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Mechanical Resistance of the Seed Coat and Endosperm during Germination of *Capsicum annuum* at Low Temperature[1]

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ABSTRACT

Decoated pepper (*Capsicum annuum* L. cv Early Calwonder) seeds germinated earlier at 25°C, but not at 15°C, compared to coated seeds. The seed coat did not appear to impose a mechanical restriction on pepper seed germination. Scarification of the endosperm material directly in front of the radicle reduced the time to germination at both 15°C and 25°C.

The amount of mechanical resistance imposed by the endosperm on radicle emergence before germination was measured using the Instron Universal Testing Machine. Endosperm strength decreased as imbibition time increased. The puncture force decreased faster when seeds were imbibed at 25°C than at 15°C. The reduction in puncture force corresponded with the ability of pepper seeds to germinate. Most radicle emergence occurred at 15°C and 25°C after the puncture force was reduced to between 0.3 and 0.4 newtons.

Application of gibberellic acid$_{4+7}$ (100 microliters per liter) resulted in earlier germination at 15°C and 25°C and decreased endosperm strength sooner than in untreated seeds. Similarly, high O_2 concentrations had similar effects on germination earliness and endosperm strength decline as did gibberellic acid$_{4+7}$, but only at 25°C. At 15°C, high O_2 concentrations slowed germination and endosperm strength decline.

Seed coats and surrounding structures may influence the ability of a seed to germinate through interference with water uptake, gas exchange, diffusion of endogenous inhibitors, or by mechanical restriction of embryo growth (5, 8). In seeds that do not have hard seed coats or require scarification for water uptake, other seed parts such as the endosperm may mechanically restrict embryo expansion, thus preventing radicle emergence (10). Junttila (7) found that in *Syringa* species, low temperature dormancy (9–15°C) could be alleviated by removal of endosperm from around the radicle. His results indicated that dormancy was primarily due to mechanical restriction of radicle emergence through the endosperm and not due to germination inhibitors.

Encasing the embryo of lettuce is a two-celled layer of endosperm, characterized by thick-walled cells and dense cytoplasm (6). The ability of this endosperm to control or to mechanically restrain lettuce radicle emergence has been debated. Ikuma and Thimann (5) proposed that red light relieved endosperm restriction on the embryo in photosensitive lettuce varieties by triggering the production of cellulolytic enzymes in the embryo, weakening the endosperm layer. Nabors and Lang (9) also studied the effect of the lettuce endosperm on restriction of radicle emergence and found that, by increasing the growth rate of the embryo, this restriction could be overcome. Red light induced growth in the lettuce embryo, enabling it to grow through the endosperm. Pavlista and Haber (10) proposed that both the mechanical force of the growing embryo pushing against the endosperm, and the enzymic weakening of the endosperm were necessary for lettuce seed germination. They observed that when weakening of the endosperm was inhibited, the embryos grew and buckled in the endosperm encasing but did not germinate.

Jones (6) determined that the cell walls of lettuce endosperm were increasingly degraded with longer incubation times, and following germination the walls were extensively broken down. Tao and Khan (12) determined that endosperm strength did not appear to be directly related to radicle protrusion in lettuce seed. Halmer *et al.* (4) reported that enzymic degradation of a mannose-containing polysaccharide in lettuce endosperm cell walls was by red light or gibberellin. Enzymic activity increased markedly only after radicle emergence and thus did not correspond with the movement of the radicle through the endosperm.

Pepper embryos are surrounded by endosperm materials which make up the bulk of food reserves for the embryo and young seedling (2). The experiments which follow test the mechanical resistance of the seed coat and endosperm during germination of *Capsicum annuum* at optimal and suboptimal temperatures.

MATERIALS AND METHODS

Decoated Seeds. Air dried pepper (*Capsicum annuum* L. cv Early Calwonder) seeds were decoated by inserting a scalpel or probe into the seed cavity and peeling back the seed coat material. Twenty-five decoated or raw (untreated) pepper seeds were placed on a moistened filter paper in a 9-cm Petri dish. The Petri dishes were placed at 15°C or 25°C constant temperature chambers for germination.

Scarified Endosperm Test. Decoated air dry seeds were scarified by removing 0.5 mm of endosperm material directly in front of the radicle tip (Fig. 1). Scarified seed and decoated unscarified (control) seeds were then germinated as described above.

Germination data for both experiments were taken at 24-h intervals and all treatments were replicated four times (25 seeds/replicate). Seeds with visible radicles were counted as germinated. Data taken for each treatment included total percent germination and MDG[3] as previously described (14):

$$\text{MDG} = \frac{\Sigma\,(\text{days to germination})\,(\text{number of seeds germinated})}{\text{total number of seeds germinated}}$$

Endosperm Strength Measurements. The Instron Universal Testing Machine (Instron Engineering Corporation, Canton, MA) was used to determine endosperm strength. Pepper seeds (50/

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[3] Abbreviation: MDG, mean days to germination.

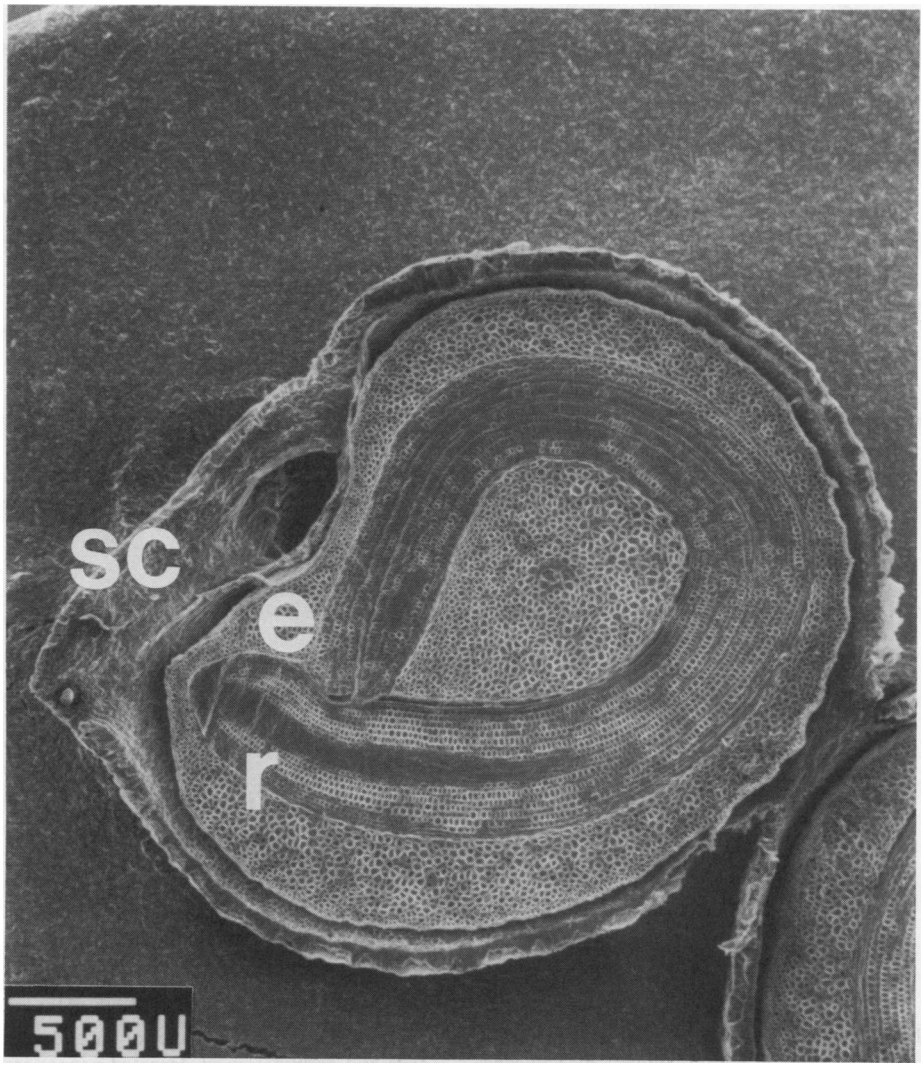

FIG. 1. Scanning electron micrograph of pepper seed. The seed coat (sc) was removed before puncture-force tests were run with the Instron on the endosperm layer (e) immediately in front of the radicle (r) (× 24).

treatment time) were imbibed in 5 ml of distilled H_2O, with or without GA_{4+7}, on filter paper in 9-cm Petri dishes at 15°C or 25°C. Seeds treated with 100% O_2 were gassed in a continuous flow system at 15°C and 25°C as previously described (15). After the appropriate treatment time, each of the seeds was prepared immediately before measurement on the Instron. The seeds were decoated and approximately one-fourth of the seed, the area of radicle emergence, was excised (see Fig. 1). The radicle was teased out of the surrounding endosperm material with a dissection needle, leaving a clean undamaged radicle cavity in the endosperm. The endosperm section was placed onto a No. 78 drill blank (diameter, 0.4 mm) attached to a basal load cell. The crosshead had a bolt placed in it with a 0.8-mm-diameter counter hole drilled into it. The load cell was set to 100 g (0.98 newton) full scale load. Crosshead and chart speeds were 1.3 and 25.4 cm/min, respectively. Each treatment consisted of five seeds and all treatments were replicated five times.

RESULTS

Decoated seeds germinated earlier than seeds with coats at 25°C but there was no difference in the final germination percentage between the two treatments (Fig. 2A). At 15°C, germination earliness and total germination were unaffected by decoating (Fig. 2B).

When decoated pepper seeds were scarified by removing 0.5 mm of endosperm directly in front of the radicle, germination rates increased dramatically at both 15°C and 25°C (Table I). The scarification treatment did not affect the total germination of pepper seeds at 15°C or 25°C.

The force necessary to puncture the endosperm directly in front of the radicle decreased with increasing incubation time at both 15°C and 25°C (Fig. 3). Endosperm resistance decreased more rapidly when seeds were imbibed at 25°C *versus* 15°C. Puncture force decreased to 0.3 newton at both 15°C and 25°C before radicle protrusion occurred in a majority of the seeds.

At 25°C, GA treatment reduced endosperm resistance and increased earliness of radicle protrusion compared to untreated seeds (Fig. 4). GA treatment had a similar effect on endosperm weakening and radicle protrusion at 15°C, except that the process was slower (Fig. 5). This treatment caused a decrease in endosperm strength by the 2nd d; whereas, endosperm strength reductions occurred by the 4th d from initiation of imbibition in untreated seeds at 15°C.

When seeds were exposed to 100% O_2 at 25°C, decreases in endosperm puncture force and radicle protrusion occurred sooner

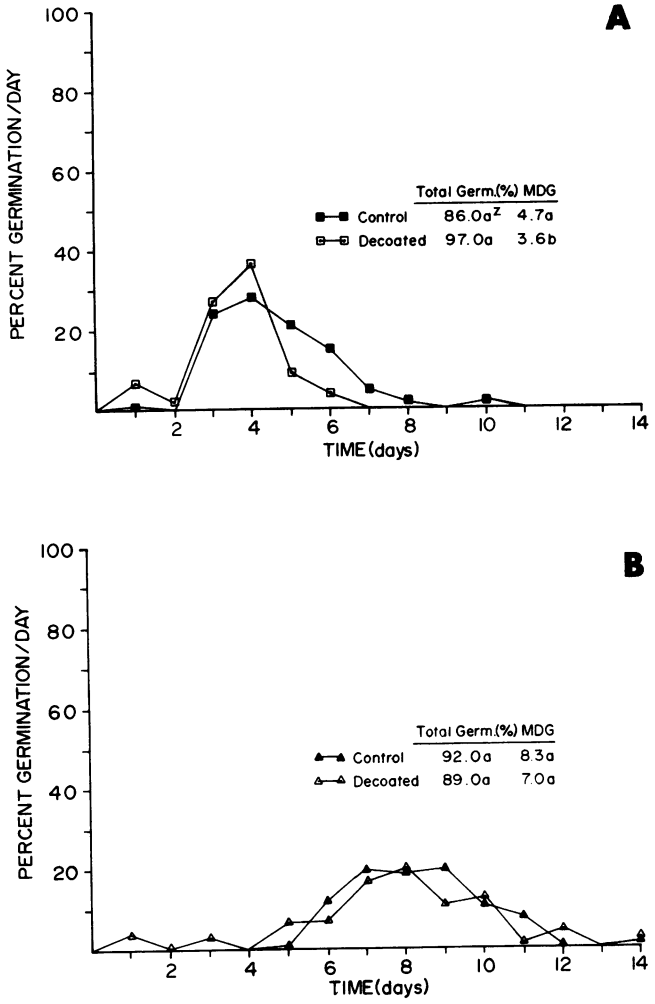

FIG. 2. Effect of seed coat removal on pepper seed germination at 25°C (A) and 15°C (B). [z]Mean separation within columns by Duncan's multiple range test, 5% level.

compared to seeds maintained at 21% O_2 (Fig. 6). The rate at which endosperm strength decreased was slowed by 100% O_2 at 15°C (Fig. 7). Germination in 100% O_2 was slower than in air. No reduction in endosperm puncture force was evident until after the

Table I. *Effect of Removing 0.5 mm of Endosperm (Scarified) Directly in Front of the Radicle on Germination and Germination Rate (MDG) of Decoated Pepper Seed at 15°C and 25°C*

Seed Treatment	Germination (25°C)		Germination (15°C)	
	Rate	Total	Rate	Total
	MDG	%	*MDG*	%
None	3.5	81	8.9	85
Decoated only	3.1	72	9.1	94
Decoated and scarified	1.7	78	3.4	90
	*[a]	NS	*	NS

[a] *, Significantly different; NS, not significant at the 5% level.

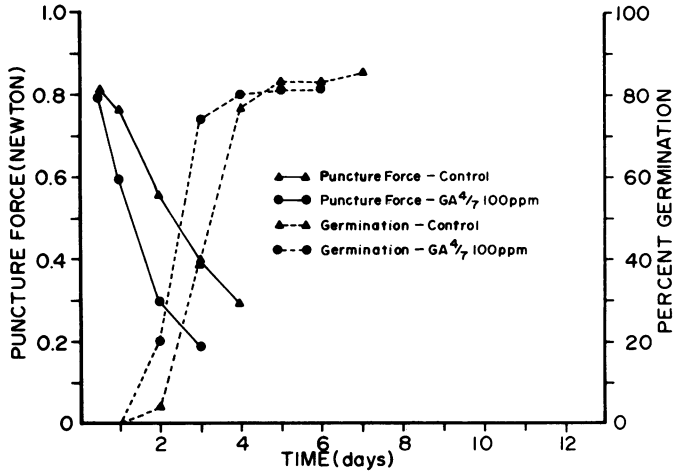

FIG. 4. Mechanical resistance of the endosperm directly in front of the radicle and germination of decoated pepper seeds as affected by GA_{4+7} (100 μl/l) at 25°C.

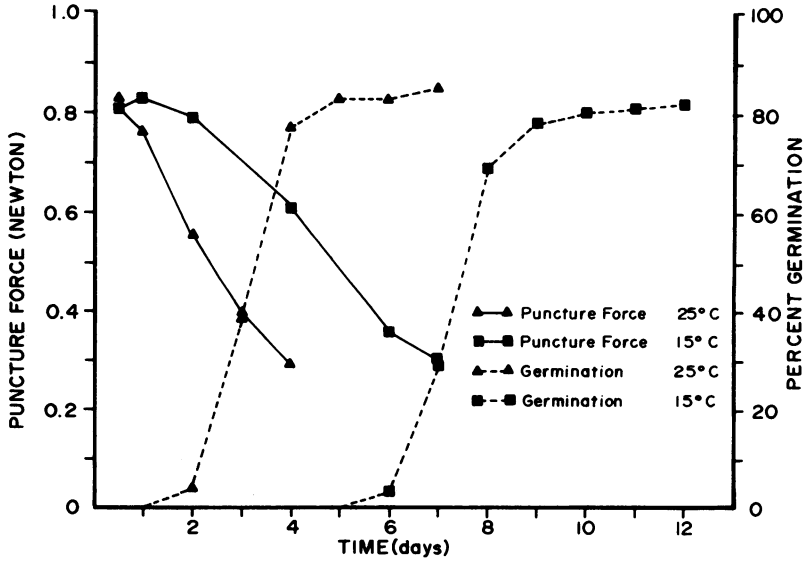

FIG. 3. Mechanical resistance of the endosperm directly in front of the radicle and germination of decoated pepper seeds as affected by germination temperature (15° and 25°C).

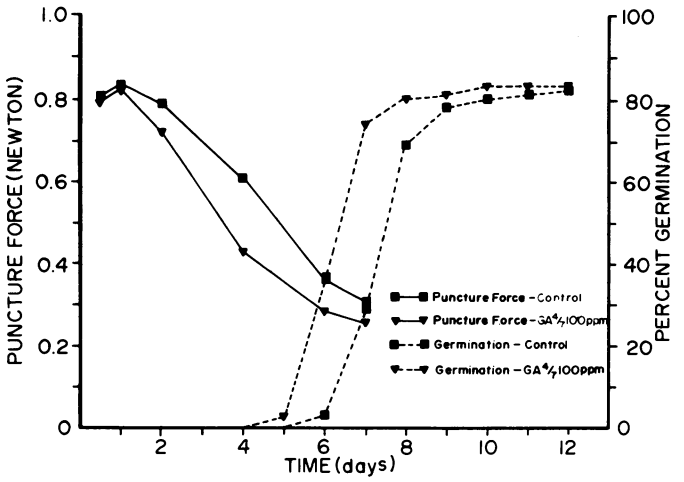

FIG. 5. Mechanical resistance of the endosperm directly in front of the radicle and germination of decoated pepper seeds as affected by GA_{4+7} (100 μl/l) at 15°C.

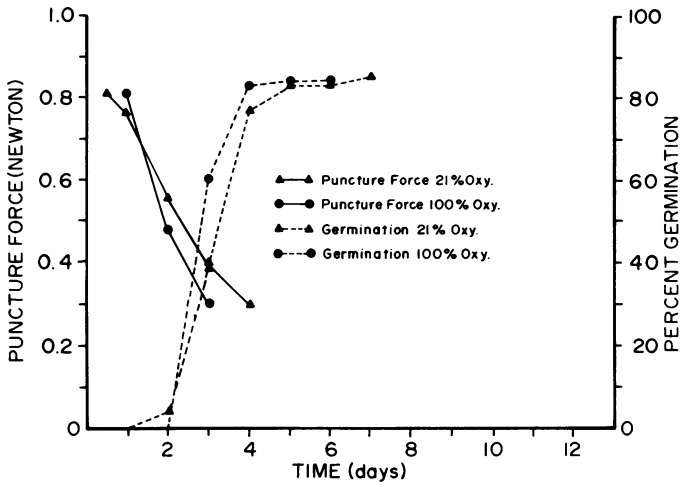

FIG. 6. Mechanical resistance of the endosperm directly in front of the radicle and germination of decoated pepper seeds as affected by 100% O_2 at 25°C.

2nd d in seeds at 21% O_2 or the 4th d in 100% O_2. Endosperm strength in 100% O_2-treated seeds was higher at all times compared with the corresponding treatments in 21% O_2.

DISCUSSION

Mechanical resistance of the endosperm as measured by the Instron Universal Testing Machine appeared to affect germination of pepper. The resistance to puncture by the endosperm in pepper seeds was dependent on imbibition time, germination temperature, and treatment. Decoated pepper seeds germinated earlier than coated seeds at 25°C. The removal of the seed coat at 25°C may remove a barrier to gas exchange which would allow germination to proceed at a faster rate as was the case in elevated O_2 environments. The removal of the seed coat at 15°C did not affect radicle protrusion possibly because there already was a sufficient O_2 supply to the embryo for maximal metabolic activities for germination at that temperature. The seed coat and endosperm were found to inhibit O_2 uptake at high temperatures but not at low temperatures in *Syringa* (7). There does not appear to be any mechanical restriction to germination imposed by the seed coat

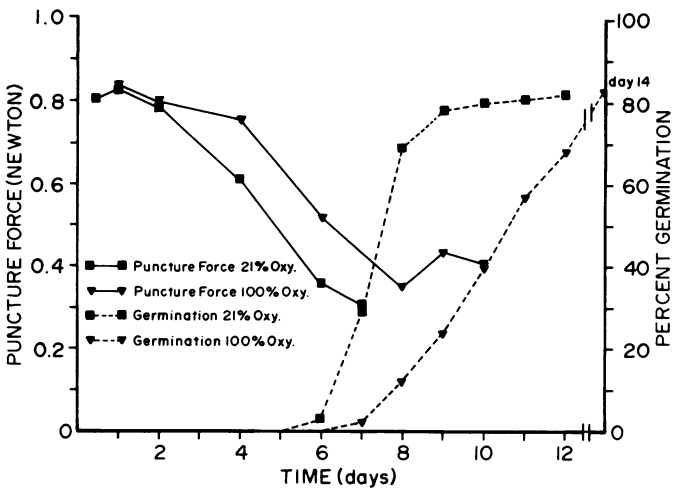

FIG. 7. Mechanical resistance of the endosperm directly in front of the radicle and germination of decoated pepper seeds as affected by 100% O_2 at 15°C.

per se. If this was the case, seed coat removal would be expected to lead to earlier radicle protrusion at 15°C as well as 25°C.

Scarification of the endosperm material directly in front of the radicle markedly reduced the time to germination. Removal of 0.5 mm of endosperm material caused the embryos to be 'pushed' from the endosperm by imbibitional pressure and usually resulted in damage to the radicle. This points to a mechanical resistance imposed by the endosperm; but, the effect of the mechanical restriction was greater at the lower temperature.

A hypothesis was proposed by Pavlista and Haber (10) that germination of lettuce seed requires (a) a mechanical force of the growing embryo pushing against the endosperm and (b) chemical or enzymic weakening of the endosperm. The studies presented here indicate that endosperm strength must be reduced before germination will occur. The reduction in endosperm resistance at 15°C and 25°C when GA_{4+7} was applied offers some evidence as to the mechanics of pepper seed germination. Stimulation of cell division by GA_{4+7} does not appear to be the cause of increased germination rates (14). Conditions which favor germination of *Syringa* embryos, *i.e.* high temperature, chilling, and GA_3, favored enhanced radicle elongation (7). GA_{4+7} stimulated earlier radicle protrusion in pepper seeds submerged in osmotic solutions which inhibited cellular expansion (14). GA_3 stimulated enzymic activity in monocot (8, 13) and lettuce seeds after radicle emergence (4). Thus, the possibility exists that GA may regulate pepper seed germination before radicle protrusion by controlling radicle elongation and/or enzymic degradation of endosperm materials. Evidence for this control by GA will be discussed elsewhere (16).

The effect of O_2 on endosperm weakening is difficult to interpret. At 25°C, pepper seeds germinated in 100% O_2 had a higher respiratory rate and thus possibly a higher metabolism than did seeds germinated in air (15). At 15°C, high O_2 did not increase respiration rate. At this temperature, endosperm strength decreased at a slower rate than occurred in air. In excised bean embryos cultured in 100% O_2, reductions in growth and enzymic activity were found (11). Eliasson (3) reported that pure O_2 inhibited the growth of wheat roots due to inhibition of cell elongation. Albaum *et al.* (1) suggested that subsequent growth of oxygenated oat seeds was inhibited due to high O_2 interfering with proteolytic breakdown of the endosperm, thereby preventing nitrogen transport from the endosperm to embryo, development of enzyme activity, and growth. Yet, high O_2 did not affect Cyt oxidase. This may indicate that the reduced endosperm weakening in pepper seeds germinated in 100% O_2 at 15°C was due to the inability of embryos to utilize more O_2 than that contained in air.

These higher levels of O_2 may then be adversely affecting enzymic breakdown of pepper endosperm, thus slowing germination (16). This reduction in enzyme activity may be directly reflected in the greater endosperm resistance of seeds without GA treatment or which were germinated in 100% O_2 at 15°C.

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