Poison ivy [Toxicodendron radicans (L.) Kuntz] is a native North American species best known for its capacity to induce allergic dermatitis symptoms after contact with human skin. The natural product responsible for the dreaded skin rashes and contact dermatitis symptoms after contact with poison ivy leaf tissue is urushiol, which is responsible for delayed hypersensitivity to poison ivy foliage with no apparent ill effects. There are few studies focused on poison ivy chemical ecology and physiology and/or ecology. Two studies are noteworthy as they establish that poison ivy is a plant species because of its production of urushiol, which is responsible for delayed contact dermatitis symptoms in humans. Poison ivy is predicted to become both more prevalent and more noxious in response to projected patterns of climate change. Future studies on poison ivy chemical ecology will require reverse genetics to investigate urushiol metabolism. A prerequisite for reverse genetic procedures is the introduction and expression of recombinant DNA into poison ivy tissues. Poison ivy leaves and cotyledons were marginally susceptible to vacuum- and syringe-agroinfiltration and expression of two firefly luciferase (LUC)-based reporter genes. The efficacy of agrobacterial infiltration and transient LUC expression was dependent on leaf age and plant growth environmental conditions, with young leaves grown in magenta boxes showing highest transient LUC expression levels. Agroinfiltrated leaves showed an Agrobacterium-dependent accumulation of brown–colored pigments. Biostatic transformation of a LUC reporter gene did not show brown pigment accumulation and readily displayed transient LUC bioluminescence in both leaves and cotyledon tissues. These studies establish best practices for introducing and transiently expressing recombinant DNA into poison ivy leaf and cotyledon tissues, on which future reverse genetic procedures can be developed.

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microparticles to high velocity (using a so-called gene gun) resulting in entry into the plant cell cytoplasm, where the nucleic acid subsequently uncoats from the microparticle, and is either transiently expressed or stably integrates into the plant nuclear genome (Klein et al., 1987). Alternatively, if combined with appropriate plastid DNA sequences the transgene can integrate into, and be expressed from, the plastid genome (Daniell et al., 1990). Biolistic transformation is the method of choice for stable transformation of major cereal crops (Klein et al., 1988; Wang et al., 1988) that are otherwise recalcitrant to Agrobacterium-mediated stable transformation.

Development of reverse genetic methods in poison ivy will enable molecular genetic investigations of poison ivy ecophysiology and chemical ecology, and provide foundational molecular genetic approaches in other members of the Toxicodendron genus (e.g., poison oak and poison sumac). As an initial step toward the implementation of such molecular genetic studies, the present report describes the introduction and transient expression of recombinant firefly LUC reporter gene constructs in poison ivy cells and tissues through agroinfiltration and biolistic transformation.

Materials and Methods

Plant material. Poison ivy (T. radicans subsp. radicans) drupes were sourced from the RocaCo-1 liana located in Catawba VA (Benhase and Jeleksn, 2013). Drupes were mechanically and chemically scarified (Benhase and Jeleksn, 2013). Axenic seedlings were germinated on petri plates of 0.5X Murashige & Skoog (MS) basal salts media (Plant Natural, Bozeman, MT), in the dark for four nights. Germinating seedlings were then transferred to either sterile 0.5X MS media in magenta boxes or pots containing nonsterile Sunshine Mix 1 (Plant Natural). The plants were grown at 28°C under 16 h light/day cycle. Poison ivy leaves were typically agroinfiltrated with a syringe at the three to four true leaf stage. Nicotiana benthamiana plants were directly germinated and grown in pots containing Sunshine Mix, under the same environmental conditions as the poison ivy seedlings. Nicotiana benthamiana plants were syringe agroinfiltrated 4 weeks postgermination.

Agroinfiltration transformation. Three LUC-containing plasmids were used in this study. Plasmid pJGJ204 contains an Arabidopsis RBCS1B–LUC gene fusion comprised of the RBCS1B promoter-exon 3, resulting in an in-frame RBCS1B–LUC chimeric fusion protein (Jeleksn et al., 1999). The firefly LUC was polymerase chain reaction (PCR) amplified from pJGJ102 (Jeleksn et al., 1999) using oligonucleotide primers (ATG)TGAAAGCAGCTTAACATACATA-3’ and ATGAAACCTGCAGCAGCGCTTCTTCTCTCTCTTTGT-3’ that incorporated 5’-attBl and 3’-attB2 sequences. The resulting LUC PCR fragment was BP subcloned into pDONR221 using BP Clonase II (ThermoFisher, Waltham, MA) to yield plasmid pJGJ404. The LUC gene from pJGJ404 was subcloned into mPCMD2 (Curtis and Grossniklaus, 2003) using LR Clonase II (Invitrogen, Carlsbad, CA) to yield plasmid pJGJ410 expressing the LUC gene from a double CaMV35S promoter. Plasmid pJGJ411 containing a LUC–INT gene was similarly subcloned, except using pLUK07 (Mankin et al., 1997) as the template in the initial PCR reaction. Plasmid pJGJ204, pJGJ410, or pJGJ411 were grown overnight (16 h) in 5 ml Luria-Bertani medium supplemented with 50 µg/ml kanamycin and 50 µg/ml gentamycin. The culture optical density (OD) at 600 nm (OD600) was estimated from a 1:10 culture dilution before initial centrifugation (15 min at 4°C, 3000 x g). Cultures were resuspended in MMA buffer [10 µM 2-N-Morpholino ethanesulfonic acid (MES), 10 mM MgCl2, and 20 units acetylseringone] by vortexing for either final OD600 of 4.0 or 0.4. The Agrobacterium strains containing a LUC–containing plasmid (pJGJ204, pJGJ410, or pJGJ411) were mixed with GV3101 harboring plasmid pJGJ411 containing a double CaMV promoter driving the expression of firefly LUC gene containing an artificial intron (LUC–INT). The intron in the LUC–INT gene abolished low expression levels in Agrobacterium cells relative to the continuous LUC open reading frame in pJGJ410 (Supplemental Fig. 1), and thus all LUC activity was derived from transient LUC expression in poison ivy cells. Poison ivy leaves from potting soil–grown plants subjected to syringe-agroinfiltration generally did not display LUC activity greater than background heat photon emission levels in qualitative pseudocolor superimposed slice photon emission images (Fig. 1A). On very few occasions weak photon emission more than background levels of syringe-agroinfiltrated leaves was observed. In contrast, syringe-Aagroinfiltrated leaves on poison ivy plants grown axenically in magenta boxes displayed small patches of consistent photon emission over regions that were syringe-agroinfiltrated (Fig. 1A). Thus, magenta box–grown poison ivy leaves were more susceptible to syringe-agroinfiltration–mediated transient DNA transformation. However, not all leaves from Magenta box grown poison ivy plants were equally susceptible to syringe-agroinfiltration. Older leaves of magenta box–grown plants were less susceptible to syringe-agroinfiltration transient LUC–INT expression compared with younger leaves (Fig. 1B). Younger leaves more readily took up the Agrobacterium-infiltration solution delivered from the blunt syringe. Consequently, young poison ivy leaves in magenta box–grown plants consistently showed higher transient LUC expression levels than older leaves from the same plants, whereas changing the bacterial concentration 10-fold had little effect on transient LUC expression levels (Supplemental Fig. 4). With that said, even the best poison ivy transient LUC expression levels were dramatically lower than N. benthamiana leaves syringe-agroinfiltrated with pJGJ411 (Fig. 1C). The poison ivy...
Transient AtRBCS1B–LUC chimeric gene fusion (pJGJ204 comprised the AtRBCS1B promoter, exons I, intron I, exon II, intron II, and partial exon III fused in-frame to the firefly LUC open reading frame), was used to vacuum-agroinfiltrate excised poison ivy leaves and cotyledons from plants grown in magenta boxes. Vacuum-agroinfiltrated leaves were placed on 0.5 × MS media plates, sprayed with luciferin at 24 h postinfiltration, and then imaged for three sequential 1 h-imaging sessions beginning at ≈48 h post agroinfiltration. The AtRBCS1B–LUC chimeric gene produces a low rate of photon emission in transgenic Arabidopsis plants. Superimposed “slice” images with complete subtraction of background photons displayed a few blue spots over leaves/cotyledons (Supplemental Fig. 2A), whereas superimposed “slice” imaging that retained some background photons displayed higher qualitative photon accumulation over leaves vacuum-agroinfiltrated with the plasmid containing the AtRBCS1B–LUC chimeric gene, relative to vacuum-agroinfiltrations with the vector control plasmid pSLK7292 (Supplemental Fig. 2B) indicating low but demonstrable induction of an apparent biotic stress response (i.e., accumulation of brown pigmentation) in vacuum-agroinfiltrated leaves and cotyledons. These results demonstrate that a heterologous Arabidopsis genomic RBCS1B promoter and intron containing RBCS1B–LUC gene fusion was expressed in poison ivy leaves and cotyledons.

**Discussion**

These studies lay the foundation for future transgenic reverse genetic approaches to investigate various poison ivy physiologies by demonstrating that exogenous DNA can be effectively introduced and expressed in poison ivy cotyledon and leaf tissues. Because the CaMV35S promoter is weakly active in *A. tumefaciens* (Supplemental Fig. 1; Mankin et al., 1997) it was essential to use intron-containing firefly LUC gene constructs (CaMV35S-LUC-INT and AtRBCS1B–LUC) to validate poison ivy-specific transient LUC activity. This was particularly germane in the case of the AtRBCS1B–LUC construct used in the vacuum-agroinfiltrated leaves and cotyledons because this reporter gene construct is expressed at relatively low levels in plant cells (Jelesko et al., 1999, 2004). The two introns in the AtRBCS1B–LUC gene ensured that all

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**Fig. 1. Imaging of agroinfiltrated leaves.** Panels A–C single photon pseudocolor images (panel A inset is pseudocolor step gradient with high photon emission shown as red to low photon emission as dark blue) superimposed on reflected light image (grayscale). Panel D, digital color image of poison ivy leaves infiltrated with either MMA buffer or MMA buffer with Agrobacterium containing the firefly LUC construct. Arrows indicate browning at site of agroinfiltration. (A and B) Composite pseudocolor single photon imaging on reflected light image of poison ivy leaves agroinfiltrated with firefly LUC construct. (C) *Nicotiana benthamiana* leaf similarly agroinfiltrated and imaged.
The age of poison ivy leaves was an important factor for the relative susceptibility of syringe agroinfiltration transient transformation. Leaves from potting soil–grown poison ivy plants were mostly recalcitrant to syringe-agroinfiltration transient transformation. On the other hand, young poison ivy leaves from plants grown in magenta boxes showed consistent syringe-agroinfiltration transient LUC–INT expression. Moreover, overall plant age was not the critical determinant, but rather the relative age of target leaves. As shown in Fig. 1B, the fifth emerged leaf (young leaf) showed greater syringe-agroinfiltration transient LUC expression levels than that of the second emerged leaf (older leaf) on the same plant. The qualitative levels of LUC–INT expression levels in poison ivy leaves were dramatically lower than the levels observed in *N. benthamiana* leaves syringe-agroinfiltrated with the same reporter gene construct. Poison ivy cotyledons and leaves were also susceptible to vacuum-agroinfiltration transient transformation using a chimeric *AtRBCS1B–LUC* transgene. Leaves infiltrated with *A. tumefaciens* strain GV3101 displayed discoloration consistent with an inducible plant biotic stress response. Both syringe- and vacuum-agroinfiltrated leaves demonstrated the accumulation of uncharacterized brown pigments (Fig. 1D; Supplemental Fig. 2C). This pigment accumulation was not observed for leaves infiltrated with MMA buffer lacking *Agrobacteria* (Fig. 1D). The relative *LUC* expression levels in the brightest poison ivy leaves was dramatically less that that observed in control *N. benthamiana* leaves syringe-agroinfiltrated with the same construct. This difference could have been due to a variety of parameters including the quantity of Agrobacteria penetrating into the apoplastic space of leaves, and/or a biotic stress response that inhibited bacterial vigor or T-DNA transfer into the plant cells. Nevertheless, poison ivy leaves and cotyledons showed significant transient *LUC–INT* and *AtRBCS1B–LUC* expression levels over controls indicating the feasibility of using agroinfiltration transient poison ivy transformation.

As expected, poison ivy cotyledons and leaves were readily transiently transformed using a biologic method. Biolistically transformed poison ivy leaves and cotyledons did not produce brown pigmented regions and thus averted a poison ivy biotic stress response against bacterial pathogen associated molecular patterns. As is typical of biologic transformation of plant tissues, the transient LUC–INT expression levels were highly variable among both leaf and cotyledon tissues. This is in large part to previously recognized inconsistencies in both plasmid DNA coating of gold micro particles (Sanford et al., 1993) and heterogenous particle spread on the macro carrier disk, resulting in heterogeneous distribution during particle acceleration toward the target tissue.

These results demonstrate the feasibility of poison ivy leaf and cotyledon transient transformation with recombinant reporter gene constructs using either agroinfiltration or biologic methods. Both poison ivy DNA transformation methods are important technical advancements enabling the introduction of recombinant DNA constructs designed for a variety of reverse genetic (e.g., RNA interference or viral induced gene silencing) and genome-editing methods (e.g., zinc finger, TALEN, or CRISPR-CAS9) to enable molecular genetic investigations of poison ivy ecophysiology and metabolism.

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Supplemental Fig. 1. Superimposed photon emission images of *Agrobacterium tumefaciens* strains GV3103/pJGJ411 (*LUC–INT* reporter gene, wells C5–6) and GV3101/pJGJ410 (*LUC* reporter gene, wells E5–6) duplicate cultures placed in a 96-well microtiter plate. Pseudo- color step gradient is the same as Fig. 1A.

Supplemental Fig. 2. Poison ivy cotyledons and leaves vacuum-infiltrated with *Agrobacterium tumefaciens* harboring either empty vector (pSLK7292) or *ArBCS1B–LUC* (pJGJ204). (A) Composite pseudocolor photon image with background heat photon levels subtracted. Pseudocolor step gradient is the same as Fig. 1A. (B) Composite pseudocolor photon image with background heat photon levels displayed. (C) Digital color image displaying mostly water-soaked infiltrated tissues and noninfiltrated tissues (indicated by arrows pointing toward lighter green noninfiltrated tissues).
Supplemental Fig. 3. Superimposed photon emission of cotyledons (A and B) and true leaves (C and D) on reflected light image. Pseudocolor step gradient is the same as Fig. 1A.

Supplemental Fig. 4. Box plots of photon emission levels from poison ivy leaves of different age and Agrobacterium inoculum concentrations. Leaves were from magenta box–grown poison ivy plants. Three leaves were independently imaged for each treatment combination. The line bisecting each box is the median value. Agrobacterium inoculum concentration was either 0.2 OD or 2.0 OD at 600 nm.