Overview of the U.S. Department of Agriculture, Forest Service's Disease Resistance Screening Center

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Abstract

The Resistance Screening Center (RSC) in Asheville, NC serves the tree improvement community by receiving progeny seed from breeding programs, exposing seedlings to disease, and evaluating resistance phenotype responses. These data provide early results that may be used to infer performance of field-grown trees in disease-prone areas and can also be used to calculate heritability estimates of resistance traits for future breeding efforts. The RSC is administered within the Forest Health Protection unit of the U.S. Department of Agriculture, Forest Service's State, Private, and Tribal Forestry division, serving any organization engaged in tree seed production, tree improvement, disease resistance, species conservation, restoration, or stewardship activities.

Inception of the Resistance Screening Center

The Resistance Screening Center (RSC) in Asheville, NC was established in response to fusiform rust caused by the native fungus *Cronartium quercuum* (Berk.) Miyabe ex. Shirai f. sp. *fusiforme* on slash pine (*Pinus elliottii* Engelm.) and loblolly pine (*P. taeda* L.). Infection by this fungus can result in swollen, spindle-shaped stem and branch galls that gravely impact pine timber quality and growth. With few options for chemical or cultural controls in agricultural forest settings and a high degree of genetic resistance within pine populations, breeding for host resistance is the primary method for growing disease-free plantations of slash and loblolly pine in the Southeastern United States (Barber 1964, Kinloch and Stonecipher 1969). Controlled breeding and traditional field selection of progeny take years to accomplish and may be confounded by variable levels of disease pressure from year to year or site to site; geographic variation in pathogen virulence; changes in host physiology due to age; environmental inputs like weather or physical site characteristics; or even unexpected damage to experimental plantings that could upend years of study (Cowling and Young 2013). By performing artificial inoculations on progeny seedlings in a controlled environment, some inconsistencies can be reduced or eliminated, and data can be produced in a fraction of the time required for field evaluations.

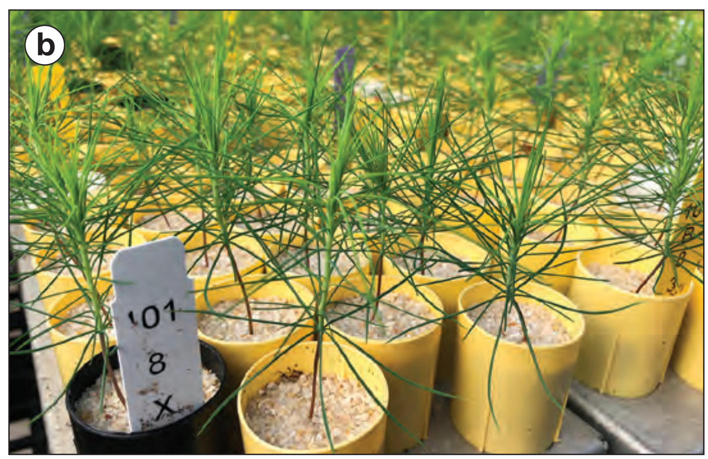
Efforts in the late 1960s by industry and U.S. Department of Agriculture (USDA), Forest Service researchers to evaluate rust resistance in field-grown pines was aided by the simultaneous development of artificial inoculation techniques and specialized equipment that would help standardize disease loads and rating techniques. With cooperation from North Carolina State University research geneticists, the idea of developing a centralized inoculation facility to perform inoculations and phenotype seedling progeny was generated (Cowling and Young 2013). Creation and administration of the RSC facility was tasked to the USDA Forest Service as a public institution that could offer continuation of long-term resources and is removed from the constraints of commercial enterprise. Because the role of the RSC is to aid in the development of management tools that can be administered on the landscape to mitigate disease, it was placed within the Forest Health Protection unit (formerly Forest Pest Management) of the State, Private, and Tribal Forestry Deputy Area, where applied sciences and service to stakeholders remain the primary foci. Operational screening of pines for rust resistance began in 1973 and today remains an integral part of the RSC's program of work. Successes with screening for fusiform rust resistance led to the development of screening programs for other tree diseases at the RSC, including pitch canker (*Fusarium circinatum* Nirenberg & O'Donnell) and brown spot needle blight (*Lecanosticta acicola* [von Thümen] Sydow) on southern pines; chestnut blight (*Cryphonectria parasitica* [Murrill] M.E.Barr) and root rot (*Phytophthora cinnamomi* Rands) on chestnut (*Castanea* spp.); dogwood anthracnose (*Discula destructiva* (Fr.) Munk ex H. Kern) on dogwood (*Cornus* spp.); and butternut canker (*Sirococcus clavigignenti-juglandacearum* [Nair, Kostichka, & Kuntz] Broders & Boland) on butternut (*Juglans cinerea* L.).

Current Programs and Organization

The RSC is a fee-for-service facility that serves the tree improvement community. Partners of the RSC include forest tree cooperatives, private industry, university researchers, nonprofit restoration groups, State agricultural divisions, and Federal entities. Services range from standard, high-throughput phenotyping using established protocols to innovative experiments designed to answer research questions. Seedlings screened at the RSC are often products of controlled breeding efforts that yield progeny on a spectrum from very resistant to very susceptible. The data produced from screening provide information about the resistance phenotype of submitted families relative to control "check" families that have known disease frequencies; check families serve to provide reference points for interpreting data as well as ensure quality of artificial inoculations.

RSC operations require horticultural and pathology expertise by staff members, who include a plant pathologist director, natural resources specialist, and biological science technician. RSC staff stratify and germinate seed, mix custom potting media, transplant germinants to containers (Ray Leach "super cell" SC10U, 10 in^3 [164 cm^3]; Stuewe & Sons, Inc., Tangent, OR), water and fertilize seedlings, and perform pest-control measures in a greenhouse setting (figure 1). RSC staff also culture fungal pathogens in a laboratory and prepare them in a form that can be applied uniformly to test seedlings.

Artificial inoculations require custom equipment to accommodate large numbers of seedlings and ensure uniform disease development. For fusiform rust,



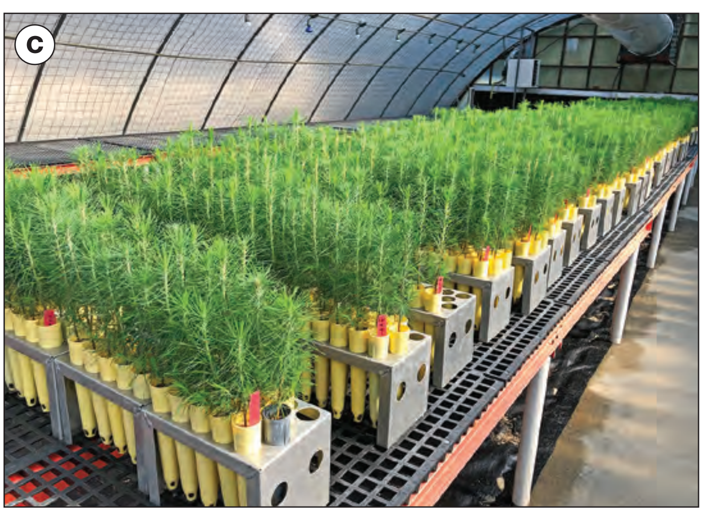


Figure 1. Disease resistance screening includes horticultural techniques such as (a) stratification and germination of seed, (b) transplanting germinants to custom soilless media in containers, and (c) seedling maintenance, including watering, fertilizing, and performing pest control measures in a greenhouse setting. (Photos by Katie McKeever, 2022)

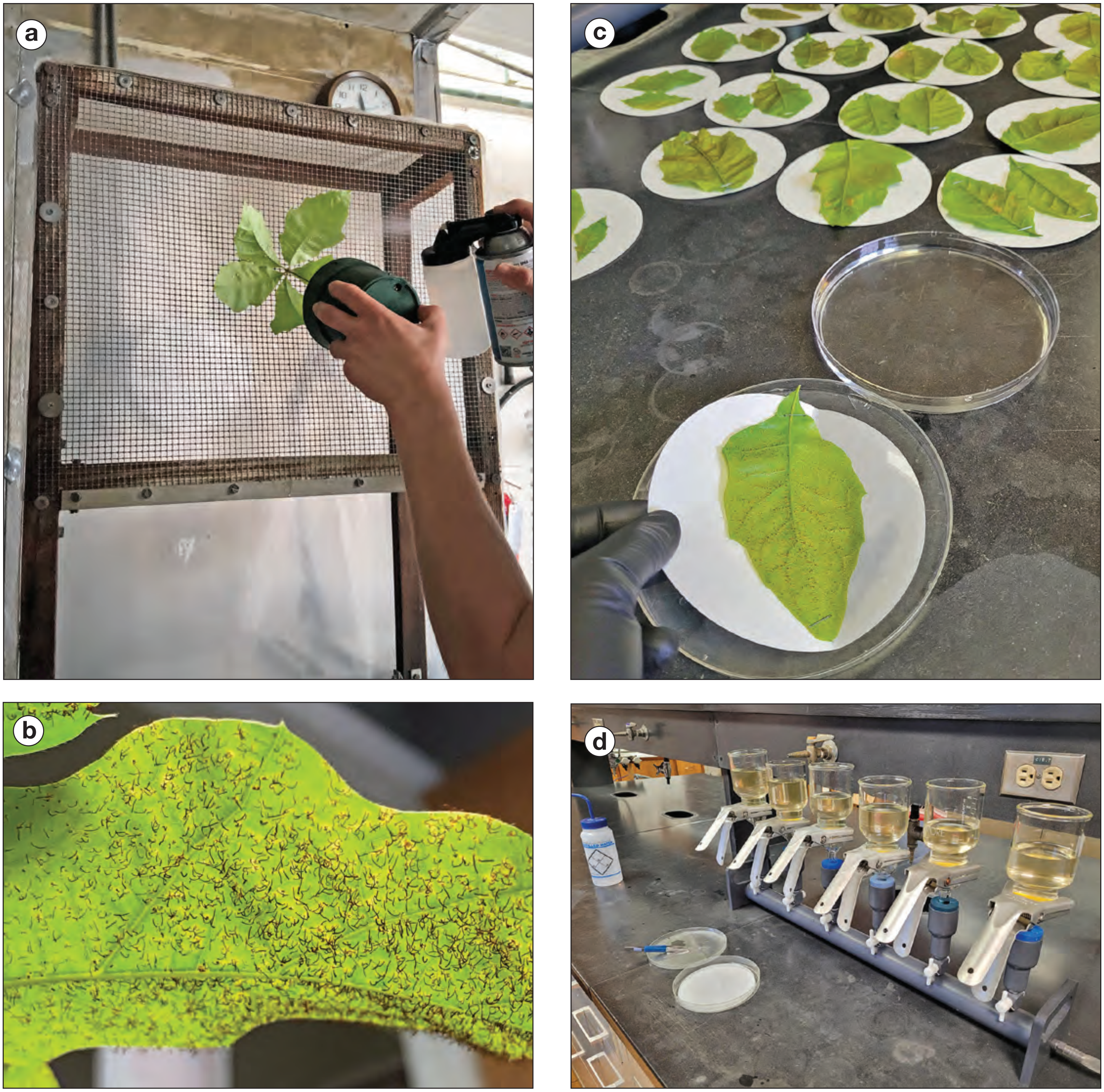


Figure 2. Fusiform rust requires two hosts to complete its lifecycle. At the RSC, oak seedlings are grown from acorns into approximately 3-week-old seedlings. (a) Spores of the fungus that have been harvested from wild pines are then applied in a suspension to the undersides of the young oak seedlings. (b) The spore type that infects pine are produced on oak leaves approximately 21 days after inoculation. (c) Infected oak leaves are suspended over acidified water to harvest spores that fall from leaves, and (d) the spore-containing acidified water is filtered through Millipore® (Millipore-Sigma, Burlington, MA) filters to obtain infectious spores that will be used for pine inoculations. (Photos by Katie McKeever and Erica Smith, USDA Forest Service, 2022)

inoculum preparation also requires rearing of oaks (*Quercus* spp.), an intermediate host of the causal fungus, and harvesting of spores derived from oak leaves (figure 2). Methods for pathogen delivery on pines include application of aqueous spore suspensions using compressed air mists or direct pipetting of droplets onto tissue. An automated, concentrated basidiospore spray system is used to mist rust spores onto pine foliage as seedlings move at a constant rate of speed on a conveyer belt (figure 3) (Cowling and Young 2013). After inoculation, pine seedlings are incubated in a dark room that has been modified to maintain

Figure 3. (a) An automated, concentrated basidiospore spray system was developed to mist rust spores onto pine seedling foliage. (b) Specialized nozzles mounted on articulating arms deliver a calibrated volume of aerosolized spore spray as trees move at a constant rate of speed on a conveyer belt. (Photo by Katie McKeever, 2021)

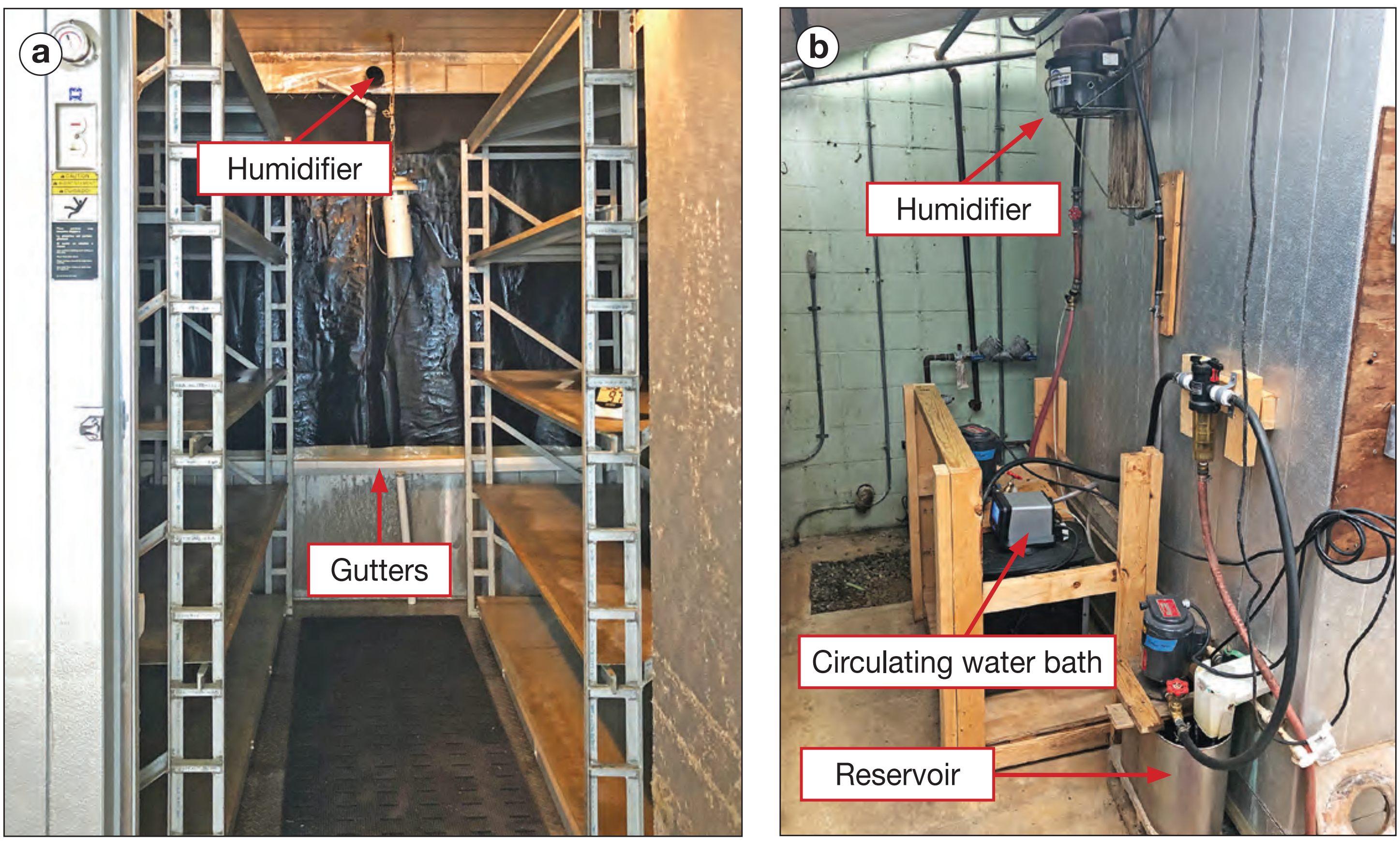


Figure 4. (a) Rust-inoculated pine seedlings are incubated for 24 hours on shelves in a dark room that is maintained at 98 percent relative humidity and 20 °C (68 °F). These conditions are favorable to spore germination and infection. (b) Distilled water is tempered in a circulating water bath to 20 °C (68 °F). This water is pumped to a humidifier and dripline that are positioned inside of the chamber. Black curtains are soaked by the dripline inside of the chamber to keep the environment moist. Gutters inside the chamber catch the dripline water and return it to the reservoir where it is pumped back into the circulating water bath in a closed loop system. (Photo by Katie McKeever, 2022)

Figure 5. (a) The RSC phytophthora root rot screening program uses a subirrigation and water-containment system. Benchtop hydroponics tubs are filled to irrigate chestnut seedlings via capillary action. (b) Tubs are connected to water collection tanks through a system of lines that allow irrigation water to be captured and disinfested prior to release. (Photo (a) by Katie McKeever, 2022 and (b) Sunny Lucas, USDA Forest Service, 2017)

high relative humidity and temperatures favorable to spore germination and infection (figure 4). This brief incubation is a requisite step for achieving disease development before seedlings are moved into the greenhouse for prolonged maintenance and observation.

The RSC partnered with The American Chestnut Foundation to screen blight-resistant hybrid chestnut seedlings for resistance to Phytophthora root rot (PRR). This partnership led to the construction of a specialized subirrigation system that serves to favor conditions for PRR development while also complying with a phytosanitary permit that dictates containment of this nonnative pathogen. In 2016, one of the RSC hoophouses was remodeled to include benchtop hydroponic tubs connected to water collection tanks (figure 5). Trays of seedlings inside the tubs are inoculated with *Phytophthora*-colonized vermiculite incorporated directly into the potting medium of individual seedlings. Seedlings are watered thrice weekly by filling the tubs and moistening the potting medium via capillary action. This technique mimics the flooding events typical in agricultural settings that stress host roots and favor sporulation and dispersal of the pathogen. Draining of the subirrigation water is managed through a series of pumps and cutoff valves to the collection tanks where the water can be disinfected with chlorine bleach prior to release into the environment. These innovative methods of inoculation and pathogen management are hallmarks of the RSC's specialization.

Figure 6. Fusiform rust phenotype data on southern pines are collected by recording the frequency of gall incidence within each family. (Photo by Josh Bronson, USDA Forest Service, 2009)

Figure 7. Pitch canker phenotype data on southern pines are collected by measuring the length in millimeters of necrotic purple lesions that extend down the stem from the inoculation site. (Photo by Katie McKeever, 2021)

Rust data are collected by recording the frequency of gall incidence within each family (figure 6). Pitch canker assessments have been adapted to include numerical measurements of necrotic lesions to augment binary lesion presence/absence data (figure 7). Chestnuts are evaluated by recording mortality over time and by a visual assessment of root rot severity on surviving seedlings at the conclusion of the trial (figure 8). Screening data are analyzed to provide rankings of family means relative to the known check families allowing inference of relative resistance among progeny populations. Field validation of RSC indices have demonstrated high correlation between artificial inoculation trials and field results, confirming utility of the seedling screening method for tree improvement efforts (Miller and Powers 1983).

Accomplishments and Future Directions

The RSC has contributed vastly to the understanding and exploitation of host resistance in southern

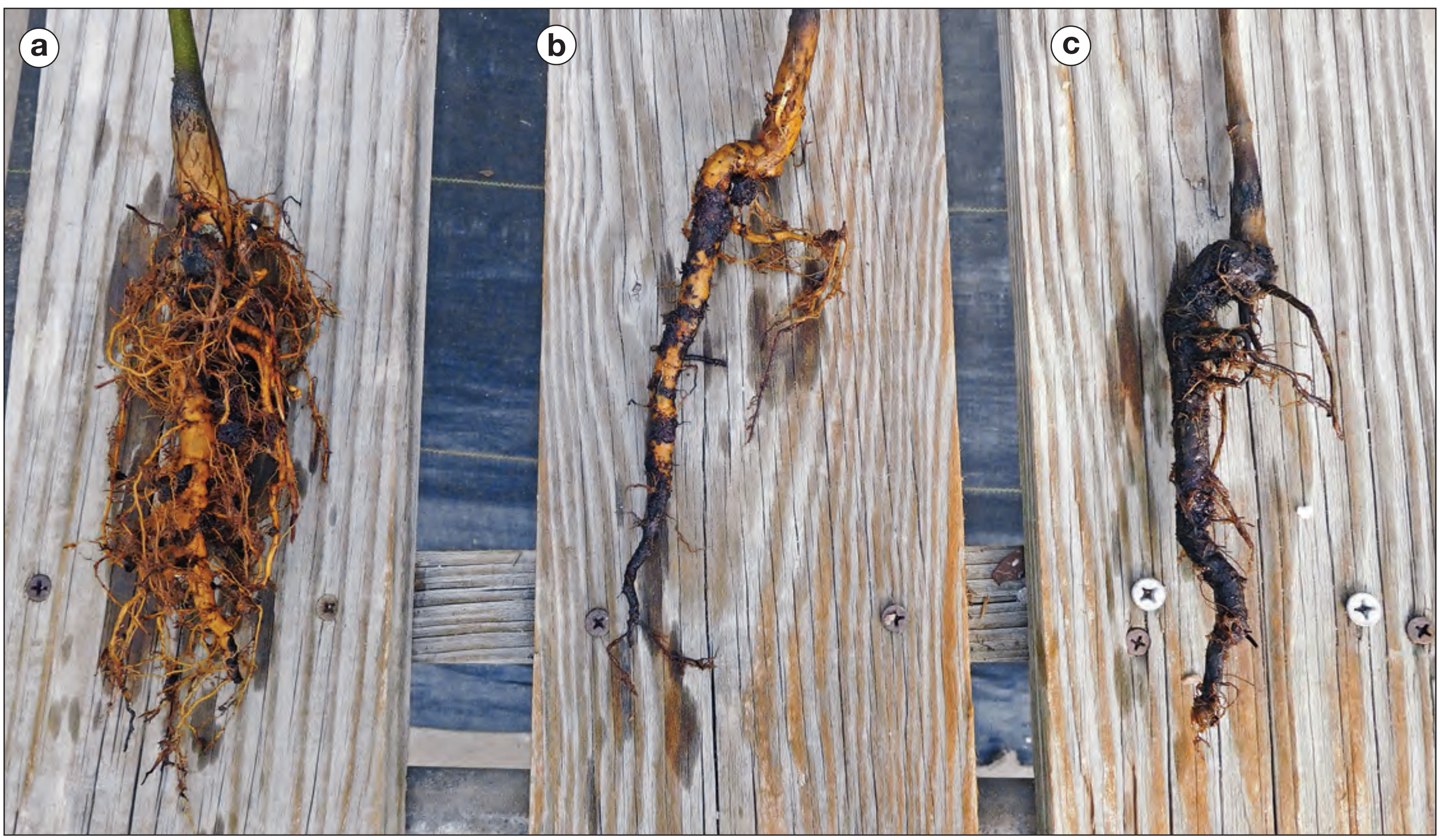


Figure 8. Phytophthora root rot on chestnut seedlings is evaluated by visually assessing root rot severity on a 0 to 3 scale at the conclusion of each trial. Seedlings with no root rot are scored as 0. The photos above show a rating of (a) 1 (disease is limited largely to feeder roots with little impact to the taproot), (b) 2 (rot evident on the tap root), and (c) 3 (root system nearly entirely rotted but seedling is still alive). (Photos by Steve Jeffers, Clemson University, 2020)

forests to native diseases. Information derived from screening has been used to structure seed orchards, select breeding parents, aid in clone deployment decisions, quantify heritability of disease resistance, evaluate fungicides for protection of nursery stock, and define molecular control of resistance traits (Cowling and Young 2013). Numerous graduate dissertations have been augmented or enabled through work at the RSC (Cowling and Young 2013). The development of the commercially available anthracnose-resistant "Appalachian Spring" dogwood cultivar (*Cornus florida* L. 'Appalachian Spring') was facilitated through cooperation with the RSC and validated through screening at the facility (Cowling and Young 2013). Selection for PRR-resistance in blight-resistant hybrid chestnut seedlings is aiding in the effort to breed populations with dual resistance to these two damaging diseases, potentially paving the way for restoration of this functionally extinct tree. New projects on the horizon include partnership with the USDA Forest Service Northern Research Station to propagate and screen American elm (*Ulmus americana* L.) seedlings for resistance to Dutch elm disease (*Ophiostoma novo-ulmi* [Fr.] Syd. & P.Syd.) and an effort to develop screening for brown spot needle blight resistance in loblolly pine.

The value of the RSC is substantial, and the impacts are diverse. Emphasis on genetic improvement to combat threats from native and nonnative invasive pests, as well as shifts in host-pathogen dynamics in a changing climate, is a cost-effective and environmentally sound strategy for sustaining forest ecosystem services. Renewed interest in plant breeding and phenotype selection as a vital element of integrated pest management underscores the indispensability of the RSC as a partner in tree improvement efforts for disease resistance in the Southeast United States.

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