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Embryo Germination in *Acer ginnala* Maxim. and the Activity of an Endogenous Exudate

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ABSTRACT

The Amur maple (*Acer ginnala* Maxim.), a small, pollution and stress-tolerant ornamental tree, is commonly planted in the northern United States. In preliminary germination studies, a reddish-brown exudate was produced from the testa surrounding the embryo. Embryos with testae in place did not germinate, whereas embryos with the testae removed, or even slightly damaged, germinated in approximately 9 days. Removal of the testa and subsequent embryo culture in total darkness permitted embryo germination, indicating that the testa may control germination by chemical means rather than as a physical barrier to light. Periodic removal of the exudate followed by subsequent analysis of newly produced exudate indicated a decrease in concentration over time, suggesting that gradual disintegration or inactivation of the testa may be a timing mechanism for germination. The exudate inhibited the development of lateral roots in lettuce seedlings, suggesting that it may posses allelochemical properties as well. Our results suggest that the testa of *Acer ginnala* provides a timing mechanism for its own embryo germination and may also influence the adjacent microenvironment through the secretion of this exudate.

Key words: Amur maple, phytochemicals, sterilization, germination, mercuric chloride, testa, exudate.

INTRODUCTION

The Amur maple (*Acer ginnala* Maxim.) is a small, pollution and stress-tolerant ornamental tree. It is native to Siberia and is planted in the northern United States (Ebinger and McClain, 1991; Norton, 1987). Amur maple fruits require a stratification time of up to 150 days (U.S. Forest Service, 1948), but investigators have demonstrated that removal or disruption of the testa, the cell layer covering the zygotic embryo in mature maple fruits (sensu Dumbroff and Webb, 1970), resulted in much faster germination (7-15 days). The presence of a testa surrounding the zygotic embryo raised questions regarding

its role in regulating the timing of germination, as no germination was observed in vitro in embryos with intact testae. The testa could inhibit germination by preventing light and water from reaching the embryo. However, Dumbroff and Webb (1970) determined that *A. ginnala* fruits were light sensitive and that germination occurred at a higher rate in fruits germinated in the dark versus those germinated in continuous light. This raises the possibility of the testa functioning as a physical barrier to light.

Alternatively the testa may produce inhibitors which act as a chemical barrier for germination. As such, the gradual degeneration of the testa following prolonged exposure to external factors could act as a timing mechanism for germination. The chemical compositions of fruits of several *Acer* species change during stratification (Pinfield and Davies, 1978; Webb et al., 1973), and the testa may contribute to these changes. Investigators have also detected chemicals which inhibit the growth of other plants in the fruits of several maples. For example, water extracts of sycamore maple, *Acer pseudoplatanus* L., strongly inhibit the growth of yellow birch, *Betula alleghaniensis* Britt. (Fisher, 1987), while similar extracts from the roots of nine other species of *Acer* were also found to be to be inhibitory (Podtelok, 1972; Maclaren, 1983).

The purpose of this study was to investigate: (1) an effective technique for surface sterilization and germination of Amur maple fruits, (2) the inhibitory role of the testa in Amur maple germination, (3) the role of Amur maple exudate in influencing the development of seedlings of other plants, and (4) preliminary physical characteristics of the exudate.

METHODS

Materials: All chemicals were purchased from Sigma Chemicals, Inc. (St. Louis, MO, USA), unless otherwise specified. *Acer ginnala* fruits were purchased from the F.W. Shumacher Co., or were collected from established open-pollinated plantings on the campus of Southern Illinois University at Edwardsville, and were stored in plastic bags at 4C. Black-Seeded Simpson Lettuce (*Lactuca sativa* L.) seeds were purchased from a local vendor. Lettuce seeds were chosen for their rapid germination rates and because they had been used previously in bioassays to determine the activity of plant extracts (Li et al., 1992).

Surface sterilization and germination of Amur maple fruits: For surface sterilization, a 20 minute 1% mercuric chloride treatment (PhytaSource, 1994) was applied to winged fruits, which were then rinsed twice in sterile distilled water. At this point, wings and pericarps were removed, leaving testa-covered embryos, which were the subjects of this study. The testa-covered embryos were then transferred to a solid medium containing Phytagar (6.5 g/L; Life Technologies, Gaithersburg, MD) in water with no nutrients, and were incubated at 25C. Only data from uncontaminated samples were included in our results.

Surface sterilized *A. ginnala* fruits were subjected to one of four treatments then examined for germination. (1) Testa-covered embryos were incubated under cool white fluorescent light (60 μE m^{-2} s^{-1}) with a 16h photoperiod. (2 and 3) The testae were gently scraped from embryos in the light using a sterile scalpel, and these embryos were either (2) exposed to light as above or (3) kept completely in the dark under aluminum foil. (4)

The testae were removed from the embryos under complete darkness (0 μE m^{-2} s^{-1}) by gently scraping the embryos while enclosed in a cardboard box (with holes for access) which was sealed with several layers of black plastic and taped to the surface of a horizontal laminar flow hood. Scraped embryos were placed on a solid medium, then the petri dishes were wrapped with aluminum foil, all under complete darkness. A Li-Cor LI-189 light meter (Li-Cor, Inc., Lincoln, NE) was used to measure light levels. Each experiment was performed twice using 20-30 embryos per treatment.

Inhibition of test plantlet root development by Amur maple exudate: Testa-covered *A. ginnala* embryos were placed in petri dishes containing Phytagar (6.5 g/L). The embryos were removed from the medium after seven days. Fifteen lettuce seeds or seedlings were used for each of the four treatments, and seeds were surface sterilized using a 0.25% solution of commercial sodium hypochlorite for 10 minutes, followed by two rinses with sterile deionized water. A lettuce seed or plantlet was then placed in the center of the circle of exudate which remained in the medium following removal of the testa-covered embryo. Day 0 seeds were removed from their commercial packaging and placed in the exudate circle. Day 1 seeds were cultured on medium containing 6.5 g/liter Phytagar for one day before being exposed to the exudate. Day 2 seeds were cultured for two days prior to exposure, etc. Control seeds and plantlets were lifted and replaced on the medium to simulate the physical handling which the other seeds or plantlets experienced. Lateral roots were counted after one week in culture. For our purpose, any root other than the primary root was considered to be a lateral root. Root count data were analyzed using Kruskal-Wallis nonparametric ANOVA followed by a nonparametric analogue to the Student-Newman-Keuls multiple range test (Zar, 1996). A $\underline{P} \leq 0.05\%$ experiment-wise error rate was used. The Systat (R) statistical package was used for the Kruskal-Wallis test and for computing rank sums (Wilkinson, 1989).

Physical analysis of Amur maple exudate: To harvest the exudate, five testa-covered embryos were placed in a modified double microcentrifuge tube apparatus. A one mm hole was made in the bottom of a 0.75 ml microcentrifuge tube and its lid was removed. This tube was placed inside a 1.5 ml microcentrifuge tube. Embryos in modified tubes were centrifuged using a microcentrifuge for 20 seconds at 10,000 x g, 10 seconds at 14,000 x g, and 30 seconds at 10,000 x g. Two approaches were taken to spectrophotometrically analyze the exudate. One method involved six plates of embryos (5 per plate). The testa-covered embryos were centrifuged after being incubated for 2, 4, 6, 8, 10, or 13 days. The exudate collected each day was diluted 1:100 with distilled water and analyzed spectrophotometrically to measure absorbance changes at wavelengths of 260nm, 280nm, 380nm, 480nm, 580nm, and 680nm. The second method involved centrifuging embryos from each plate, returning them to their plates of origin, then spinning these same embryos again on days 2, 4, 6, 8, 10 and 13. Exudates were diluted 1:100 with distilled water and analyzed as above.

RESULTS AND DISCUSSION

Surface sterilization of Amur maple fruits: A 1% mercuric chloride treatment of fruits followed by two rinses with sterile water was effective in reducing contamination (from virtually 100% contamination of untreated control fruits to < 10% contamination).

Treatment with mercuric chloride did not affect fruit viability, germination, or exudate production (data not shown).

Observation of exudate production: Testa-covered embryos of *A. ginnala* were incubated on a solid medium. A dark reddish-brown exudate was observed on the upper surface of the testa in "pools" as well as in a dark "halo" which diffused into the medium under the embryo. Because the upper surface was not in contact with the medium, the presence of the exudate on this surface indicates that the exudate is not simply being washed off the surface of the embryo.

Effect of testa removal on embryo germination: Two experiments were conducted to analyze the effect of light on embryo germination following removal of the testa. The combined data from these experiments are presented here, as the acquisition of useful data under these conditions without introducing contaminating microorganisms was very difficult. When the pericarp and testa were removed from embryos, followed by incubation in the light, embryos germinated in seven to nine days (94% in uncontaminated experiments). A second set of embryos, from which the pericarp and testa had been removed in the light, were transferred to the dark. These germinated in 10-11 days at a slightly lower rate (67% in uncontaminated experiments) than embryos which germinated in the light. Despite an initial etiolated appearance, these seedlings grew and thrived. A third set of embryos from which the pericarp and testa had been removed in the dark were incubated under conditions of complete darkness and were also able to germinate (71% in uncontaminated experiments), but required subsequent light treatment for plantlet development. Embryos covered with intact testae did not germinate (0%) in light or darkness within a 120 day period, although slight physical nicking of the testa layer resulted in rapid germination. Dumbroff and Webb (1970) suggest that the role of the testa may be in restricting the rate of water uptake. While our observations do not contradict these results, we have observed that *A. ginnala* embryos germinating in vitro do not remain in physical contact with the exudate "halo" in the medium. This suggests that perhaps the exudate is affecting germination through chemical inhibition. We plan to examine the interaction between germinating *A. ginnala* embryos and the exudate.

Inhibition of lettuce root development by Amur maple exudate: The timing of exposure of germinating lettuce seeds and seedlings to the exudate significantly affected the mean number of lateral roots developed after one week of germination. Lettuce seeds exposed to the exudate on Day 0 [$\bar{x} = 0.57 \pm 0.20$], Day 1 [$\bar{x} = 0.00 \pm 0.00$], or Day 2 [$\bar{x} = 0.73 \pm 0.36$] had significantly fewer lateral roots (Figure 1) as compared to an untreated control [$\bar{x} = 1.61 \pm 0.31$]. The most severe lateral root inhibition occurred in seeds exposed to the exudate one day after being plated (beginning the germination process). Treated seedlings showed both a marked decrease in the number of lateral roots per plant and a noticeable reduction in root hairs (Figure 2; photograph of Control vs. Day 1 Lettuce). The longer the plantlets were allowed to develop (beyond Day 1) before exposure to the exudate, the less affected they were by the exposure (data not shown). These data suggest that the test seeds are vulnerable to the effects of the exudate, but that soon after the seeds germinate, the seedlings are no longer significantly affected. Other investigators made a comparable observation that "(chemical) control (of competitors) is restricted to germination and establishment. Once the plants are established, they generally survive" (Pickett and Baskin, 1973).

Physical analysis of Amur maple exudate: Spectrophotometric analyses of exudates provided significant peaks only at 260 and 280 nm. Analyses of exudates collected only once from testa-covered embryos on a solid medium after various lengths of time revealed that the absorbance of the exudate did not substantially change over time (data not shown). However, when exudate from a set of testa-covered embryos was collected every 2-3 days for 13 days, exudate absorbance decreased over time at 260 and 280 nm (Figure 3). This decrease in concentration may be due to exudate production decreasing over time, exudate being hydrolyzed or photolyzed over time, or exudate being diluted with water taken up from the medium. One of these hypotheses, that absorption of water from the medium leads to a dilution of the exudate, is analogous to the effect of moisture uptake from the soil on the concentration of the exudate in nature.

This study provides additional information (1) on germination physiology in *Acer ginnala* through the assessment of the role of the testa, (2) by documenting a reduction of lateral root formation in lettuce following exposure to the exudate, and (3) by initiating a spectrophotometric analysis of the exudate. We conclude that the testa plays a role in inhibiting the germination of Amur maple as more than just a physical barrier to light. Also, the exudate may function in inhibiting early seedlings of competitive companion species in the wild.

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Figure 1. Mean number and standard error of lateral roots in lettuce seedlings at specific times during their development. Day 0 samples were exposed as seeds, Day 1, one day after beginning germination in culture, and so on. Asterisks are used to distinguish among groups which differ from the control at the $\underline{P} \leq 0.05$ experiment-wise. Control seeds were not exposed to the exudate.

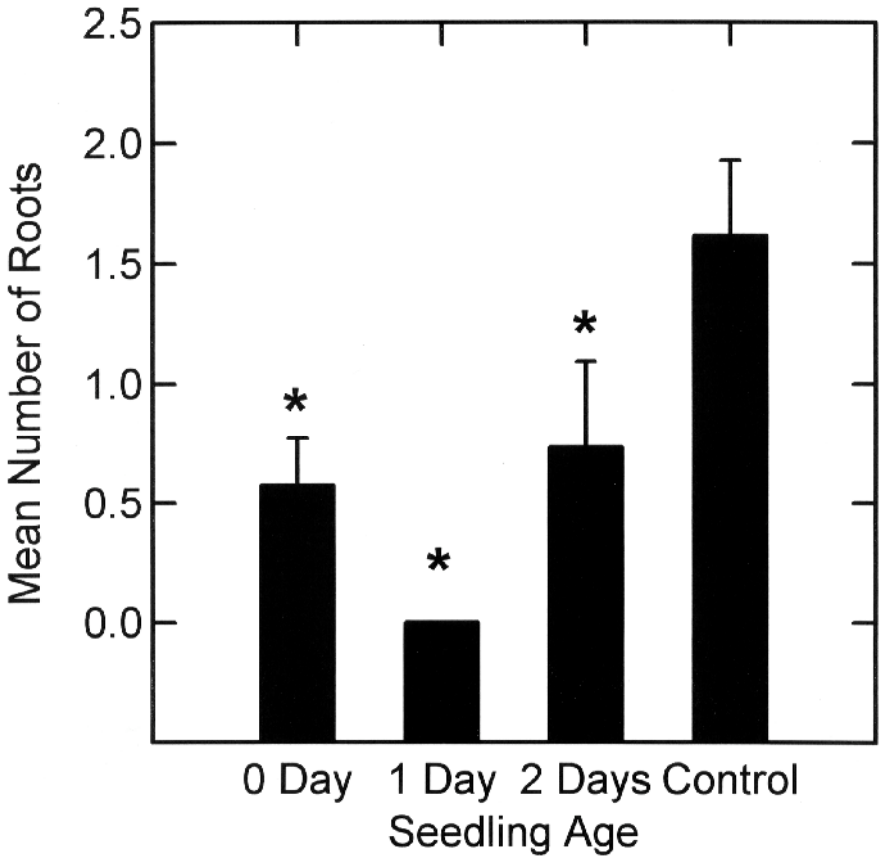

Figure 2. Photograph of lettuce control (left) versus Day 1 lettuce treated with *A. ginnala* exudate (right), taken four days after the initiation of the experiment. Note also the difference in the size of the seedlings (plate diameter 95 mm for reference).

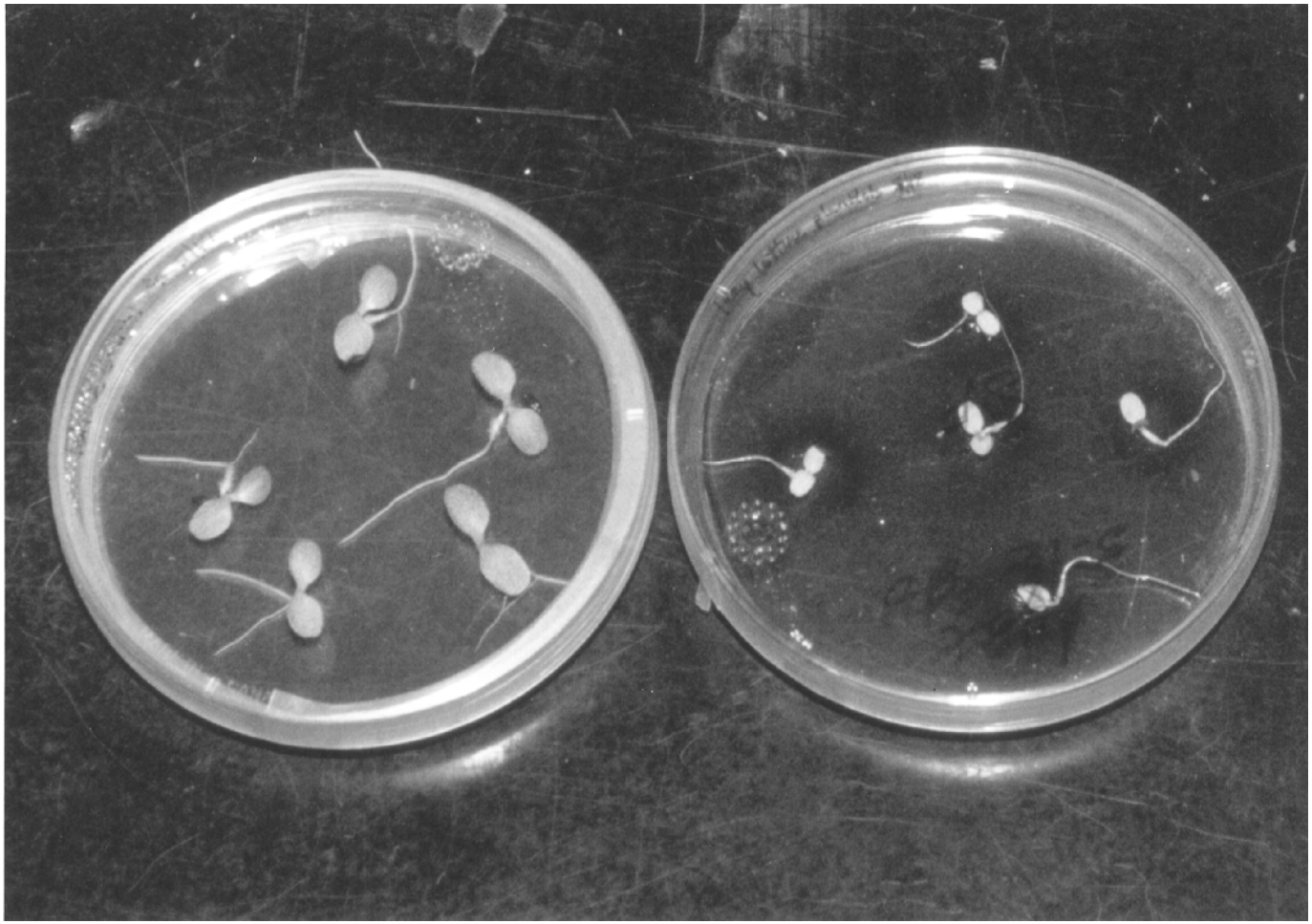

Figure 3. Optical density of testa exudate. Seeds were centrifuged following two days of being cultured on solid medium. The same seeds were then returned to culture and centrifuged again after 4, 6, 8, 10, and 13 days.