

Germinating and Culturing Axenic Poison Ivy Seedlings

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Abstract. Urushiols are the chemical constituents responsible for causing the characteristic skin rash resulting from contact with poison ivy [*Toxicodendron radicans* subsp. *radicans* (L.) Kuntze] plant tissue. Future detailed physiological and molecular studies of *T. radicans* urushiol metabolism will require the production and cultivation of axenic *T. radicans* plants in controlled environments. To this end, the present study focused on treatments to enhance germination and reduce microbial contamination to obtain axenic *T. radicans* seedlings. *Toxicodendron radicans* drupes treated singularly with water, bleach, cold, or gibberellic acid showed very low germination frequencies. In contrast, concentrated sulfuric acid strongly promoted seedling germination by removing exocarp, mesocarp, and causing pitting of the brachysclereid and osteosclereid layers of the endocarp. Most *T. radicans* drupes harbored significant amounts of fungal and bacterial contaminants. Although the serial mechanical scarification, sulfuric acid, and bleach treatments promoted seedling germination, this serial treatment regime was not adequate to render the majority of drupes microbe-free. Nevertheless, ≈25% of treated *T. radicans* drupes were axenic, and these needed to be promptly separated from adjacent fungal-contaminated drupes to avoid cross-contamination. The isolated axenic *T. radicans* drupes germinated at high frequency producing viable seedlings that grew well in sterile plant culture conditions.

Poison ivy is best known for its ability to cause irritating skin rashes called *Rhus* dermatitis. Poison ivy belongs to the family Anacardiaceae, which includes other species producing sap capable of causing skin reactions, including poison oak (formerly *Rhus toxicodendron diversilobum*), poison sumac (formerly *Rhus toxicodendron vernix*), mango (*Mangifera indica*), cashew (*Anacardium occidentale*), and the Asian lac tree (*Rhus verniciflua*). Gillis proposed a systematic revision of poison ivy, poison oak, and poison sumac from the genera *Rhus* to *Toxicodendron* (*Toxicodendron radicans*, *Toxicodendron diversilobum*, and *Toxicodendron vernix*, respectively) (Gillis, 1971). Despite the moniker “poison” ivy, the manifested dermatitis is an immunologically based allergic reaction, delayed contact hypersensitivity, not an acute toxicity or poisoning (Kurtz and Dawson, 1971).

The natural product responsible for inducing the delayed contact dermatitis is generically called urushiol. Urushiol refers to a number of pentadecylcatechol or heptadecylcatechol congeners with varying degrees of unsaturation ranging from one to three double bonds (Symes

and Dawson, 1953, 1954). Urushiol triolefin congeners correlate with increased severity of contact dermatitis symptoms compared with those with less unsaturation (Johnson et al., 1972). The principal urushiol congeners in *T. radicans* are the pentadecylcatechols. Although the chemical composition of *T. radicans* urushiol and clinical immunology of the delayed contact dermatitis is well documented, urushiol physiology and metabolism in *T. radicans* plants are poorly understood.

Urushiol levels and composition vary in poison ivy plants. In one report, young leaves, fresh young stems, fruits, and bark showed high urushiol levels, and the triolefin congener comprised over half of the total urushiols present (Craig et al., 1978). A different study examining leaves of different ages also showed high urushiol levels in young leaves with lower urushiol levels in the oldest leaves (Baer et al., 1980). In the latter study, the diolefin was the most abundant congener, whereas the triolefin was the least abundant. Both reports used tissues obtained from unmanaged *T. radicans* plants in which genetic, abiotic, and biotic factors were neither determined nor controlled. From these two studies it is clear that urushiol composition and levels are not determinate traits in poison ivy. Therefore, similar to other defensive plant secondary metabolites, *T. radicans* urushiol levels and composition are likely to change in response to developmental, environmental, and biotic factors. As a prerequisite for future detailed urushiol physiological and metabolism studies, it will be necessary to grow *T. radicans* plants under well-controlled environmental conditions.

Axenic plantlets grown on synthetic media provide optimal experimental control over most abiotic and biotic parameters. However, because *T. radicans* has no economic value other than being a noxious invasive plant, there is rather limited knowledge about *T. radicans* germination patterns. Two prior studies suggest that *T. radicans* drupes require scarification to initiate seedling germination. One study focused on *T. radicans* seed dispersal by birds and squirrels demonstrated that sandpaper scarification significantly increased seedling germination frequencies relative to untreated drupes (Penner et al., 1999). A different study oriented on *T. radicans* interactions with host tree species used a combination of physical (pounding) and chemical (sulfuric acid) treatments to obtain adequate seedling germination frequencies to support studies into the effects of host tree allelochemical effects on *T. radicans* seedling germination and growth (Talley et al., 1996).

These limited *T. radicans* findings are consistent with more extensive seed germination studies of closely related *Rhus* species (Anacardiaceae). Untreated drupes from five *Rhus* species (*Rhus glabra*, *R. typhina*, *R. virens*, *R. aromatica*, and *R. trilobata*) show very low seedling germination rates during permissive germination conditions (Li et al., 1999c). However, drupes from all five species showed significantly increased germination (albeit to differing degrees) after sulfuric acid scarification. The five *Rhus* species differed in whether the embryo dormancy was enforced by physiological or physical mechanisms. Only *R. aromatica* showed physiological dormancy that was broken by gibberellic acid treatment (Li et al., 1999b). In contrast, treatments that disrupted the physical integrity of the endocarp were sufficient to induce seedling germination in the other four *Rhus* species, indicating physically enforced seed dormancy (Li et al., 1999c). In the case of *R. glabra*, physical seed dormancy is maintained by the water-impermeable endocarp tissue, in particular the outermost brachysclereid and internally proximal osteosclereid cell layers (Li et al., 1999a, 1999c). The water-impermeable brachysclereid and osteosclereid layers are sensitive to disruption by acid treatment, thereby allowing water to penetrate the underlying macrosclereid layer resulting in embryo imbibition and dormancy release.

Given our interest to investigate urushiol metabolism and physiology during sterile tissue culture conditions in the future, it is currently necessary to develop foundational methods to germinate and culture axenic *T. radicans* seedlings. To this end, the primary objective of the present study was to identify physical, chemical, and cultural treatments suitable for producing axenic *T. radicans* seedlings cultured on synthetic media.

Materials and Methods

Toxicodendron radicans drupe collection. Two lianas of *T. radicans* subsp. *radicans* were the source of drupes used in this study. Drupes from the RoaCo-1 liana, located in

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Catawba, VA, at GPS coordinates latitude 37°22'57.19" N, longitude 80°6'43.07" W, were harvested in August of 2012. Drupes from the Huckleberry-1 liana, located on the Huckleberry Trail in Blacksburg, VA, at GPS coordinates latitude 37°12'7.29" N, longitude 80°24'9.00" W, were harvested on 1 Sept. 2012. The panicles were separated from the lianas, leaves removed, placed in black 113.5-L plastic bags in an outdoor shed for 3 d, and then moved to an air-conditioned research facility to allow the panicles and drupes to fully dry.

Drupe scarification treatments. *Toxicodendron radicans* drupes were subjected to several types of scarification. Initially drupes were removed from the panicles and manually scarified between two blocks of 60 grit sandpaper for ≈ 1 min. In later experiments, drupes attached to panicles were mechanically scarified by placing them into a "3 lb rock tumbler" (Chicago Electric Power Tools, Chicago, IL) together with nine small rocks and 50 mL of all-purpose fine sand (Quickrete, Atlanta, GA) and continuously tumbled for a duration spanning four nights. The manual and mechanically scarified seeds were separated from detached exocarp, mesocarp, stem tissue, sand, and rocks using a No. 25, 710- μ m mesh screen (W.S. Tyler, Mentor, OH) and stored at room temperature in paper bags. Chemical scarification comprised treating no more than 100 seeds in 20 mL of 13 N sulfuric acid (SA) (Fisher Scientific, Waltham, MA) in a 50-mL BD Falcon tube (Becton Dickinson, Franklin Lakes, NJ) placed in a horizontal position on an orbital shaker at 100 rpm for 30 min at room temperature. During the SA treatment, the tubes were vortexed for 15 s at 10-min intervals during the 30-min acid treatment. The SA was removed and the drupes washed three times with 25 mL sterile distilled water per wash in a laminar flow sterile cabinet (Labconco, Kansas City, MO).

Initial chemical and temperature drupe treatments. Drupes were treated with 50% commercial liquid bleach, 3% sodium hypochlorite final, (Clorox Co., Oakland, CA), and washed with sterile water as described previously for the SA treatment. Bleach pre-treated seeds were incubated with 10 mL 1 mg·mL⁻¹ gibberellic acid G3 (Alfa Aesar, Ward Hill, MA) for 0.5 h and then washed with three rinses of 25 mL sterile distilled water. For the cold treatments, bleach-treated drupes were incubated at 4 °C for 2, 4, or 6 weeks before transfer to a growth chamber for seedling germination. The cold-treated seeds were assessed for seedling germination after 4 weeks in a long-day growth chamber.

Seedling germination conditions. All treated *T. radicans* drupes were placed on sterile 0.5 × Murashige and Skoog (MS) basal salts media (Murashige and Skoog, 1962) solidified with 0.3% w/v Phytigel (Sigma-Aldrich Co., St. Louis, MO) in plastic petri plates (either 50 × 100 mm or 150 × 100-mm petri plates depending on the experiment). Because the overarching objective of this study was to obtain sterile *T. radicans* seedlings, unless explicitly stated, treated drupes were also

subjected to a 30-min 50% bleach treatment before plating with the goal of drupe surface sterilization. The initial drupe treatment experiments used 50 treated drupes on 100 × 15-mm petri plates containing 0.5 × MS basal salts solid media. After initial chemical treatments, the seeds were stored at room temperature in the dark for 7 d and then transferred to a Percival CU-3614 growth chamber (Percival Scientific, Perry, IA) set for constant 28 °C and 16 h light. Seedling germination was scored 4 weeks post-drupe treatment. Germination was defined minimally as the emergence of the radical. In subsequent experiments comprised of sulfuric acid and bleach treatments, only 20 drupes were placed on larger 150 mm × 10-mm petri plates with solidified 0.5 × MS media. Drupe sterility was initially assessed on Day 4 posttreatment using a Leica Zoom 2000 illuminated stereo microscope (20× magnification) examining closed petri plates. Drupes lacking visible fungal hyphae and/or bacterial growth were scored as sterile drupes. Individual sterile drupes were transferred to 0.5 × MS basal salt media in either Magenta boxes or Phytatray II boxes (Sigma-Aldrich Co.) in a laminar flow sterile cabinet. At 10 d posttreatment drupes were scored for seedling germination. Drupes were imaged using a Zeiss Stemi, SV11 Apo (Zeiss, Thornwood, NY) dissecting microscope fitted with a Syncrosopy (Synoptics Inc., Frederick, MD) digital camera. Axenic individual seedlings either in Magenta boxes or Phytatrays continued to grow well for several weeks. The sulfuric acid-bleach serial treatment experiments were replicated three times, each on a different day. All other treatments were replicated four times with each replication initiated on a different day. The resulting data were analyzed using a General Linear Model analysis of variance (ANOVA) with Tukey correction, or *t* test using Minitab Version 14 (State College, PA) using $\alpha \leq 0.05$.

Results

Sulfuric acid promoted seedling germination. *T. radicans* drupes were subjected to four treatments often used to promote seedling germination. Drupes from two *T. radicans* lianas (Huckleberry-1 and RoaCo-1) were either unscarified or sandpaper-scarified and

then subsequently treated with sterile water, cold (2 to 6 weeks at 4 °C), 1 mg·mL⁻¹ gibberellic acid (GA), or SA treatments. One-way ANOVA results indicated that both replication and sandpaper scarification were not significant factors ($F = 0.45, P = 0.50$; and $F = 0.75, P = 0.53$, respectively), so these two parameters were removed from subsequent ANOVA analyses. Fig. 1 illustrates *T. radicans* seedling germination frequencies in response to these treatments. Relative to water-treated controls, in which 1% and 3% germination frequencies were observed for Huckleberry-1 and RoaCo-1 ecotypes, respectively, there were no significant increases in seedling germination frequencies as a result of either cold or GA treatments. In contrast, SA treatment resulted in significantly greater *T. radicans* seedling mean germination frequency (11% for Huckleberry-1 and 42% for RoaCo-1) relative to water-treated controls (ANOVA, Tukey correction $T = -3.349, P < 0.019$; and $T = -9.756, P < 0.0001$, respectively). When factored overall treatments, the RoaCo-1 drupes consistently showed greater seedling germination frequencies than Huckleberry-1 (ANOVA, Tukey correction, $T = 3.81, P = 0.0003$), indicating ecotype-specific differences.

Extensive microbial contamination. All drupe treatments resulted in substantial microbial contamination. The treated drupes typically showed extensive fungal hyphal growth and, to a much lesser degree, bacterial growth. The microbial contamination was particularly acute in the 4 °C-treated plates, which showed increasing amounts of fungal growth on plates with longer durations of cold treatment. Extending the duration of 50% bleach treatment did not result in any consistent improvement in fungal surface sterilization (data not shown). Nevertheless, bleach treatment was advantageous. Drupes treated only with sterile water showed extensive bacterial contamination with little evidence of fungal contamination, suggesting that bacterial growth suppressed fungal growth. Compared with water treatment alone, bleach treatment resulted in considerably less bacterial contamination such that fungal contamination then predominated the observed microbial contaminants. Interestingly, the

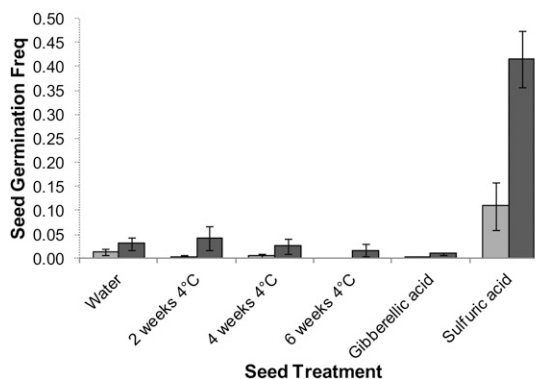


Fig. 1. Poison ivy seedling germination frequency in response to various treatments. Shaded columns indicate drupes from lianas of Huckleberry-1 (light gray) or RoaCo-1 (dark gray). Gibberellic acid (G3) concentration was 1 mg·mL⁻¹. Error bars indicate SE; n = 4 independent replications per mean.

harsh SA treatment was not effective at eliminating fungal contamination (data not shown). In summary, neither bleach nor SA treatment alone was effective in producing axenic drupes.

Optimal mechanical and chemical scarification treatments. Several aspects of the initial seedling germination studies might have contributed to the high levels of fungal contamination. The sandpaper scarification generally removed the brittle exocarp but left much of the mesocarp tissue intact. This was particularly the case for small drupes interspersed with larger drupes, the latter of which prevented the sandpaper from coming into direct contact with the smaller drupes. The failure to efficiently remove mesocarp tissue meant there was little abrasion of the endocarp tissues. In general, drupes with more intact mesocarp typically showed greater amounts of fungal contamination, suggesting endophytic fungi residing within the mesocarp. Another disadvantage of sandpaper scarification included the occupational exposure of researchers to fine particulates generated during the “sanding” of poison ivy drupes. Lastly, the drupe plating density on standard sized petri plates combined with the 4-week incubation period before scoring seedling germination might have obscured the presence of initially sterile drupes that were subsequently infected by adjacent fungal-contaminated drupes on the solid media. Therefore, several refinements in both drupe scarification and cultural methods were made to minimize the presence of mesocarp and minimize fungal cross-contamination between plated drupes. To obtain more efficient and even abrasion of exocarp and mesocarp tissues, we mechanically scarified *T. radicans* RoaCo-1 drupes attached to panicles by using a combination of small rocks and fine sand in a rock tumbler for ≈ 96 h. This mechanical scarification resulted in drupes that were separated from the panicles, more evenly scarified, and much less occupational exposure to airborne fine particulates. Interestingly, the sand acquired a novel gray color after mechanical drupe scarification that differed from both the initial sand color and the color of *T. radicans* drupes. Likewise, the mesocarp of mechanically scarified drupes displayed a gray color and had a more friable texture that was different from both non-scarified and sandpaper-scarified drupes.

The mechanically scarified *T. radicans* drupes were subjected to combinatorial bleach and SA treatments to identify optimal conditions for initiating seedling germination. Drupes first treated with sterile water and then subsequently with bleach [Fig. 2 (water, bleach)] showed a comparable low seedling mean germination frequency like in Fig. 1 (water), demonstrating that bleach treatment did not promote seedling germination. Again, SA significantly increased seedling germination (Fig. 2). The order of bleach and SA treatment had a small but significant effect on seedling germination frequency. Drupes sequentially treated with SA and then

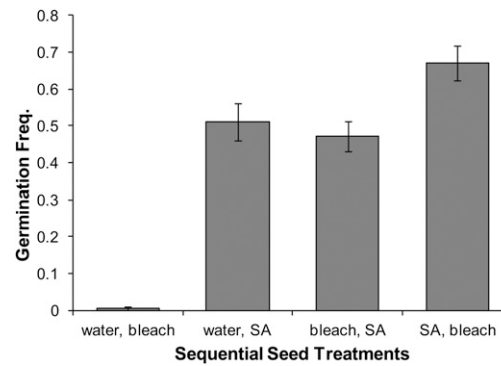


Fig. 2. Mean germination frequency of RoaCo-1 poison ivy drupes after pairwise sequential treatments involving combinations of water, concentrated sulphuric acid (SA), and bleach (3% sodium hypochlorite). Pairwise treatment order is listed from left to right. Error bars indicate SE; $n = 3$ independent replications per mean.

bleach (SA, bleach) germinated at a mean frequency of 0.67 compared with those treated with bleach and then SA, which showed a germination frequency of 0.47 (ANOVA, Tukey correction, $T = 3.51$, $P = 0.03$). In these sequential dual treatments, most (though not all) of the drupes displayed evidence of fungal and, to a much lesser degree, bacterial contamination. These results together with the aforementioned results demonstrated that concentrated SA alone, bleach alone, nor sequential SA and bleach treatments were adequate to efficiently surface-sterilize most drupes. However, it was also noted that after a few days, some drupes were apparently not contaminated with fungi or bacteria. These later observations led to additional cultural practices used to identify and isolate axenic *T. radicans* seedlings.

Axenic seedling isolation by cultural methods. To promote the identification and isolation of sterile *T. radicans* drupes, we either modified or introduced several cultural practices. Mechanically scarified RoaCo-1 *T. radicans* drupes were serially treated with SA and then bleach to promote efficient seedling germination. All treated drupes were plated at a lower density (20 drupes on larger 150×10 -mm $0.5 \times$ MS plate). Approximately one-fourth of the treated drupes were buoyant during the sterile water washes, and these were plated separately from the non-buoyant drupes. After four nights of incubation in the dark, each drupe was visually inspected using a dissecting microscope and scored as either sterile or contaminated. At this time, many drupes showed evidence of embryo imbibition, which was comprised of increased drupe size and often cracking of the endocarp. Apparent sterile drupes were removed from the large $0.5 \times$ MS petri plates and transferred to $0.5 \times$ MS media in Magenta boxes or Phytatrays and placed in a long-day growth chamber. The plates containing the contaminated drupes were also transferred to the same growth chamber. After 10 additional days of incubation, both the Magenta boxes and petri plates were scored for seedling germination. Fig. 3A illustrates that there was no significant difference in mean drupe sterility frequency between non-buoyant and

buoyant drupes (one-way ANOVA, $F = 0.26$, $P = 0.78$). However, Fig. 3B illustrates there was a dramatic difference in seedling mean germination frequencies between non-buoyant and buoyant drupes (ANOVA, Tukey correction $T = 8.928$, $P < 0.0001$). None of the buoyant drupes germinated nor did they show evidence of embryo imbibition. Thus, buoyant drupes most likely contained inviable embryos even before treatments were applied.

The non-buoyant drupes contained viable embryos and produced germinating seedlings. Fig. 3B shows that initially scored sterile (i.e., microbe-free) drupes resulted in a mean germination frequency of 0.80 ± 0.07 SE. The contaminated drupes showed a reduced mean germination frequency of 0.59 ± 0.05 SE. Although there were consistent differences in the mean germination frequency between sterile and contaminated drupes, the observed difference in means was not statistically significant (paired t test, $T = -2.68$, $P = 0.075$). The majority of initially scored sterile drupes resulted in axenic poison ivy seedlings that grew well in Magenta boxes or Phytatrays for several weeks, as evidenced by the production of many lateral roots, several true leaves, and the absence of visible microbial contamination. Although the majority of initially scored sterile drupes produced fully axenic seedlings, some resulted in fungal-contaminated seedlings that typically manifested as hyphae-growing within the $0.5 \times$ MS media associated with the roots. The frequency of initially scored sterile drupes that produced fungal-contaminated seedlings was relatively low at 0.15 ± 0.05 SE. We could not determine whether these fungal-contaminated seedlings were the result of initial inaccurate assessment of sterility or the result of secondary fungal contamination occurring during the transfer of a sterile drupe from the petri plate to a Magenta box or Phytatray. In summary, the serial process of mechanical scarification, SA then bleach chemical treatments followed by early visual scoring and separation of microbe-free drupes produced many axenic *T. radicans* seedlings that grew well in tissue culture conditions.

T. radicans embryo dormancy is maintained by enforcing embryo desiccation. Embryo

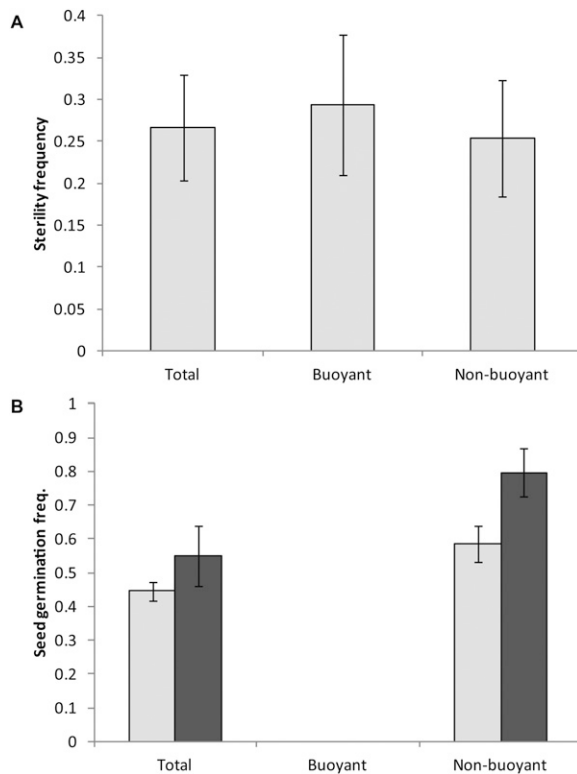


Fig. 3. Microbial contamination and seedling germination patterns of poison ivy drupes after rock/sand scarification, sulphuric acid (SA), and bleach treatments. (A) Frequency of sterile/microbe-free drupes. Total indicates combined buoyant and non-buoyant drupes. (B) Germination frequency of drupes of differing buoyancy and differing state of microbial contamination: contaminated drupes (light gray) and sterile drupes (dark gray). Error bars indicate SE; n = 4 independent replications per mean.

dormancy can be enforced by either physiological or physical mechanisms. Gibberellin treatment will frequently break physiologically enforced embryo dormancy. However, 1 mg·mL⁻¹ GA treatment did not significantly increase *T. radicans* mean seedling germination frequency (Fig. 1) relative to water treatment (ANOVA, Dunnett test, $T = -0.52$, $P = 0.98$), suggesting that *T. radicans* embryos were not under physiologically enforced embryo dormancy. Similarly, treatments that removed the exocarp (water or bleach, Fig. 4A–B) and partial mesocarp removal (sandpaper or mechanical scarification with or without bleach, Figs. 4C and 4D, respectively) did not significantly increase seedling germination (Figs. 1 and 2). In contrast, in addition to the removal of exocarp and most mesocarp tissue, SA treatments also resulted in pitting of the outer endocarp (Fig. 4E) that was in turn associated with significantly increased seedling germination frequencies (Figs. 1 and 2). Endocarp pitting specifically removed the impermeable exterior brachysclereid and adjacent osteosclereid cell layers (Fig. 4E), responsible for keeping water from passing through the endocarp. The thick and more porous internal macrosclereid cell layer remained mostly intact at the pitted endocarp regions (Fig. 4F). Over most of the endocarp, the macrosclereid cell layer was relatively thick. The notable exception was the micropylar region where the macrosclereid layer was much thinner and from which the germinating

seedling typically emerged. In conclusion, based on both the ineffectiveness of GA to promote seedling germination together with the effectiveness of SA treatment to erode the brachysclereid and osteosclereid cell layers, *T. radicans* drupes maintained embryo dormancy mostly by physical, not physiological, mechanisms.

Discussion

Poison ivy (*T. radicans*) is well known for its ability to cause the distinguishing delayed contact dermatitis symptoms. Although the chemical composition of *T. radicans* urushiols is documented, there are vast gaps in knowledge about the physiology and metabolism of urushiol in *T. radicans* and other closely related plant species. To date, all studies used *T. radicans* tissues harvested from unmanaged plants growing in natural environments, in which important genetic and environmental parameters were not controlled. Based on that experimental approach, it is difficult to establish whether observed differences in urushiol accumulation levels and/or composition were the result of any of a wide range of parameters including plant tissue type, age, genotype, environmental (e.g., light quality/quantity, moisture, and mineral nutrients) and biotic or abiotic stress. Thus, more exacting studies of *T. radicans* urushiol physiology will necessitate controlling as many of the aforementioned experimental

parameters as possible. Growing axenic *T. radicans* plants in sterile plant culture media would provide well-controlled conditions in which to characterize urushiol metabolism. Toward this end, the primary objective of this study was to establish a reliable protocol for efficient poison ivy seedling germination and cultivation in axenic conditions.

In the present report, sandpaper scarification did not increase *T. radicans* seedling germination frequency. This is in contrast to a previous report that showed significantly increased *T. radicans* germination rates resulting from sandpaper scarification (Penner et al., 1999). Efficient *T. radicans* seedling germination was achieved by combining mechanical scarification (rock tumbler, rocks, and sand) with sequential SA and bleach treatments. Mechanical scarification removed all of the brittle exocarp and much mesocarp tissue, including most of the apparent resin ducts. The prior removal of these outer drupe tissues made the SA-mediated removal of the remaining friable mesocarp more efficient, because SA-treated drupes without mechanical scarification would often still retain mesocarp tissue on the drupe. With nearly all/most mesocarp removed, the exposed endocarp was therefore more susceptible to SA-mediated dissolution of brachysclereid and osteosclereid cell layers (i.e., endocarp “pitting”). The stimulation of *T. radicans* embryo imbibition and seedling germination by SA treatment is similar to some Anacardiaceae members such as *Rhus glabra* in which embryo dormancy is enforced by water-impermeable brachysclereid and osteosclereid cell layers that maintain embryo desiccation and hence dormancy (Li et al., 1999a, 1999b, 1999c). Thus, *T. radicans* embryo dormancy is largely enforced by physical isolation of the embryo from environmental moisture.

The stimulation of *T. radicans* seedling germination by strong acid treatment may have ecological implications. Certain bird species eat poison ivy drupes (Martin et al., 1951; Senchina, 2008). It follows that *T. radicans* drupe dispersal and seedling germination may be adapted to passage through avian intestinal tracts, where the combination of mechanical grinding in the gizzard coupled with strong stomach acid may promote endocarp pitting, thus priming the drupe for environmental moisture to reach the embryo. A study examining the effects of *T. radicans* drupe herbivory by squirrels and Ruffed Grouse demonstrated that *T. radicans* drupes isolated from the feces of Ruffed Grouse showed seedling germination rates that were not significantly different from drupes that were not eaten by the birds (Penner et al., 1999). With that said, the mean germination frequency of control water-treated drupes in the present study ranged from 1% to 3% (Fig. 1), whereas the untreated drupe germination frequency in Penner et al. (1999) ranged from 12.5% to 16%, indicating the drupes were substantially more prone to germinate before being eaten by the Ruffed Grouse. Poison ivy shows a high degree of anatomical polymorphism in different geographical locations

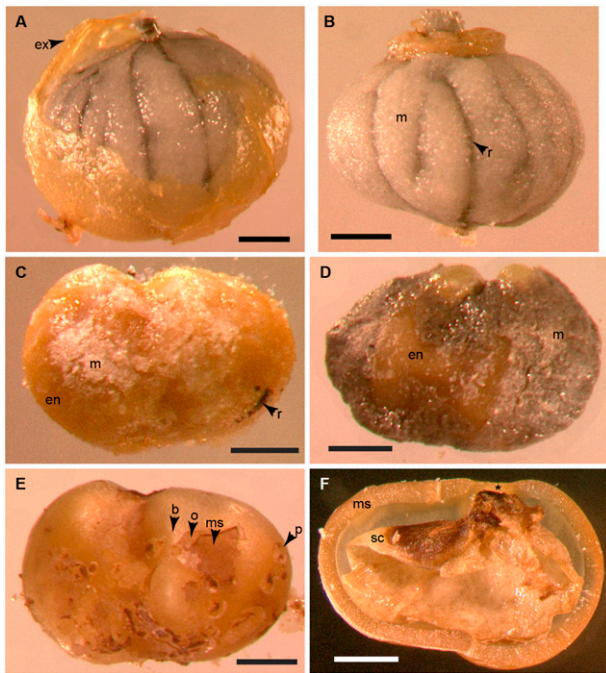


Fig. 4. Effects of treatments on subsequent poison ivy drupe anatomy. (A) Unscarified with water treatment; (B) unscarified with bleach treatment; (C) sandpaper scarified with bleach treatment; (D) sand/rock scarified with water treatment; (E) sand/rock scarified with sulphuric acid (SA) and then bleach treatment; and (F) medial section of endocarp released from germinated seedling in response to sand/rock scarification with SA and bleach treatment. Drupe features indicated by arrows and/or letters: ex = exocarp; m = mesocarp; r = resin duct; en = endocarp; b = brachysclereids; o = osteosclereids; ms = macrosclereid; p = pitting; sc = seedcoat; and asterisk = carpellary microphyllar region. Bars equal 1 mm.

(Gillis, 1971), so the degree to which differences in environmental and/or genetic factors contributed to the different patterns of *T. radicans* embryo dormancy in the present study and Penner et al. (1999) are currently unknown. On the other hand, *T. radicans* drupes eaten by squirrels were non-viable because squirrels chew through the endocarp, to remove and then eat the embryo, whereas birds swallow the drupes intact and do not separate the embryo from the endocarp. Based on the particulars of that study, birds are at least efficient vectors for *T. radicans* drupe dispersal.

Microbial contamination of *T. radicans* drupes was a chief impediment for attaining axenic *T. radicans* seedlings. *T. radicans* drupes treated only with sterile water showed extensive bacterial contamination as evidenced by copious slimy microbial growth. Although 50% bleach treatment was effective at greatly reducing bacterial growth, it was not adequate to efficiently sterilize fungal spores or hyphae. Likewise, combining 50% bleach with concentrated SA was also inefficient at surface sterilization of either sandpaper-scarified or mechanically scarified drupes. The recalcitrance of the drupe-associated fungi to 30 min of concentrated SA treatment is particularly notable, suggesting that the fungi might be present as mesocarp endophytes rather than mere surface saprophytes.

An appreciable portion of *T. radicans* drupes were effectively surface-sterilized, resulting in axenic seedlings. In the RoaCo-1 *T. radicans* ecotype, $\approx 25\%$ of the drupes were initially scored as sterile and needed to be physically removed from adjacent fungal-contaminated drupes before fungal hyphal growth spread to contaminate the sterile drupes. Thus, the visual identification of sterile drupes at low plating density combined with their timely removal were essential cultural practices for producing axenic *T. radicans* seedlings. Because *T. radicans* is a dioecious species (Gillis, 1971), harvesting drupes from a single female plant ensures that the drupes are all genetically related as half-sibs. Thus, the ability to culture axenic *T. radicans* half-sib seedlings provides unprecedented opportunities for well-controlled studies in urushiol metabolism and physiology. Given the demonstrated effects of increased atmospheric CO₂ levels resulting in increased poison ivy growth and the production of more allergenic urushiol congeners (Mohan et al., 2006; Ziska et al., 2007), detailed knowledge of urushiol metabolism and physiology is required to develop novel *T. radicans* control measures.

Literature Cited

Baer, H., M. Hooton, H. Fales, A. Wu, and F. Schaub. 1980. Catecholic and other constituents of the leaves of *Toxicodendron radicans*

- and variation of urushiol concentrations within one plant. *Phytochemistry* 19:799–802.
- Craig, J.C., C.W. Waller, S. Billets, and M.A. Elsohly. 1978. New GLC analysis of urushiol congeners in different plant parts of poison ivy, *Toxicodendron radicans*. *J. Pharmacol. Sci.* 67:483–485.
- Gillis, W.T. 1971. The systematics and ecology of poison-ivy and the poison-oaks (*Toxicodendron*, *Anacardiaceae*). *Rhodora* 73:72–159, 161–237, 370–443, 465–540.
- Johnson, R.A., H. Baer, C.H. Kirkpatrick, C.R. Dawson, and R.G. Khurana. 1972. Comparison of the contact allergenicity of the four pentadecylcatechols derived from poison ivy urushiol in human subjects. *J. Allergy Clin. Immunol.* 49:27–35.
- Kurtz, A.P. and C.R. Dawson. 1971. Synthesis of compounds structurally related to poison ivy urushiol. 3. 3-n-pentadecylcatechol and 3-n-alkylcatechols of varying side-chain length. *J. Med. Chem.* 14:729–732.
- Li, X., J.M. Baskin, and C.C. Baskin. 1999a. Anatomy of two mechanisms of breaking physical dormancy by experimental treatments in seeds of two North American *Rhus* species (*Anacardiaceae*). *Amer. J. Bot.* 86: 1505–1511.
- Li, X., J.M. Baskin, and C.C. Baskin. 1999b. Physiological dormancy and germination requirements of seeds of several North American *Rhus* species (*Anacardiaceae*). *Seed Sci. Res.* 9:237–245.
- Li, X., J.M. Baskin, and C.C. Baskin. 1999c. Seed morphology and physical dormancy of several North American *Rhus* species (*Anacardiaceae*). *Seed Sci. Res.* 9:247–258.
- Martin, A.C., H.S. Zim, and A.L. Nelson. 1951. *American wildlife & plants*. Dover Publications, Inc., New York, NY.
- Mohan, J.E., L.H. Ziska, W.H. Schlesinger, R.B. Thomas, R.C. Sicher, K. George, and J.S. Clark. 2006. Biomass and toxicity responses of poison ivy (*Toxicodendron radicans*) to elevated atmospheric CO₂. *Proc. Natl. Acad. Sci. USA* 103:9086–9089.
- Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiol. Plant.* 15:473–497.
- Penner, R., G.E.E. Moodie, and R.J. Staniforth. 1999. The dispersal of fruits and seeds of Poison-ivy, *Toxicodendron radicans*, by Ruffed Grouse, *Bonasa umbellus*, and squirrels, *Tamiasciurus hudsonicus* and *Sciurus carolinensis*. *Can. Field Nat.* 113:616–620.
- Senchina, D.S. 2008. Fungal and animal associates of *Toxicodendron* spp. (*Anacardiaceae*) in North America. *Perspect. Plant Ecol. Evol. Syst.* 10:197–216.
- Symes, W.F. and C.R. Dawson. 1953. Separation and structural determination of the olefinic components of poison ivy urushiol, cardanol and cardol. *Nature* 171:841–842.
- Symes, W.F. and C.R. Dawson. 1954. Poison ivy 'urushiol.' *J. Amer. Chem. Soc.* 76:2959–2963.
- Talley, S.M., R.O. Lawton, and W.N. Setzer. 1996. Host preferences of *Rhus radicans* (*Anacardiaceae*) in a southern deciduous hardwood forest. *Ecology* (Wash. D. C.) 77:1271–1276.
- Ziska, L.H., R.C. Sicher, K. George, and J.E. Mohan. 2007. Rising atmospheric carbon dioxide and potential impacts on the growth and toxicity of poison ivy (*Toxicodendron radicans*). *Weed Sci.* 55:288–292.