A STUDY OF THE EARLY FRUIT CHARACTERISTICS OF PONDBERRY

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Abstract—Pondberry [*Lindera melissifolia* (Walt.) Blume] is an endangered, dioecious, clonal shrub that grows in forested wetlands in the Southeastern United States. Because pondberry is endangered, presence of this plant could limit silvicultural options available to managers of public lands. Interest in pondberry has focused on the clonal nature of this species, and little has been published about the early physical and biochemical characteristics of the fruit as they mature. Four fruits from each of 40 plants were subsampled on a 30-day schedule after flower anthesis. Three months (90 days) after flowering, a complete seed had formed within the fruit. Of the total fruit weight (average 0.228 g), seed tissue accounted for 33 percent of the mass gained from 2 months (60 days) after flowering. Preliminary lipid analysis revealed the presence of myristic, palmitic, stearic, oleic, linoleic, and linolenic fatty acids; lauric acid was not found in any of the early seed samples but was plentiful at later stages in seed development. Preliminary results from seed longevity and persistence studies indicate that seeds without pulp and seeds left on the soil surface germinate more rapidly than buried seeds or those with the pulp intact.

INTRODUCTION

Pondberry [*Lindera melissafolia* (Walt.) Blume] is an endangered, dioecious, deciduous shrub that grows in southeastern forested wetlands in seasonally flooded areas, or along the margins of sinks and ponds. Stands consist of numerous stems (Devall 2004). The species has probably always been fairly rare in occurrence (Radford and others 1968). Pondberry stems can flower when only 2 or 3 years of age. Female plants can produce many bright red fruits, but it is commonly stated that seeds are of little or no value in stand formation because there is little evidence of new seedling establishment (Tucker 1984). Information on the ecology of the species is sparse, and little is known about the development and biochemistry of fruit and seed or of the fate of pondberry seeds once shed from the plant. Preliminary tests have shown that pondberry seeds are orthodox, and that excellent germination can be achieved when laboratory germinators are set on a cycle of 35 °C for 16 hours with light and 30 °C for 8 hours without light. We have used this information to begin our next series of studies, the purpose of which is to document fruit and seed growth rates, changes in fruit and seed biochemistry, and seed longevity and persistence in the field.

MATERIALS AND METHODS

Fruit collection and seed longevity and persistence studies were conducted at two sites within the Delta National Forest, MS. In fruit and seed longevity and persistence studies, both entire (with pulp) and peeled drupes were used as treatments.

Fruit and Seed Morphological and Biochemical Development

The length and diameter of drupes were measured with digital calipers (model CD 6″CS, Mitutoyo America Corporation) within 3 hours after drupes were removed from the plants from April through October. After removal of the pulp, seed diameter was measured. Samples of pulp and seed material were freeze dried, ground in a Wiley mill, and stored in liquid nitrogen pending lipid analysis. Lipids were later extracted from seed and pulp by first stirring the seed tissue for 30 minutes in a mixture consisting of two parts chloroform and one part methanol. The mixture was filtered, and the filtrate was measured volumetrically, washed, and purified in the manner described by Folch and others (1957) using one wash of 0.9 percent NaCl in distilled water and two washes of a 1:1 mixture of methanol and 0.9 percent NaCl in distilled water. Lipids were esterified using 1.5 percent concentrated H_2SO_4 in methanol (Christie 1990). Samples were placed in a 50 °C water-bath overnight, cooled, and vortexed for 15 seconds with 3 ml water and 2 ml hexane (Murrieta and others 2003). The hexane phase was removed, dried over anhydrous Na_2SO_4, and analyzed on a Hewlett-Packard® 5890 gas chromatograph using an HP-5 column. The initial oven temperature, for 5.2 minutes, was 110 °C. Oven temperature was then increased at 30 °C per minute to 140 °C and held for 22 minutes; temperature was then increased 30 °C per minute to 170 °C and held for 18 minutes. Total run time was 47.2 minutes. Injector and detector temperatures were 200 °C. Response factors were calculated from injections of low erucic rapeseed oil and AOCS oil reference mix number 3 (Sigma Chemical Co., St. Louis, MO).

Fruit and Seed Longevity

For this study, seed longevity is defined as the longest period of time that a seed will remain viable in the field. In September 2004, we installed a study to examine the longevity of seeds with and without pulp. Seeds were bagged in plastic screen. The bags were attached to wire flags (to aid location) and buried approximately 5 cm below the soil surface. Each bag held 25 seeds and counted as one replicate. Four bags of each treatment (100 seeds total) were to be collected after 1, 2, 4, 6, 12, 18, and 24 months of burial in the soil. After the seeds were brought into the lab, they were examined to determine if any had germinated in the field. Those that were ungerminated were rinsed free of dirt and placed in a germinator for 16 weeks to determine if they would germinate. Seeds were germinated in a Stultz® germination cabinet set

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at 35 °C for 16 hours with light and 30 °C for 8 hours without light. We report here on the 1- and 2-month collections.

Fruit and Seed Persistence

Persistence is a measure of the fate of seeds that have been dispersed. The persistence study was established at the same time as the longevity study. Both peeled and entire drupes were put in mesh bags. As before, each bag (replicate) contained 25 seeds and was attached to a wire flag. Each flag marked the location of both a bag of seeds with pulp and a bag of seeds without pulp. The bags rested on the soil surface. Four bags of seeds both with and without pulp were to be sampled after 1, 2, 4, 6, 12, 18, and 24 months of exposure. We report here on the 1- and 2-month collections. Sample seeds were germinated as above.

RESULTS AND DISCUSSION

Fruit and Seed Morphological and Biochemical Development

Pondberry drupes reached their mature size by July. While the average dimensions were larger for August, the difference was not significant. The fruits collected in July averaged 10.9 mm in length and 8.1 mm in diameter. The seeds reached their mature size by August. Seeds were, on average, 6.6 mm in diameter and weighed 0.18 g at that time. Lauric acid was the dominant fatty acid in the pondberry seeds (fig. 1A). Although none of this acid was found in earlier seed samples, concentrations reached 231 mg/g dry seed tissue by August. Oleic and linoleic acids were also found in fairly large quantities, and traces of palmitic, stearic, and linolenic fatty acids were present (fig. 1B). The fruit pulp is very low in lauric acid

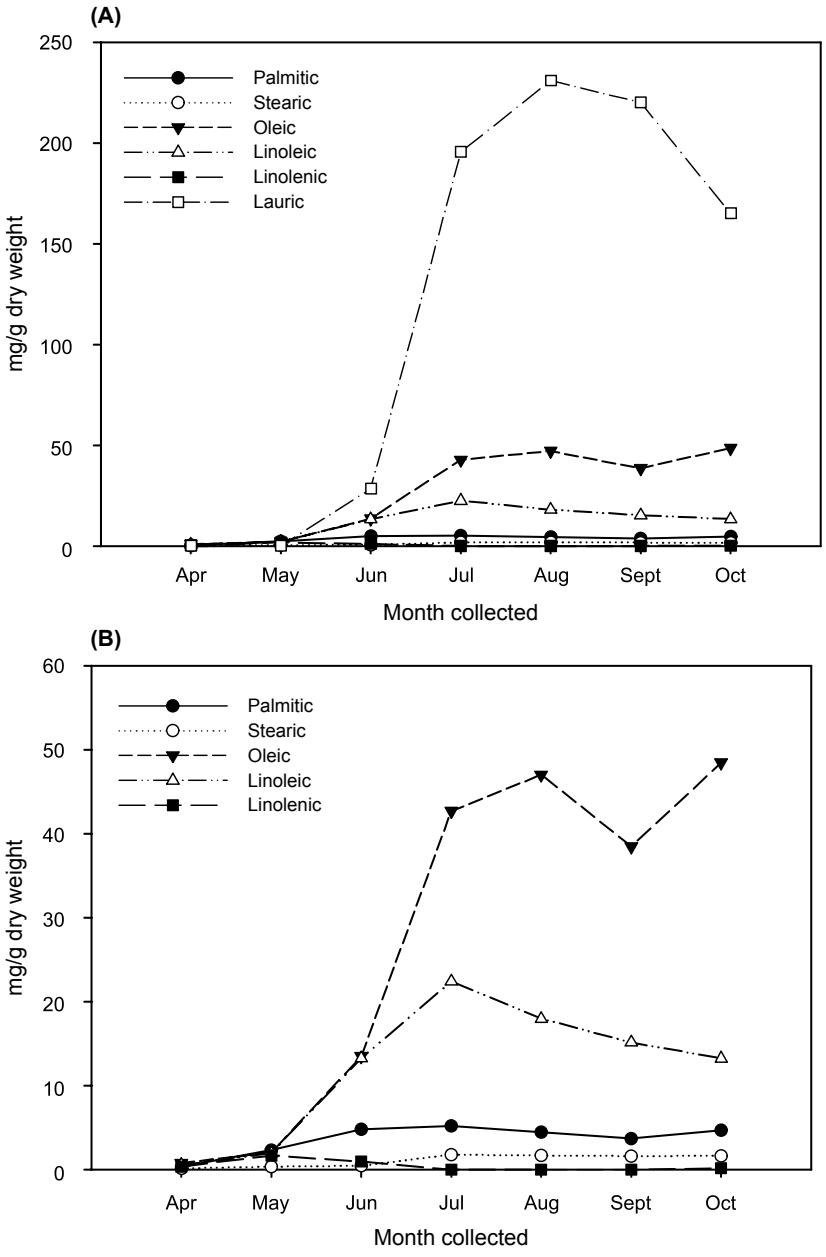

Figure 1—Pondberry seed collected from the Delta National Forest, MS: (A) Fatty acid profile. (B) Fatty acid profile without lauric acid.

throughout its development. Figure 2 profiles the fatty acids that were present in the pulp; oleic acid was the most prevalent, reaching > 300 mg/g dry seed tissue in October. Small amounts of octanoic acid were found in pulp but were absent from seed tissue.

Fruit and Seed Longevity

Seeds with pulp, buried for 1 month and kept in a germinator for 9 weeks, had a germination rate of 0 percent (fig. 3A). Seeds with pulp, buried for 2 months and kept in a germinator for 6 weeks, had 39 percent germination (fig. 3B). Also, the percentage of seeds that rotted was lower for seeds that were buried for 2 months than for those that were buried for 1 month. Seeds without pulp, buried for 1 month and kept in a germinator for 9 weeks, had 18 percent germination (fig. 4A). Those buried for 2 months and kept in an incubator for 6 weeks had 53 percent germination (fig. 4B). There was a 4 percent reduction in rotten seeds. These results show that, in the laboratory, seeds without pulp have a higher viability than seeds with pulp, and we would expect that they would move out of the seed bank faster than those with pulp left intact. Among the unknowns, however, are the mechanism by which intact pulp may affect seed decay, how flooding will affect seed viability, and how long seed will remain viable in the remaining field samples.

Fruit and Seed Persistence

Fresh seeds with pulp intact that were deposited directly on the litter layer and remained there for 1 month had 6 percent germination after 9 weeks in the germinator (fig. 5A). Those that remained on the litter layer for 2 months had 59 percent germination after 6 weeks in the germinator (fig. 5B). The

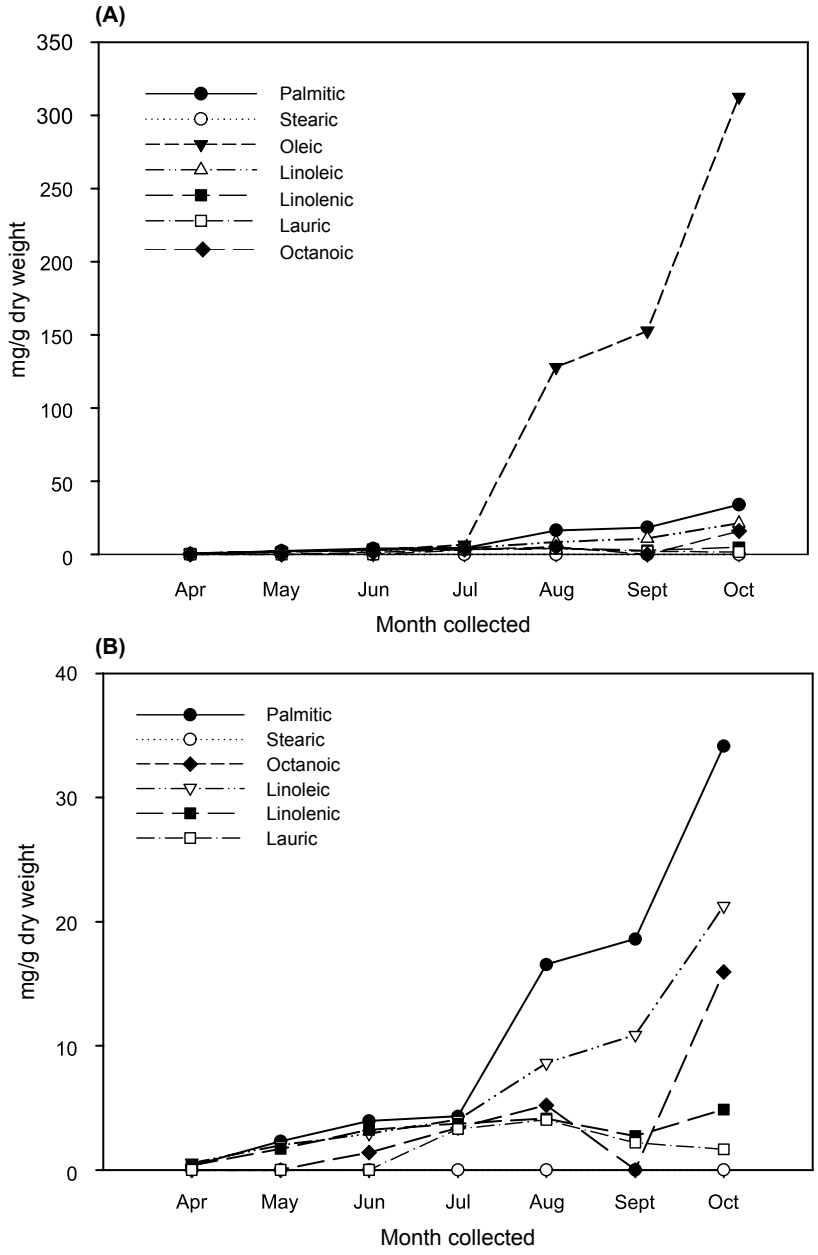

Figure 2.—Pondberry fruit pulp collected from the Delta National Forest, MS: (A) Fatty acid profile. (B) Fatty acid profile without oleic acid.

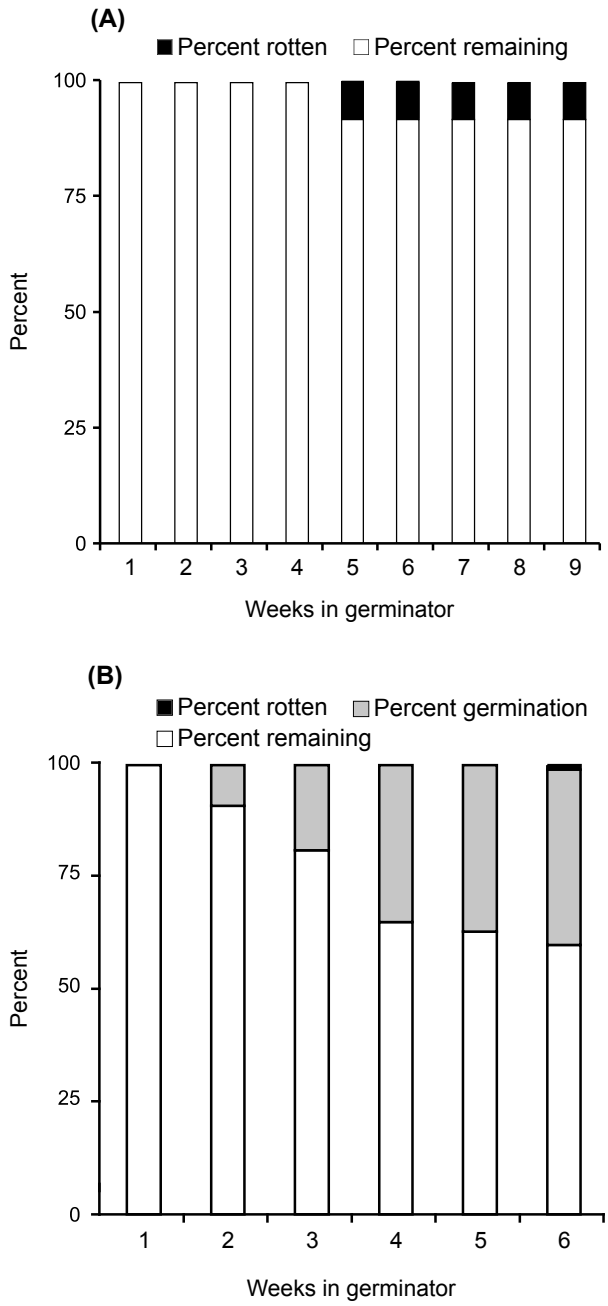

Figure 3—Seed with pulp from the pondberry longevity study after (A) 1 month and (B) 2 months in the field.

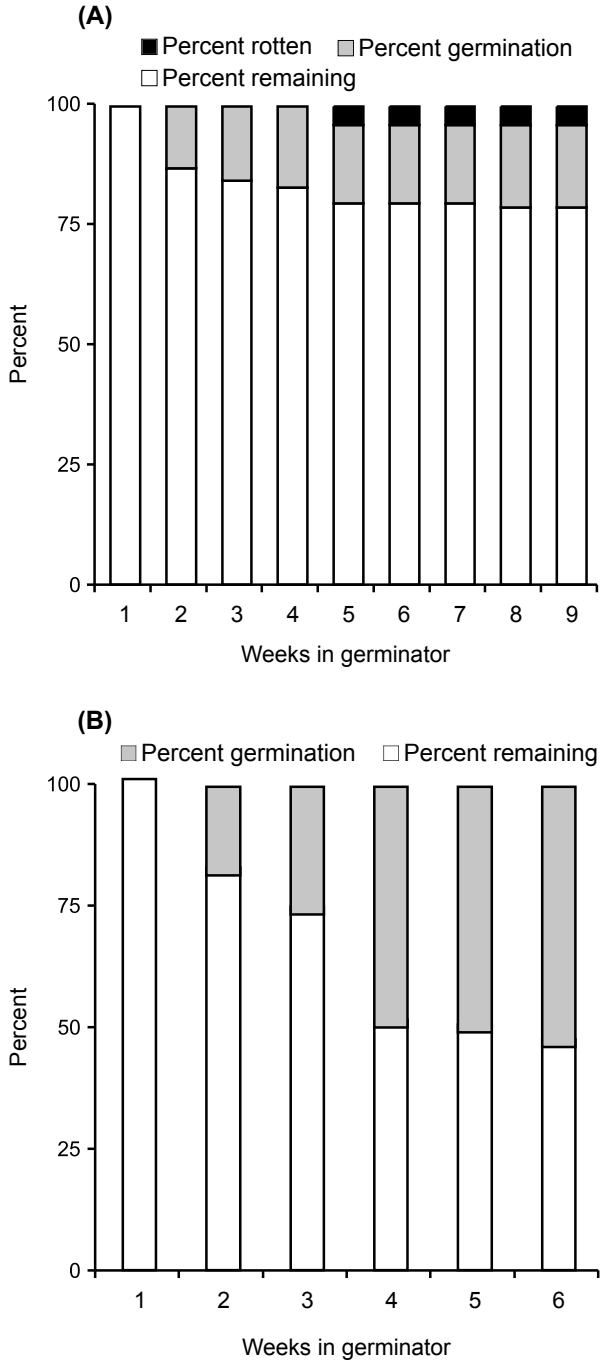

Figure 4—Seed without pulp from the pondberry longevity study after (A) 1 month and (B) 2 months in the field.

proportion of rotten seeds was 3 percent for each group (figs. 5A and 5B). Seeds without pulp that were deposited directly on the litter layer and remained there for 1 month had 20 percent germination after 9 weeks (fig. 6A). Those that remained on the litter layer for 2 months had 90 percent germination after 6 weeks (fig. 6B). One percent of the pulpless seeds that remained in place 1 month rotted, and none of the pulpless seeds that remained in place for 2 months rotted (figs. 6A and 6B). These preliminary laboratory results suggest that seeds on the soil surface might germinate faster than buried seeds and move out of the seed bank faster than buried seeds. Seeds without pulp also have a higher germination percentage than seeds with pulp.

Both the longevity and persistence studies show that seeds freshly deposited in the field do not germinate immediately. This study is still in progress, and although seeds are doing well in the laboratory germinators, we have yet to see any germination in the field. Conclusions are pending completion of the study.

ACKNOWLEDGMENTS

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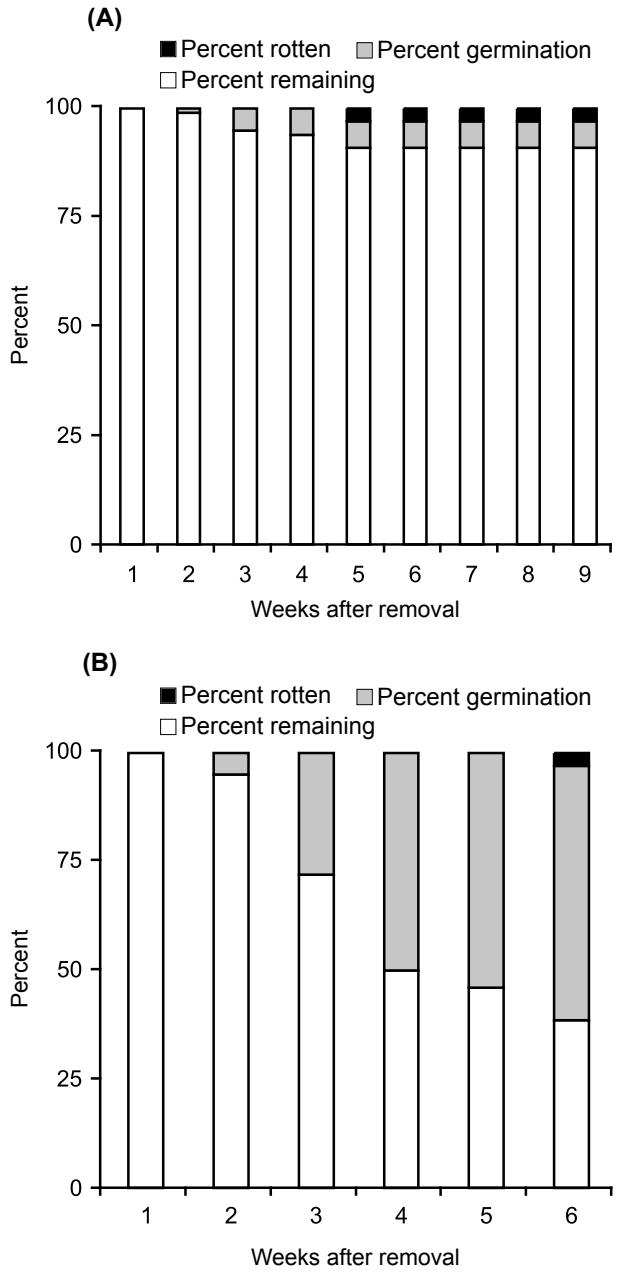

Figure 5—Seed with pulp from the pondberry persistence study after (A) 1 month and (B) 2 months in the field.

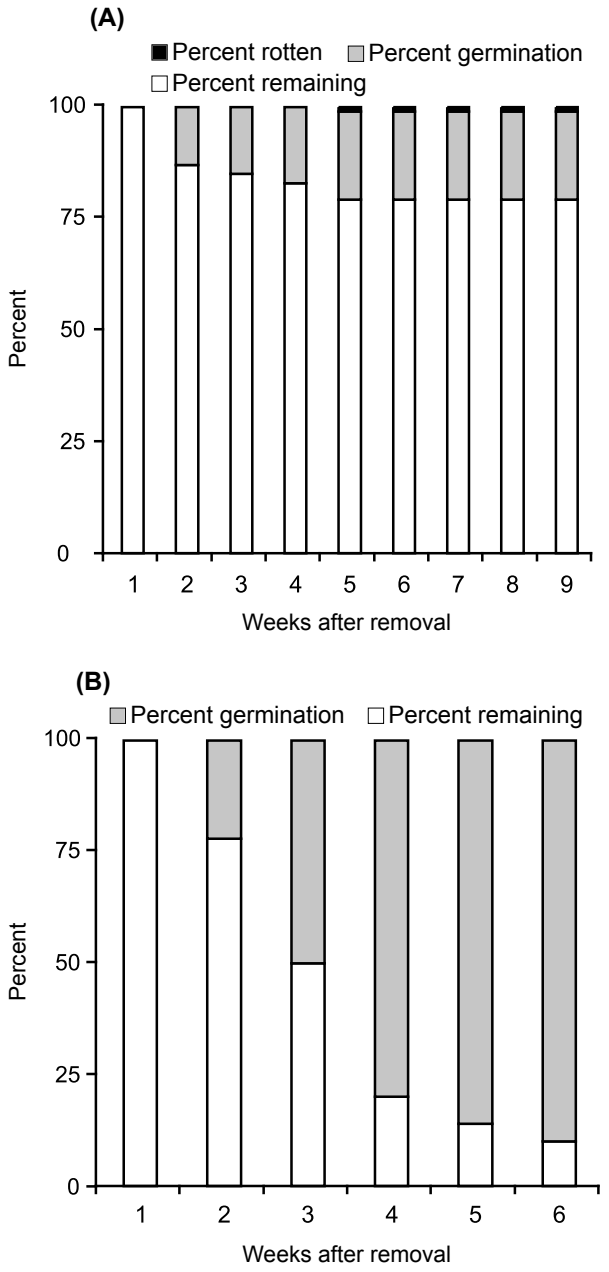

Figure 6—Seed without pulp from the pondberry persistence study after (A) 1 month and (B) 2 months in the field.

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