

## Seed dormancy in striped maple<sup>1</sup>

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Striped maple (*Acer pensylvanicum* L.) seeds showed delayed germination unless they were buried. With no pericarp but with intact, papery testae, seeds germinated at 5°C after 3–6 months cold, moist stratification. They did not germinate at 23°C even after stratification. With testae removed from the root tip germination was 20–30% at 23°C and 100% after 3 months stratification. Germination with testae removed (but not with testae intact) was accelerated by benzyladenine. Gibberellic acid had no effect. Testa-imposed dormancy did not seem to be related to inhibitors or restriction of water uptake but may have been due to mechanical restriction of radicle elongation.

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La semence de l'érable de Pennsylvanie (*Acer pensylvanicum* L.) a présenté un délai de germination qui a été éliminé par l'enfouissement. Privée de son péricarpe mais munie de ses téguments à texture de papier la semence a germé à 5°C après une stratification de 3–6 mois au froid et à l'humidité. Les semences n'ont pas germé à 23°C même à la suite d'une stratification. L'excision des téguments à l'apex de la radicule a permis un taux de germination de 20–30% à 23°C et de 100% après 3 mois de stratification. La germination qui s'est opérée chez des graines démunies de leurs téguments (phénomène non observé pour les graines à téguments intacts) s'est vue accélérée par la benzyladénine. L'acide gibbérellique n'a pas eu d'effet. La dormance imposée par les téguments n'a pas semblé être reliée à des inhibiteurs ou à une restriction de l'apport en eau mais a pu être causée par des contraintes mécaniques vis-à-vis l'élongation de la radicule.

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Seeds of most *Acer* species are dormant when shed and will not germinate without a period of cold, moist stratification. Striped maple (*Acer pensylvanicum* L.) has one of the longest stratification requirements in the genus, 3–6 months (United States Department of Agriculture 1974). Physiological aspects of seed dormancy have been studied in *A. saccharum* (Janerette 1978; Simmonds and Dumbroff 1974; Van Staden *et al.* 1972; Webb and Dumbroff 1969; Webb *et al.* 1973a), *A. pseudoplatanus* (Pinfield and Stobart 1972; Webb *et al.* 1973b; Webb and Wareing 1972a, 1972b), *A. platinoides* (Pinfield *et al.* 1974), *A. ginnala* (Dumbroff and Webb 1970), and *A. negundo* (Irving 1968). Each species is different in its mechanisms for maintaining dormancy but factors frequently contributing are the presence of inhibitors, usually in the embryo,

and the action of the testa in reducing either the rate of imbibition or the rate of leaching of inhibitors. We investigated the role of these factors in seed dormancy of striped maple and how they might be related to germination behaviour in the field.

### Materials and Methods

Mature seeds were collected in October of 1975–1977 from trees near Amherst, MA, and Grafton, VT. Seeds were air dried for 2 days and stored in bottles at 5°C. Most experiments used single-tree seed sources but three sources were combined for testing germination under different cover types.

One set of experiments was conducted in the field. Seeds on two trees at Mt. Toby, University of Massachusetts Experimental Forest, and three trees at the Holyoke Range, both near Amherst, were painted using commercial spray cans and left to fall naturally. Germination under different cover types was tested with lots of 40 seeds, replicated four or eight times for each cover type, placed directly on the litter surface after leaf fall. Seed burial was tested with lots of 20 seeds with five replicates at each depth. Each lot was covered with a wire cone to keep out rodents. Even so, two

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of the lots were eaten. Field germination was defined by shoot emergence above the litter and was checked in April, May, and June.

A second set of experiments was conducted in the laboratory using seeds with the pericarp removed. Seeds were imbibed by immersing in water for 2 days. Germination was tested at 23°C in 9-cm plastic petri dishes with a single layer of Whatman No. 1 filter paper moistened with 2 cm<sup>3</sup> of water or a solution of benzyladenine 10<sup>-4</sup> M (BA) or gibberellic acid 10<sup>-4</sup> M (GA). Tests were conducted in the dark except for measurement periods every 3–5 days. Our preliminary tests showed no effect of these light exposures. Germinated seeds were counted after 20 days if the radicle had elongated more than 0.5 mm. Each germination test had a minimum of three replicates of 6 seeds each and a maximum of five replicates of 10 seeds each. Differences between means were tested with Duncan's new multiple range test (Steel and Torrie 1960) at  $p = 0.05$ .

Seeds that were stratified were kept dark and moist, between layers of filter paper, in a refrigerator at 5°C for up to 6 months. In some experiments the papery testa was removed from over the radicle (about 10% of the total testa) or from over the cotyledons (the other 90% of the testa). Testae were removed after soaking the seed for 2 days in water at room temperature.

Inhibitory effects of whole isolated testae or whole isolated embryos were tested using the technique of Webb and Wareing (1972a). The respective parts of the striped maple seeds were placed in the same petri dish as the lettuce seed, var. Grand Rapids, and germination was counted after 2 days. The effects of testae were also tested by adding 6–15 whole isolated testae to the standard germination tests already described using unstratified striped maple seed with the testa over the radicle removed.

The effect of testae on BA uptake was tested by imbibing seed with intact testae for 2 days in either BA 10<sup>-4</sup> M solution or water. Seeds were then washed for 5 min to remove any BA on the surface of the testa. Germination was tested at 23°C with the testa over the root tip removed, both before and after 3 months stratification with intact testae.

## Results

### *Germination in the Field*

Seeds matured in the autumn and fell from late autumn throughout the winter. All sites we studied were snow covered for at least 3 months. Naturally dispersed, painted seed found in litter samples did not germinate the first spring (no germination from 80 seeds). They did germinate after 3–6 months additional stratification in the laboratory. In the second spring 23 seedlings were found that had originated from painted seed. Of 960 seeds placed under different cover types (white pine, hemlock, oak, northern hardwood) only 0.03% germinated the 1st year but 24.8% germinated the 2nd year. There were no significant differences in germination among cover types. Of all seedlings, 93% emerged in May, so radicle elongation presumably started in April and early May.

We observed good 1st year germination of seeds

TABLE 1. Percent germination, by June, of striped maple seeds planted the previous October at different depths in organic horizons of the soil profile. The F layer (01) is partially decomposed leaves, the H layer (02) is decomposed humus. Values followed by the same letter are not significantly different

Planting depth			
0 cm (on top of litter)	2–3 cm (under 1st year's leaves)	2.5–4 cm (under F layer)	3.5–6 cm (under H layer)
0a	1a	15a	41

planted in a garden under 5–10 mm of soil. Seeds planted under northern hardwoods did not germinate the 1st year if placed directly on the newly fallen leaves, or underneath them, some did germinate under the layer of 2- to 3-year-old leaves (under the F horizon), and 41% germinated when placed under the humus layer on top of the A horizon (Table 1).

### *Laboratory Experiments*

Seeds with intact testae did not germinate without stratification. Germination started after 3 months stratification and was complete after 6 months. Germination occurred at 5°C. In three different experiments ungerminated seeds were removed after up to 5 months stratification and tested for germination at 23°C. None of these seeds germinated.

BA or GA applied to seeds with intact testae during the 3-month stratification period did not decrease the required stratification time for germination at 5°C. Seeds treated with GA or BA during the 23°C testing period did not germinate even after 5 months stratification. Unstratified seeds leached under running water for 4 days did not germinate at 23°C.

Germination of unstratified seed at 23°C was significantly increased (to 20–30%) by removing the testa over the radicle. There was no significant effect of removing only the testa over the cotyledons (only 1 of 54 seeds germinated). Stratification of seeds with the testa removed from the roots increased the amount of germination at both 5 and 23°C (Table 2). Germination was nearly complete at both temperatures after 3 months stratification, the minimal stratification time for seed with intact testae.

BA-treated, unstratified seed, with the testa over the root removed, germinated 100% at 23°C, a significant increase over the 20–30% germination of similar seeds germinated in water or GA. When seeds were first imbibed in water, germination after BA treatment was completed in 7 days.

Dry seeds weighed an average of 21 mg. After 2 days of imbibition seeds with intact testae weighed 40

TABLE 2. Percent germination of striped maple seed with the testae removed over the radicles after 2 days imbibition. Germination at 5°C occurred during stratification. Germination at 23°C was in tests on samples taken at monthly intervals from the stratified, ungerminated seed. There were not enough ungerminated seeds left after 3 months stratification to run a test at 23°C

Length of stratification (months)	Termination	
	at 5°C	at 23°C
0	0	30
1	1	50
2	11	76
3	82	—

TABLE 3. Germination of striped maple seeds in tests at 23°C. Testae were intact up to the time of testing, but the testa over the radicle was removed before testing.

Imbibing solution	Before stratification	After stratification
Water	0% in 6 days	100% in 3 days
Benzyladenine, 10 <sup>-4</sup> M	100% in 6 days	100% in 3 days

TABLE 4. Effect of striped maple embryos and testae (six per treatment) on lettuce seed germination (percent). Values followed by the same letter are not significantly different

Treatment	Lettuce seed germination
Control	64.0a
Unstratified	
Testae	28.6b
Embryos	60.0a
Stratified	
Testae	22.0b
Embryos	53.4a

mg and those with the testa removed (corrected for the weight of the testa) weighed 38 mg. After 7 days imbibition, when the first seeds with the testa removed started to germinate, seeds with intact testae weighed 40 mg and those with testae removed weighed 43 mg.

Unstratified seeds with the testa intact were imbibed in BA and then washed to remove any BA from the surface of the testa. If the testae were left intact the seeds would not germinate at 23°C but if the testa over the radicle was removed there was 100% germination at 23°C, significantly faster and more complete germination than seeds imbibed in water (Table 3). After 3 months stratification both BA imbibed and water imbibed seeds germinated totally within 3 days at 23°C.

Testae from striped maple inhibited germination of lettuce seed (Table 4). The amount of inhibition was not significantly lower using testae from seed that had been stratified for 3 months. Striped maple embryos did not significantly affect lettuce seed germination (Table 4). Adding testae (up to 14 per treatment) from striped maple seed to striped maple seed with the testae removed over the radicle did not reduce the germination of the striped maple seed. In several experiments there was even a slight increase in germination when the extra testae were present.

### Discussion

Striped maple seed seemed to have both embryo dormancy and testa-imposed dormancy. Unstratified seed did not germinate if the testae were intact but 20–30% would germinate when the testae were removed from the radicles. After 1–3 months stratification or BA treatment, seeds would not germinate if the testae were intact, but there would be rapid and total germination if the testae were removed from the radicles. Apparently the 70–80% of the embryos that were originally dormant were ready to grow after stratification or BA treatment but were now restricted by testa-imposed dormancy. The testa-imposed dormancy could be broken by removing the testa over the radicle, when germination occurred at 5 or at 23°C, or by additional stratification, when germination occurred at 5 but not at 23°C. The testa presumably remains intact in the field because it is protected by the pericarp. Therefore, a low temperature requirement for germination after stratification plus the cold temperatures required for stratification would account for the predominant germination in April and early May when the ground is still cold.

Much work on testa-imposed dormancy implicates inhibitors. Striped maple testae inhibit lettuce seed germination but this inhibition may not be involved in testa-imposed dormancy for the following reasons: (1) the inhibitory effect of testae on lettuce seed germination did not decrease with stratification; (2) BA and GA did not cause germination in striped maple if the testae were intact, yet they can overcome inhibitors in some *Acer* species and other genera (Khan 1975; Webb and Wareing 1972b); (3) testae from striped maple did not inhibit the germination of striped maple seed (the cotyledons of *A. saccharum* did inhibit germination of the same species (Janerette 1978); (4) leaching seed with intact testae did not permit germination.

The testa does not appear to prevent leaching of an inhibitor from the embryo, as proposed for *A.*

*pseudoplatanus* by Webb and Wareing (1972a). Striped maple embryos have no effect on lettuce seed germination so they may not contain inhibitors. In addition, seed with the testa removed over the cotyledons, which should allow leaching from the embryo as it did in *A. pseudoplatanus* (Webb and Wareing 1972a), had no effect on germination of striped maple seed.

The testa in striped maple seeds did not seem to interfere with water uptake as proposed for *A. saccharum* (Webb and Dumbroff 1969) and for *A. ginnala* (Dumbroff and Webb 1970). Seeds with intact testae imbibed almost as much water as did those with the testa over the root removed.

The testa in striped maple may mechanically restrict growth of the radicle. Webb and Wareing (1972a) felt that the testa of *A. pseudoplatanus* might restrict growth mechanically near the end of the stratification period. Villiers (1972), however, states the prevalent view of mechanical restriction that "it is only among those seeds which remain enclosed within an extremely hard endocarp that one would look for causes of dormancy imposed solely by such a mechanism." The testa of striped maple seed is thin and papery but our results are consistent with the hypothesis of mechanical restriction. Removing the testa over the root will permit some germination of unstratified seed. Removing the testa over the cotyledons is ineffective. Only stratification permitted germination if the testa was intact. If the testa is acting mechanically, then stratification must either reduce its strength or increase the pressure of the embryo against it.

Our observation of delayed germination in two separate experiments disagrees with the results of Marquis (1975) who found in Pennsylvania that 90% of the *A. pensylvanicum* seed that germinated in the first 2 years germinated during the 1st year. We found essentially no germination in the first spring and summer when seeds were sown on the surface of the forest floor. Germination did occur the 1st year in buried seed. There are several factors which may account for our results: (1) seed fall is spread throughout the autumn and winter so that seeds that fall late do not get a long enough stratification time; (2) the required length of stratification is extremely variable and few of our sites would provide stratifying conditions for that portion of the seed population that requires as long as five months in one winter; (3) most seeds that fall on top of the litter require deeper burial by at least another year of litter accumulation before conditions are attained that permit germination.

Delayed germination of nonburied seeds in the

field may be due either to light inhibition or to moisture conditions near the surface of the forest floor. Continuous light reduced, but did not totally inhibit, the amount of germination in *A. ginnala*, *A. saccharum*, and *A. pseudoplatanus* (Dumbroff and Webb 1970; Webb and Dumbroff 1969; Webb and Wareing 1972a). Recently fallen litter may not retain enough moisture to provide adequate stratifying conditions until it is mostly decayed to form humus. Further experiments are needed to separate light and moisture effects.

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