was recorded using an automatic seed analyzer computer (ASAC-1000, Neogen Corp., Lansing, Mich.) with a 4-V reference. Conductivity was expressed in μA.

The rate of seed weight and volume increase was rapid during initial stages of hydration then slowed as the seed imbibed (Fig. 1). The weight quotients for control and heat-killed seeds were 2.65 and 3.25, respectively, after an 8-hr soak; thus, heat-killed seeds imbibed 23% more water than the controls. The volume quotients for control and heat-killed seeds after 8 hr were 2.80 and 3.54, respectively, with the resulting increase being 26%.

In both control and heat-killed seeds, the dynamics of imbibition appear to fit first-order reaction kinetics (7). Transformation of the x-axis (time) to log₁₀ scale was performed and correlation coefficients were calculated from 1 hr through 8 hr. Positive linear correlations were calculated for each physical characteristic and for seed quality against time; r = 0.99 for weight and volume quotients from control seed, and r = 0.97 for weight and volume quotients from heat-killed seed, both being significant at P = 0.01.

Volumetric changes of control and heat-killed seeds appeared to depend on the integrity of the endosperm (Table 1). After an 8-hr soak, intact seeds of both classes had higher swelling quotients than punctured seeds. Puncturing the heat-killed seeds decreased swelling by 41% compared to the intact heat-killed seeds, with the difference for control seed being 19%. A slight decrease in swelling was measured in punctured heat-killed compared to punctured control seeds.

Electrical conductivity of the steep water was similar for both intact seed classes (Table 1). However, puncturing the seeds resulted in an increased conductivity for both control (54%) and heat-killed (140%) seeds.

Endosperm integrity also affected the density distributions after imbibition (Fig. 2). Seeds from both controls tended to be denser than heat-killed seeds, and, likewise, punctured heat-killed seeds tended to be denser than nonpunctured heat-killed seeds. The percentage of punctured heat-killed seeds with a density of 1.08 g·cm⁻³ was 64%, compared to 2% for the nonpunctured heat-killed seeds. The 1.08 g·cm⁻³ density class contained 90% and 95% of the intact and punctured control seeds, respectively.

Seed density is the quotient of the seed weight to the seed volume. The initial bulk seed density of dry lettuce seed was 1.2 g·cm⁻³, and, after an 8-hr soak, the densities of control and heat-killed seeds were 1.073 and 1.058 g·cm⁻³, respectively (4). The loss in density associated with imbibition is attributed to water (with a density of 1.0 g·cm⁻³) uptake. The lower density of imbibed heat-killed seeds compared to the control also is attributed to their greater water uptake than by the control seeds (Fig. 1). Puncturing the control seeds resulted in an increase in the proportion in the 1.08-g·cm⁻³ density class distribution of up to 18% (Table 1).
density class (Fig. 2). This shift in the density profile may be related to the initial seed quality of the control seedlots. A small percentage of nonviable seeds (4%) was present with the control seeds. Assuming these seeds swelled in a similar manner as heat-killed seeds, then puncturing these seeds would decrease water uptake, as measured by decreased seed swelling, and result in increased density.

Heat-killed seeds would be expected to leak more solutes into the soak water than control seeds because of reduced compartmentalization of solutes by cell membranes (8). However, no differences were observed in the electrical conductivity of the steep water between control and heat-killed seeds (Table 1). The intact endosperm acts as a semi-permeable barrier and has been shown to have a molecular exclusion limit of about 300 kDa (10). Exclusion may also depend on other factors, such as molecular shape (6, 10). Osmotically active solutes have been shown to accumulate in the extra-embryonic fluid that occupies the space between the embryo and the endosperm (10). A qualitative analysis of the extra-embryonic fluid revealed the presence of proteins and carbohydrates; sucrose and ribose were the major carbohydrates, with lesser amounts of glucose and fructose (10).

Puncturing the endosperm increased electrolyte leakage, especially in heat-killed seeds (Table 1). It can be surmised that dead seeds with an intact endosperm would have a greater accumulation of solutes in the extra-embryonic fluid during soaking than control seeds. This higher concentration of solutes would decrease the water potential of the extra-embryonic fluid and cause a water potential gradient from the water to the seed. This gradient would increase water uptake by the seeds and result in greater seed swelling (Fig. 1).

Previous reports have shown that lettuce seeds distend and appear to be gorged with water when imbibed in solutions containing organic solvents, detergents, or metabolic inhibitors (1, 9, 10). The formation of osmotically distended seeds has been shown to be temperature-dependent and appears to require enzymatic activity (10). We have shown that the increased seed swelling and decreased density of nonviable seeds is a consequence of seed death that may be induced by autoclaving (Fig. 1) and can occur after natural seed deterioration (4).

In summary, differences in volume between imbibed control and heat-killed seeds were due to the semi-permeable nature of the endosperm. The endosperm allowed water movement, but restricted electrolyte leakage to the environment. The higher concentrations of solutes in the extra-embryonic fluid of the endosperm of heat-killed seeds decreased the water potential, which, in turn, resulted in additional water uptake and increased seed swelling. We attribute lower density in the heat-killed than in the control seedlots to the greater uptake of water by the former.

**Literature Cited**


**Ultrastuctural Morphology of ‘Nonpareil’ Almond Pollen**

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**Additional index words.** taxonomy

**Abstract.** The stability of some morphological and biometric characteristics of ‘Nonpareil’ almond (Prunus dulcis (Mill.) D.A. Webb, syn. P. amygdalus Batsch.) pollen was evaluated to determine its usefulness in distinguishing among different cultivars. The influence of rootstock, irrigation, geographical location, and year on pollen grain size and pollen ultrastructural microsculpture was followed. No parameter was influenced by irrigation or rootstock, but grain size and pore diameter class distribution were significantly different for the years and localities studied.

The scanning electron microscope allows observation of pollen grain exine morphology under high magnification with a three-dimensional image. This has allowed collection of more detailed information on the morphology and biometric characters of pollen of fruit species (Roselli, 1977; Fogle, 1977a; Maas, 1977; Westwood and Challice, 1978; Reforgiato Recupero and Russo, 1980; Ciampolini et al., 1986). In particular, researchers have concentrated on the possibility of using pollen characteristics to distinguish among cultivars within given species (Roselli, 1977; Ahmedullah, 1983; Maruccu et al., 1984; Castelli et al., 1985). This has been done successfully on olive (Roselli, 1979), grape (Ahmedullah, 1983), apple (Fogle, 1977b; Maruccu et al., 1984), and peach (Fogle, 1977b).

There are no detailed studies of almond pollen to the best of our knowledge. This species poses many problems concerning classification of cultivars, clones, and hybrids. Consequently, it is important to establish easily determined and stable auxiliary taxonomic characters.

This study was undertaken with two objectives: a) to verify the stability of elements characterizing the pollen of a specific cultivar with respect to certain phenotypic variables of particular importance (rootstock, irrigation, geographic location, and year of observation); and b) to examine and quantify the characters of exine microstructure that usually are defined in terms of qualitative judgements based on the greater or lesser conspicuousness of pores and ridge trends. Irrigation and rootstock effects were measured by taking samples of pollen from ‘Nonpareil’ trees in an experimental field near Sassari in 1986 and 1987. The trees were on irrigated or nonirrigated plots and divided...