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Weed Management—Techniques

Mechanical Scarification of Dodder Seeds with a Handheld Rotary Tool

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Dodder seeds are physically dormant because of hard seed coats and do not readily germinate without scarification. Reliable methods of scarification for small lots of dodder seed are needed to facilitate laboratory, greenhouse, and field research projects. Dodder seed was scarified for varying times using a handheld rotary tool at the 10,000 rpm setting with a conical grinding-stone bit attached. Percentage of germination and weight change of seeds were assessed using scarification times between 0 and 4 min at 0.5-min increments. Mean seed weight loss and mean number of germinated seeds increased quadratically as scarification time increased. Scarifying for 2.5 min was judged the shortest time with maximal germination. Another study evaluated the effect of seed number (100 to 400 seeds sample^{-1}) on the efficacy of rotary tool scarification when scarification time was held constant at 2.5 min. Percentage of germination decreased linearly as seed batch size increased. The handheld rotary tool provides consistent and repeatable scarification of dodder seed with germination rates greater than 80%.
Nomenclature: Dodder, *Cuscuta* spp.; cranberry, *Vaccinium macrocarpon* Ait.
Key words: Electric scarifier, impermeable seed coat, parasitic weed, persistent seeds, physical dormancy, presowing treatment, seed germination.

Las semillas de *Cuscuta spp.* tienen latencia física debido a que sus testas son duras y no germinan fácilmente sin escarificación. Se necesitan métodos confiables de escarificación para lotes pequeños de semillas de *Cuscuta* para facilitar los proyectos de investigación de laboratorio, invernadero y campo. La semilla de *Cuscuta* fue escarificada por períodos diferentes usando una herramienta de rotación manual a 10,000 RPM a la cual se le colocó una piedra cónica de esmeril. El porcentaje de germinación y el cambio en el peso de las semillas fueron evaluados usando tiempos de escarificación entre 0 y 4 min, con incrementos de 0.5 min. La pérdida promedio en el peso de la semilla y el número promedio de semillas germinadas se incrementó cuadráticamente conforme aumentó el tiempo de escarificación. La escarificación por 2.5 min fue considerada como el tiempo más corto con máxima germinación. Otro estudio evaluó el efecto del número de semillas (de 100 a 400 semillas por muestra) en la eficacia de la herramienta rotativa cuando el tiempo de escarificación se mantuvo constante a 2.5 min. El porcentaje de germinación disminuyó linealmente conforme se incrementó la cantidad de semillas en la muestra. La herramienta rotativa manual proporciona escarificación consistente y repetible de las semillas de *Cuscuta* con índices de germinación superiores al 80%.

Reliable methods to promote seed germination are needed in many areas of weed science research. The seeds of dodder do not readily germinate without scarification (Meulebrouck et al. 2010), especially after field collection and storage in the laboratory or other facilities. The physical dormancy of dodder seeds is attributed to hard seed coats, preventing water from being imbibed, and is believed to be associated with the drying out of the seed coat (Dawson et al. 1994; Gaertner 1950; Jayasuriya et al. 2009). It has been suggested that when dormancy is broken under natural conditions, water enters dodder seeds exclusively via the hilum (Jayasuriya et al. 2009). However, laboratory studies that used concentrated sulfuric acid to break physical dormancy by causing damage to the seed coat have demonstrated that water can be absorbed though the entire dodder seed coat (Hutchison and Ashton 1979; Jayasuriya et al. 2009).

The search to find adequate germination methods for dodder extends back many decades, often with minimal success. The acid scarification method developed in the 1950s to enhance dodder seed germination has retained favorable prominence for seed germination work. The conventional approach is to soak the seeds in concentrated (96 to 98%) sulfuric acid (Buhler and Hoffman 1999; Gaertner 1950). In our experience, treatment with concentrated sulfuric acid has generated germination rates less than 50% (H. Sandler, unpublished data). In addition, potential injury to laboratory personnel from the use of a strong acid and base (needed to neutralize the pH after acid treatment) reduces the utility of this approach. The use of acid to scarify seeds requires the use of proper ventilation, protective clothing for the body and face, and appropriate disposal of hazardous chemicals after the scarification process is complete.

Mechanical scarification has been used with varying degrees of success with many other types of seeds. Methods have included scarifying annual wildrice (*Zizania aquatica* L.) seeds with granite (Oelke and Albrecht 1978), puncturing ornamental cycad (*Zamia* L. spp.) seeds (Smith 1978), piercing trailing crownvetch (*Coronilla varia* L.) seed (McKee et al. 1979), and rubbing seeds with sandpaper (acacia [*Acacia* Mill. spp.] and chinaberry [*Melia azedarach* L.])(Azad et al. 2010; Padma et al. 1993). High-capacity (high-volume) commercial mechanical seed scarifiers are available, such as the Forsberg Huller/Scarifier (Forsberg, Inc., Thief River Falls, MN), but

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they are expensive and generally impractical for modifying small samples typically needed for scientific studies. Smaller machines have been successfully used to scarify seeds, such as small rock tumblers lined with sand paper (Stabell et al. 1998) and a single-speed electric scarifier (horizontally mounted rotary propeller within a steel drum lined with 40-grit sandpaper)(Olszewski et al. 2010).

Sandpaper (with a mechanical scarifier as described in Stabell et al. 1998 and Olszewski et al. 2010) was used to break dormancy of smallseed alfalfa dodder (*Cuscuta approximata* Bab.) and largeseed dodder (*Cuscuta indecora* Choisy) seeds; the effectiveness of scarification varied by dodder species (Tingey and Allred 1961), but, in some studies, it was found to be at least as effective at promoting germination as acid scarification for dodder seeds (Hutchison and Ashton 1979). In our laboratory, screened sand has been used as an abrasive (grinding in a mortar and pestle) to encourage dodder seed germination but rates rarely exceeded 35% (H. Sandler, unpublished data). A handheld rotary sanding tool has not, to our knowledge, been evaluated for improvement of dodder seed scarification. If effective, this type of tool would be a time-saving and practical technique for scarifying dodder seed.

To facilitate research into other aspects of dodder biology, we needed a method that would easily and consistently promote scarification and germination of small batches of dodder seed. Our objective was to develop a simple mechanical method to scarify the seed coat and enhance dodder seed germination that could be easily used for small sample sizes like those typically needed for laboratory or greenhouse studies.

Materials and Methods

Laboratory studies were conducted at the University of Massachusetts Cranberry Station, East Wareham, MA. Dodder seeds were collected on September 25, 2008, from a commercial cranberry bog in Carver, MA. Dodder on Massachusetts cranberry bogs has classically been identified as swamp dodder (*Cuscuta gronovii* Willd. ex J.A. Schultes). Dodder species are very difficult to visually differentiate, and some taxonomy is controversial (Stefanovic et al. 2007). Recent and ongoing genetic work with dodder populations on Massachusetts cranberry bogs suggests that multiple species are present (Kim et al. 2004; K. Ghantous, unpublished data). Because of these new developments, we refer to the identity of the seed used in this experiment to the genus only.

Handheld pruners were used to remove all plant material that was attached to dodder seed pods from the collection area. Plant material was placed into paper bags and brought back to the laboratory where the seed pods were sorted from other plant material. The seed pods were crushed by hand to release seeds from the pods. Seeds were culled from the chaff by hand with a magnifying lens and tweezers and were then viewed under a microscope to visually assess seed condition. Seeds that were discolored or misshapen were removed from the sample. The remaining seeds were stored in closed glass scintillation vials out of direct light until use.

A handheld rotary tool (Dremel 4000 High Performance Rotary Tool, Robert Bosch Tool Corporation, Racine, WI 53406) was vertically mounted with a clamp stand (Figure 1). A conical aluminum oxide grinding stone bit (Dremel part 953, aluminum oxide grinding stone, Robert Bosch Tool Corporation) was used. The tool was set to the 10,000 rpm setting. Dodder seeds intended for scarification were placed into microcentrifuge tubes (Fisherbrand Snap-Cap microcentrifuge tubes, 2 ml, part 02-681-258, Fisher Scientific, Pittsburg, PA 15205). Mechanical scarification was done by placing the grinding bit into the microcentrifuge tubes, keeping the rim of the tube flush with the tool chuck to avoid seed loss (Figure 1). The tool was turned on, and the length of scarification time was measured using a stopwatch. Seeds were weighed before and after treatments, and weight loss was attributed to the amount of seed coat physically removed by the grinding process.

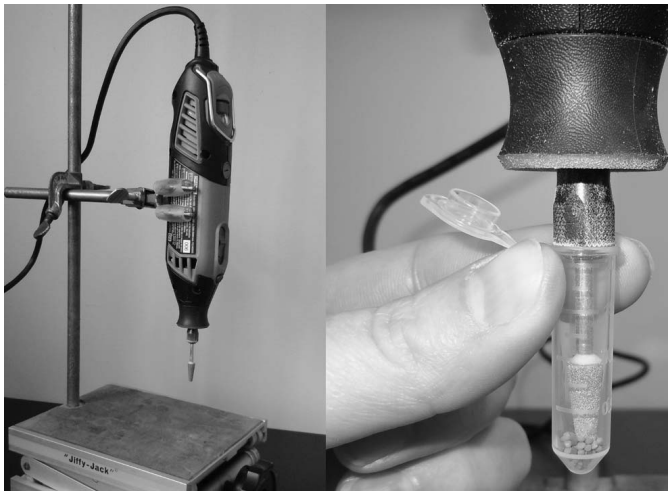

Figure 1. Dremel 4000 in clamp stand and depiction of dodder seeds in 2-ml microcentrifuge tube held flush with tool chuck ready for mechanical scarification. A clamp may be used to hold the microcentrifuge tube to reduce risk of injury.

After scarification, seeds were transferred from the microcentrifuge tube into a fine-mesh strainer. The strainer was gently shaken to remove dust particles generated during scarification before the samples were weighed. Seeds were not rinsed before weighing to avoid any possible weight increase because of absorption. After weighing the seeds, they were rinsed with water to remove any remaining seed coat particles that were not removed by the shaking process. Each sample was germinated individually in a separate petri dish. All the dodder seeds from a sample were placed on top of round filter paper that was inserted into glass 9-cm-diameter petri dishes and moistened with water. Glass covers were placed over dishes and sealed with Parafilm (Parafilm M, Pechiney Plastic Packaging, Menasha, WI 54952) strips to reduce evaporative water loss. Dishes were incubated (Fisher Low Temperature Incubator Model 307, Fisher Scientific) in the dark at 23 C and were checked periodically for 3 wk after scarification to evaluate seed germination (Benvenuti et al. 2005; O'Connell et al. 2011). Germinated seeds were removed from the dishes and counted, and the percentage of germination was calculated. Upon each evaluation, filter paper was remoistened as needed, and dishes were resealed and returned to the incubator.

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Scarification Duration. Seventy-two samples of sets of seed were counted and placed into microcentrifuge tubes. Each sample consisted of 100 dodder seeds. Samples were weighed before and after treatment. The experiment used a randomized complete-block design (RCBD) with four replications. Treatment was the length of time the seeds were mechanically scarified by the rotary tool. The nine scarification treatments were 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, or 4 min. Seeds were scarified and incubated in blocks to account for any possible difference in conditions inside the incubator in different areas. The entire experiment was repeated twice. Seed germination was determined as described above.

Seed Number. An experiment was conducted to evaluate dodder germination in response to the number of seeds scarified per batch. Based on data from the previous experiment, we established that the average weight for 100 dodder seeds was 0.100 g. Seed number for samples in this experiment was measured by weight rather than by counting the seeds. Scarifying for 2.5 min was judged the shortest time with the best germination, based on results of the scarification duration experiment. The experiment used an RCBD with four replications. Treatment was the number of seeds per vial (100, 200, 300, or 400 seeds vial^{-1}), and all samples were scarified for 2.5 min. Seeds were scarified and incubated in blocks to account for any possible difference in conditions inside the incubator in different areas. Samples were weighed before and after treatment. The entire experiment was repeated twice. Seed germination was determined as described above. After the 3-wk germination period, all the seeds remaining in the petri dishes were squeezed with tweezers to establish how many hard, nonimbibed seeds remained for each seed batch.

Data Analysis. The proportion of weight change for samples was calculated by subtracting the postscarification weight from the original weight and dividing by the original sample weight (both experiments). The proportion of nonimbibed seeds was calculated by dividing the number of hard seeds remaining after the 3-wk germination period by the total number of seeds in the seed number experiment. The proportion of dodder seeds that germinated was calculated by dividing the number of seed that germinated in each set by the total number of seeds in that set (both experiments). Proportions were multiplied by 100 to convert them into percentages for presentation.

Data were analyzed in SAS software, version 9.2 (SAS Institute Inc., Cary, NC 27513). For each study, treatment-by-run interactions were tested using Proc Mixed with blocks (replicates) nested within repetition. Normality was tested using Proc Univariate, and nonnormal data were transformed. The proportions of seeds germinated in the scarification duration experiment were arcsine-transformed, and the proportion of weight change and the proportion of nonimbibed seeds were arcsine-square root–transformed in the seed-number experiment to conform to the assumptions of normality for ANOVA. Data were back-transformed for presentation as needed and presented as percentages. Germination counts and changes in seed weights were assessed for significant regression trends at the linear and quadratic levels using orthogonal contrasts and Proc GLM. Coefficients of determination (r^2) values were calculated by dividing the sums of squares associated with the linear contrast by the sums of squares associated with the effect of scarification time for linear trends or by dividing the sums of squares associated with the linear contrast plus the sums of squares associated with the quadratic contrast by the sums of squares associated with effect of scarification time for quadratic trends (Damon and Harvey 1987).

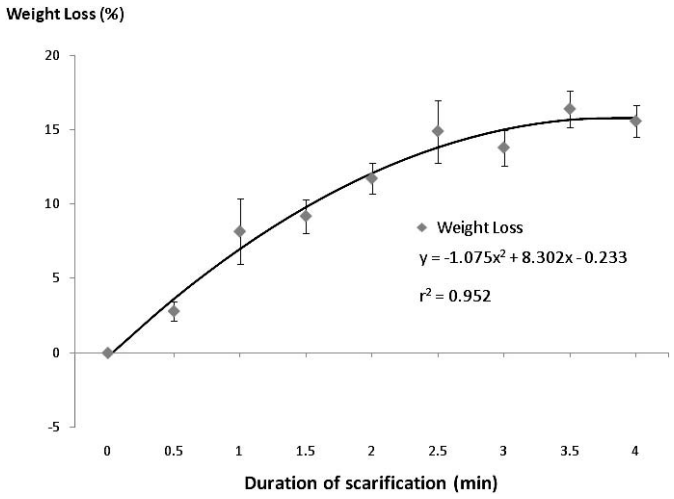

Figure 2. Average percentage of weight loss per sample of 100 dodder seeds after variable durations of mechanical scarification by rotary tool (n = 8). Error bars are ± standard error.

Results and Discussion

Scarification Duration. The effect of scarification duration by run interaction was not significant; therefore, data from both runs were pooled. Average percentage of weight loss from samples increased quadratically as exposure increased ($P \leq 0.001$)(Figure 2). A visual inspection of the seeds after scarification with the rotary tool showed that the seed coat appeared to be worn through by the grinding process (Figure 3). Although any possible loss from seed-to-seed contact cannot be discounted, the weight loss likely corresponds to the amount of seed coat material removed by the grinding action of the rotary tool: longer grinding times removed more seed coat. The anatomy of dodder seed coats consists of several different layers of cells beneath the epidermis (Hutchison and Ashton 1979; Lyshede 1984; Lyshede 1992). The quadratic nature of the trend may indicate that deeper layers of the seed coat are harder than outer layers and not as easily removed by grinding, but further work is needed to test the hypothesis.

The average percentage of seeds that germinated also increased quadratically as the duration of the scarification increased ($P \leq 0.001$)(Figure 4). If grinding provides the seed coat with more areas by which water can enter, more seeds are likely to germinate because imbibition is crucial for germination to occur.

The quadratic nature of the response to scarification duration indicates that, at some point, increasing the duration

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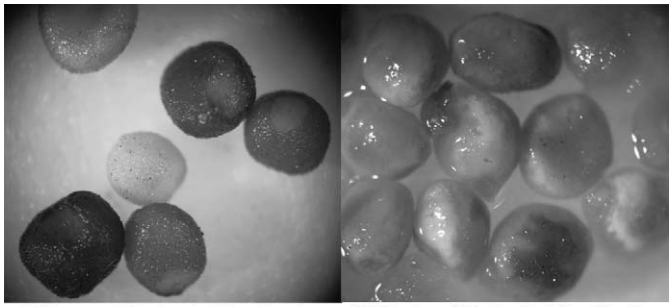

Figure 3. Visual comparison of dodder seed coats with and without scarification.

of the scarification no longer increases germination. Non-replicated, longer scarification durations were conducted up to 8 min long (data not shown). No obvious decrease in germination rates was seen; however, the seedlings appeared thinner than seeds scarified for shorter times. The purpose of this experiment was to test whether germination rates could be enhanced with this novel scarification technique. Only germination was examined, which was an indicator of whether viable embryos were released from physical dormancy. The fitness of seedlings after germination and survival rates were not evaluated. Longer scarification durations may damage the seeds by removing some of the endosperm underneath the seed coat. Future research could help to address any change in fitness in the scarified seedlings. Since the completion of this experiment, this technique has been used to scarify dodder seeds for 2.5 min and has been able to reliably rear dodder on host plants from seeds scarified for this duration (K. Ghantous, unpublished data).

There could be differences in the seed coats of dodder seeds obtained from different populations. The optimum length of time for soaking dodder seeds in concentrated sulfuric acid

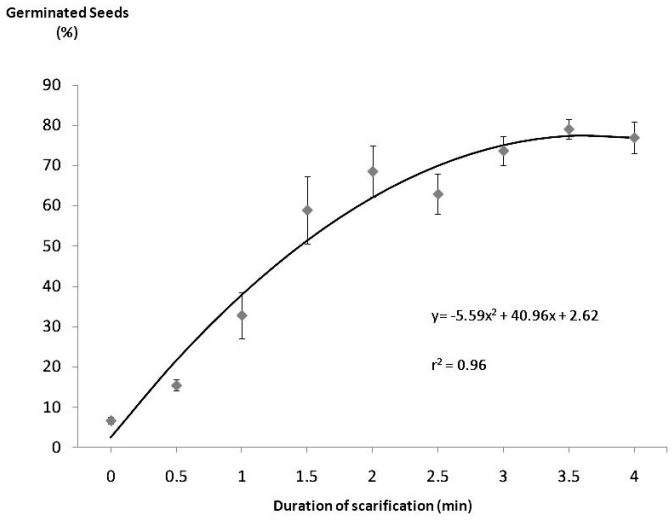

Figure 4. The average percentage of seeds that germinated in the 3-wk interval following variable durations of mechanical scarification by the rotary tool ($n = 8$). Error bars are ± standard error.

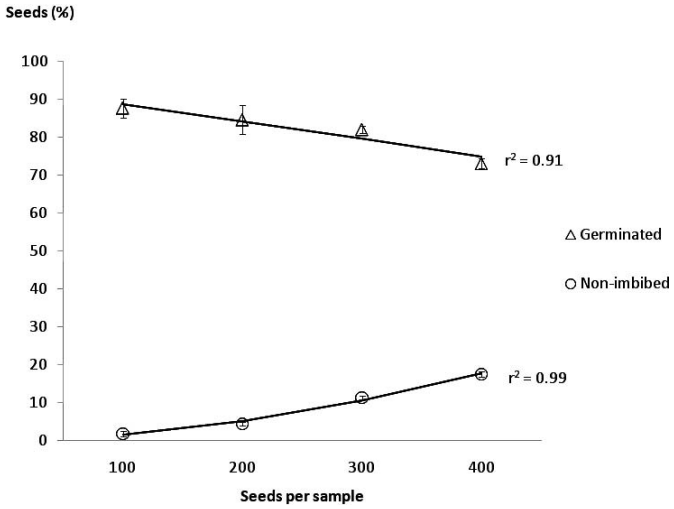

Figure 5. The average percentage of seeds that germinated in the 3-wk interval following 2.5 min of mechanical scarification by rotary tool, and the average percentage of nonimbibed seeds remaining ($n = 8$). Germination: $y = -0.046x + 93.343$. Nonimbibed seeds: $y = 0.00008959x^2 + 0.00937x - 0.33281$. Error bars are ± standard error.

depends on the age and species of the dodder seed (Gaertner 1950). The efficacy of mechanical scarification may also depend on those factors, but more research is needed before definitive recommendations can be made.

Seed Number. The effect of seed number–by-run interaction was not significant; therefore, data from both runs were pooled. After 2.5 min of scarification, the change in the percentage of weight was not affected by the number of seeds in a sample (data not shown). The average percentage of germination decreased linearly as the number of seeds per sample increased (Figure 5). The decrease was slight (87 and 72% for batches of 100 and 400 seeds, respectively) but was highly significant ($P \leq 0.01$).

The decrease in germination, coupled with no impact on seed weight loss, could indicate that that either our measurements were not sensitive enough to detect the weight change or that, within the batches, some seeds were more scarified, whereas others were less scarified. As seed number increased, bit-area to seed-surface-area decreased. There was also less room for seeds to move inside the tubes, perhaps leading to seeds spending unequal time in contact with the grinding bit (100 seeds occupied approximately 10% of the 2-ml tube volume, whereas 400 seeds occupied approximately 40% of the tube volume).

The percentage of nonimbibed seeds increased quadratically ($P \leq 0.001$) as the number of seeds per sample increased (Figure 5). The imbibed seeds that did not germinate were easily crushed by tweezers and considered not viable (Borza et al. 2007). The nonimbibed seeds likely did not have enough scarification to permit them to imbibe. Although seed number has no effect on weight loss after scarification, the seeds are not being scarified evenly when number of seeds per sample is increased. The percentage of imbibed seeds that did not germinate (nonviable seeds) was not significantly different between treatments.

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The objective of our experiment was to investigate whether mechanical scarification of dodder seeds could be a reliable, efficient, and effective way to break the physical dormancy of dodder seeds. Subjective methods of manually, physically scarifying seeds, such as the use of sandpaper or nicking, have the potential to introduce many sources variation based on the person performing the task. It has been successfully demonstrated that the use of the handheld rotary tool yields a consistent technique for breaking the physical dormancy associated with dodder seeds, as evidenced by the small standard error for each length of time. This novel technique is less time-consuming than acid scarification, which may include soaking seeds for 30 to 75 min in concentrated sulfuric acid (Gaertner 1950). It is an appealing choice for scarifying small batches of dodder seed, does not require use and disposal of hazardous chemicals, and may have applications for other types of seeds.

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