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Contributed Paper

Using Citizen Science Programs to Identify Host Resistance in Pest-Invaded Forests

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Abstract: *Threats to native forests from non-native insects and pathogens (pests) are generally addressed with methods such as quarantine, eradication, biological control, and development of resistant stock through hybridization and breeding. In conjunction with such efforts, it may be useful to have citizen scientists locate rare surviving trees that may be naturally pest resistant or tolerant. The degree of resistance of individual trees identified in this way can be tested under controlled conditions, and the most resistant individuals can be integrated into plant breeding programs aimed at developing pest-resistant native stock. Involving citizen scientists in programs aimed at identifying rare trees that survive colonization by pests provides a low-cost means of maximizing search efforts across wide geographic regions and may provide an effective supplement to existing management approaches.*

Keywords: citizen science, forest management, hemlock woolly adelgid, invasive species, plant breeding, policy, resistance, tolerance

Utilización de Programas Científicos Ciudadanos para Identificar la Resistencia de Hospederos en Bosques Invadidos por Plagas

Resumen: *Las amenazas de insectos no nativos y patógenos (plagas) a los bosques nativos generalmente son atendidas con métodos como la cuarentena, erradicación, control biológico y desarrollo de variedades resistentes mediante la hibridación y cultivo. Conjuntamente con estos esfuerzos, puede ser útil que científicos ciudadanos localicen árboles raros sobrevivientes que pueden ser naturalmente resistentes o tolerantes a las plagas. El grado de resistencia de los árboles identificados de esta manera puede ser probado bajo condiciones controladas, y los individuos más resistentes pueden ser integrados a programas de cultivo de plantas enfocados a desarrollar formas nativas resistentes a plagas. La participación de científicos ciudadanos en programas orientados a identificar árboles raros que sobreviven la colonización por plagas proporciona medios de bajo costo para maximizar los esfuerzos de búsqueda en regiones geográficas extensas y puede proporcionar un suplemento efectivo a los métodos actuales de manejo.*

Palabras Clave: adélgido del abeto, ciencia ciudadana, cultivo de plantas, especies invasoras, manejo de bosques, política, resistencia, tolerancia

Introduction

Several hundred non-native insects and pathogens occur in forests in the United States, resulting in economic losses of over US$4.2 billion per year (Liebhold et al. 1995; Pimentel et al. 2005). Non-native insects and pathogens (henceforth, pests), such as chestnut blight (*Cryphonectria parasitica*), Dutch elm disease (*Ophiostoma novo-ulmi* and *O. ulmi*), blister rust (*Cronartium ribicola*), and the emerald ash borer (*Agrilus planipennis*), can extirpate their host plants and modify ecological processes (Schlarbaum et al. 1999; Kinloch 2003; Lovett et al. 2006). In response to the failure of early attempts to control and eradicate such pests, a high priority has been

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placed on developing a comprehensive strategy for dealing with invasions (Hobbs & Humphries 1995; Vermeij 1996). In 2004 the U.S. Forest Service (USFS) released a national strategy and implementation plan intended to standardize responses to forest pests while promoting communication between managers and researchers (Reis et al. 2004).

Although early detection and eradication and chemical and biological treatment efforts are incorporated into virtually all forest pest-management plans, the USFS strategy also recommends developing native plant stock that is tolerant of or resistant to the pests (Reis et al. 2004). This is especially important in situations in which the pest cannot be eradicated. Development of resistant or tolerant stock traditionally involves testing the resistance of existing cultivars of the susceptible native tree species; developing resistant-hybrid breeding programs that cross the susceptible native species with a closely related resistant species, usually from the pest's native range; and producing and screening seedlings of the threatened native species for pest resistance (Becker & Townsend 1996; Bentz et al. 2002). We suggest that involving volunteer citizen scientists and forestry professionals in extensive searches for surviving trees can be an inexpensive but effective means of finding resistant individuals in forests. The search for rare native survivors of pest colonization is not a new idea, and such an approach has been used previously in response to several forest pests (Bingham et al. 1953; Ostry et al. 1996; Griffin et al. 2005). Finding resistant native trees may substantially improve the speed and efficacy of breeding programs aimed at developing resistant stock. We use the term *resistant* to refer to individual trees that sustain minimal reduction in growth or reproduction in response to pest colonization. Although there are a wide array of mechanisms by which rare individuals might survive pest colonization, we focus on whether a plant has survived rather than how it has done so.

Host Resistance in the Context of Pest Management

In cases where a pest that is lethal to native tree species cannot be suppressed by biological control or other means, researchers often search the native range of the pest for resistant host species for use in breeding programs (Becker & Townsend 1996; Rebek et al. 2008; Montgomery et al. 2009). Such efforts are sometimes pursued in parallel with ex situ plantings and seed storage to prevent the extinction of the native tree species. Although hybridization with non-native trees may ultimately preserve the functional role of the native tree species, there are advantages and disadvantages to both interspecific and intraspecific crossings. Interspecific hybridization integrates known resistance in hybrid specimens, but viable hybrids can be very difficult to produce (e.g., Bentz et al. 2002), and successful programs require a long-term commitment of resources and the importation of non-native species (Bingham et al. 1953). We emphasize this last point because the resistant species or its hybrids may do poorly in the native ecosystem because of climate, geographic, or other differences. Breeding programs often address this challenge by backcrossing individuals to maintain the adaptive traits of the native species while incorporating the resistant traits of the non-native species (Hayes et al. 1955). In contrast, intraspecific breeding of native survivors preserves phenotypic characteristics and compatibility with the native ecosystem (Bingham et al. 1953).

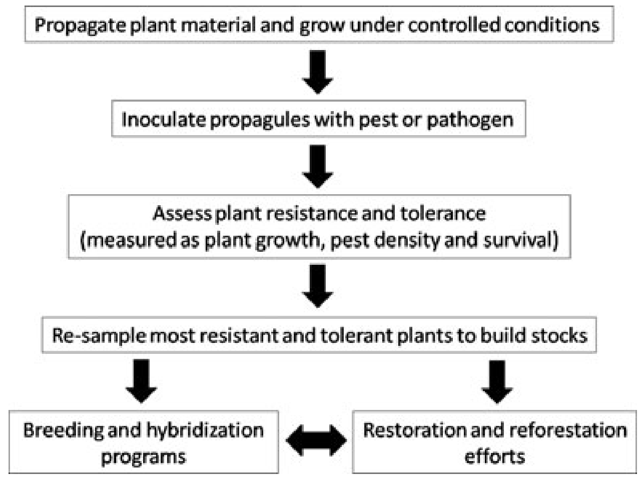

Figure 1. Steps in determining whether surviving trees are resistant to a pest.

A major impediment to identifying individuals or populations of native tree species with some degree of resistance is that, if they exist at all, they are rare and thus difficult to locate. Hiring personnel to systematically survey large portions of the invaded range is both logistically challenging and expensive. In contrast, the approach we describe in our case study of hemlock woolly adelgid (*Adelges tsugae*) used email, pamphlets, and media outlets to enlist volunteer professionals and citizens to survey invaded forests for trees that fit a strict set of criteria (Supporting Information).

Volunteers who locate trees that may be resistant contact a project coordinator who collects additional information, such as location and growing conditions, and arranges the collection of plant cuttings for propagation at a central facility (Fig. 1). Cuttings are treated to induce rooting and grown in controlled conditions alongside cuttings from susceptible trees until both groups can be experimentally inoculated with pests and their relative degrees of resistance assessed. The metrics used to assess resistance vary according to the particular situation and may include pest density, survival, and plant growth.

Additional cuttings are collected from the least affected individual trees and retested for resistance. If these subsequent tests confirm the presence of pest resistance, the individuals are then included in breeding programs and restoration efforts.

Our approach has several advantages, especially when undertaken in concert with already established breeding or hybridization programs. First, trees are generally large and long-lived organisms that are unlikely to repeatedly escape pest colonization in areas with high pest densities. This fact reduces the possibility that a single healthy tree within a stand of colonized trees has fortuitously escaped pest colonization and increases the probability that such survivors will possess some degree of resistance. Second, the use of professionals and interested citizens in the search for native survivors minimizes the cost of surveying large areas while involving participants in the scientific process. Third, the considerable cultural and economic importance of trees provides powerful motivation for volunteer involvement and makes it likely that even modest outreach efforts will be met with an enthusiastic response.

Importance of Pest Virulence

A high level of pest virulence (mortality in the host population caused by the invading organism) characterizes many of the most damaging biological invasions in eastern North America (Lovett et al. 2006). Our approach is most effective with highly virulent pests that spread quickly across the landscape and kill almost all host trees. From an evolutionary perspective, such pests act as a selective filter that reliably removes susceptible individuals and increases the probability that survivors will possess traits conferring pest resistance. As a result, searching for surviving host trees is most effective when levels of pest infestation and host mortality are high. This contrasts with management strategies that are most effective at the onset of an invasion, such as pest eradication, chemical or biological control, and quarantines of affected areas. When searching for native host resistance, sites with high pest densities produce fewer false positives and maximize the likelihood of identifying individual trees with traits that confer pest resistance.

Role of Forest Surveys

Cultivated varieties of trees affected by non-native pests are often the initial focus of investigations into pest resistance (Becker & Townsend 1996; Lagalante et al. 2007; Rebek et al. 2008). These varieties are often easily procured from nurseries and arboreta, and protocols for their propagation and growth are available. Their well-documented lineage also makes it easy to assess the relative effect of genetic versus environmental factors on plant phenotype. Although they offer many advantages, varieties may be selected for phenotypic traits unrelated to or negatively correlated with pest resistance (e.g., selection for rapid growth often reduces the plant's energetic expenditure on pest defense). In contrast, forest trees are generally much more abundant and possess greater population-level genetic variability than do commercially grown cultivars. Individuals with traits conferring some degree of pest resistance may be especially likely to occur at range boundaries, in isolated populations, or growing under less than ideal conditions. Such environments may impose selective pressures (Kawecki 2008) that favor novel adaptations that confer some degree of pest resistance. Finally, the selective pressures imposed on forest trees by pests are generally greater than those on cultivated varieties grown with fertilizer and pesticides.

It would be both inefficient and prohibitively expensive for a single group or agency to survey the native range of a host species for individual survivors of a virulent pest. Nevertheless, numerous forestry professionals, conservation groups, and individual citizens are concerned with the preservation of native ecosystems and eager to help with such efforts. Several long-term and large-scale research programs have used such groups to search for and census amphibians, birds, and other organisms (Wall et al. 2001; Turner 2003; Lepczyk 2005). These citizen science programs require long-term commitments from participants, detailed data collection, and statistical analyses to compensate for the fact that data collected by volunteers can vary considerably in quality (Cooper et al. 2007).

In contrast to replicated surveys for birds or amphibians, searching for surviving trees in pest-affected forests requires relatively little training and can be conducted in combination with activities such as hiking or camping. Volunteers provide information on potentially resistant trees to professionals who evaluate the information, identify promising candidates for sampling, and may even provide volunteers with the guidance necessary to collect samples themselves. Volunteer-driven programs of this sort allow data to be collected across large geographic areas. Members of native plant societies, conservation groups, and similar organizations may be particularly interested in such programs. The involvement of volunteer professional foresters or resource managers, individuals whose expertise makes them especially valuable contributors, is also important.

Potential Obstacles and Weaknesses

Citizen-science programs rely on the ability of volunteers to identify tree species accurately, which can be difficult with some genera. In the northeastern United States, for instance, several species of ash (*Fraxinus* spp.) co-occur and are difficult to distinguish. Organizers of programs involving such species may need to train volunteers and make special efforts to recruit highly trained individuals. More generally, any program that assesses native host resistance may encounter difficulties with propagating potentially resistant plant stock. Because the parents of seeds collected from surviving plants can be difficult to identify, host resistance is often assessed most quickly and effectively with material that is genetically identical to the surviving tree. Although a variety of methods exist for producing clonal material (e.g., vegetative propagation, grafting, micropropagation, and tissue culturing; MacDonald 2006), each requires specialized equipment and expertise and may be expensive. Season and plant age also affect propagation, and even when plant material can be propagated, it may be years before it can be assessed. For example, juvenile American elms (*Ulmus americana*) are highly resistant to Dutch elm disease (Smalley & Guries 1993). Furthermore, the use of only pest-resistant individuals in breeding programs can decrease population-level genetic diversity. The potential for a genetic bottleneck is inversely proportional to the number of surviving individuals; when only a few individuals are resistant, reducing the probability of a bottleneck requires carefully designed breeding programs and extensive searches for additional survivors.

Case Study: Hemlock Woolly Adelgid

Hemlock woolly adelgid (HWA) was introduced from Asia to the state of Virginia (U.S.A.) in the 1950s and has caused high mortality in eastern and Carolina hemlocks (*Tsuga canadensis* and *T. caroliniana*, respectively) throughout their native range (Souto et al. 1996). After a few surviving eastern hemlocks were located (Preisser et al. 2008), we expanded the search for resistant hemlocks by enlisting volunteers from throughout the northeastern United States to survey HWA-invaded hemlock forests.

We developed a brochure that described the adelgid, its effects on its hosts, and the likely phenotypic characteristics of resistant trees (Supporting Information). The brochures asked readers to contact us via telephone or email if they knew of resistant trees (Ingwell 2007). We mailed the brochure to conservation and environmental groups in HWA-invaded areas and to columnists who wrote for local newspapers about nature and the environment. Our project was highlighted in at least 8 local newspapers and in approximately 20 newsletters of groups such as the Audubon Society, Conservation Alliance, and Rhode Island Tree Council. A single mailing of 100 brochures yielded contacts from over 200 forestry professionals and citizens who provided information on possibly resistant trees and small stands in Connecticut, Massachusetts, New Jersey, New York, Pennsylvania, and Rhode Island. The brochure cost approximately US$300.00 to design, print, and mail and effectively enlisted volunteers to survey hundreds of hemlock forests for rare healthy trees.

We took cuttings of the newest growth from the 20 most promising trees for propagation in a controlled greenhouse setting. We propagated these cuttings alongside control cuttings from eastern (HWA susceptible) and western (*Tsuga heterophylla*; HWA resistant) hemlocks in a 1:1 solution of Dip-N-Grow (Griffin Greenhouse Supplies, Tewksbury, Massachusetts). Over 2 years, these cuttings overcame transplant stress and developed healthy root networks. In summer 2009 we assessed HWA resistance in 37 rooted cuttings from 8 potentially resistant eastern hemlocks found in Connecticut, New Jersey, and Pennsylvania, in 28 rooted cuttings from 5 eastern hemlocks in Rhode Island and Connecticut, and in 5 rooted cuttings from western hemlocks.

Rooted cuttings from all groups were inoculated with HWA from hemlock branchlets that had high HWA densities just prior to the emergence of the adelgid's mobile crawling phase (see Butin et al. 2007). We counted the number of crawlers of the sistens generation that settled (i.e., had inserted their stylet into plant tissue) and were still alive after 3 months (see Supporting Information for detailed methods). Density of HWA differed among groups (repeated measures analysis of variance: $F_{2,63} = 0.4911$, $p < 0.0001$) and over time ($F_{1,63} = 0.7177, p < 0.0001$). Although initial HWA sisten settlement did not differ significantly between potentially resistant and control trees, adult sisten densities were significantly (Tukey's HSD; $p < 0.05$) lower on cuttings from the potentially resistant trees than on cuttings from the controls (Fig. 2). These results suggest that some eastern hemlocks are resistant to HWA. In addition to growing and testing cuttings from the remaining candidate trees, we are collecting additional cuttings from individual trees with high degrees of HWA resistance for testing in outdoor field trials. We hope to use these cuttings in breeding programs aimed at developing highly HWA-resistant eastern hemlocks.

The relatively small number of candidate trees (approximately 30 as of 2010) we have identified suggests the potential for a genetic bottleneck. The effective population size of areas reforested with these individuals may be even lower than the number of candidate individuals would indicate. We have attempted to address both issues by searching for surviving trees throughout the invaded range of eastern hemlock. Our current stock of possibly resistant trees comes from nine locations in five states. In addition, the number of putatively resistant individuals exceeds the 20 suggested by Soulé et al. (1985) as a general guideline for minimizing potential founder effects (also see Atangana et al. 2010).

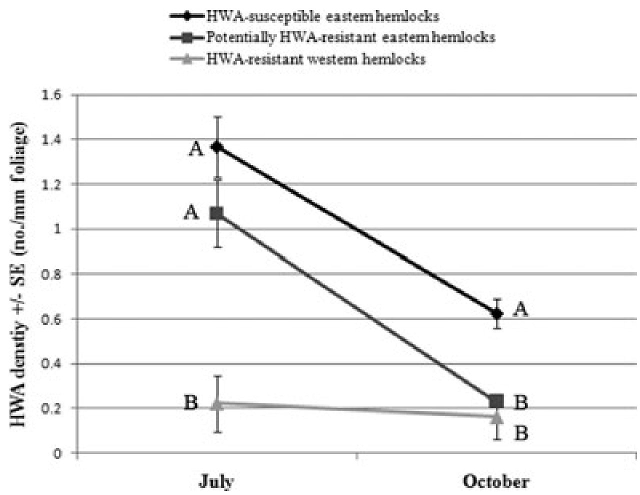

Figure 2. Density of sistens-generation hemlock woolly adelgids (HWA) on cuttings from HWA-susceptible eastern hemlock trees, potentially HWA-resistant eastern hemlock trees, and HWA-resistant western hemlocks.

Other Case Studies

Researchers of forest pathogens such as chestnut blight, Dutch elm disease, and butternut canker, have long searched for native trees with some degree of resistance. In the early 20th century, Kelley (1924) reported several cases of mature American chestnut (*Castanea dentata*) trees that had survived chestnut blight and appeared relatively healthy. At the time, land owners were hastily removing chestnut trees in hopes of slowing spread of the blight and maximizing their profits. This practice continued despite Kelley's suggestion that land owners protect surviving groves in the hope of preserving blight-resistant chestnut populations. Although the spread of chestnut blight resulted in the nearly complete removal of mature American chestnuts, anecdotal reports of survivors eventually led to the creation of a propagation and evaluation program (Griffin et al. 1983). Surviving American chestnuts have since been used in breeding programs designed to reintroduce the species (Anagnostakis & Hillman 1992; Brewer 1995; Griffin et al. 2005) Most of the surviving trees were found through surveys conducted by local foresters and searches for reports of surviving trees in garden club newsletters, reports of conservation organizations, and newspapers (G. Griffin, personal communication). The cultivation of offspring from these survivors and their evaluation for blight resistance continues.

Butternut canker (*Sirococcus clavigignenti-juglandacearum*) has been present in the United States since 1967 and is killing native butternut trees (*Juglans* spp.) throughout the southeast (Renlund 1971; Schlarbaum et al. 1999). A few surviving butternut trees have been found, primarily in riparian zones, and efforts have begun to identify more survivors in order to incorporate them in breeding programs (Ostry et al. 1994, 1996; Schlarbaum et al. 1999). A U.S. Forest Service publication was developed that provides citizen scientists with guidelines for the identification of potentially resistant survivors (e.g., presence of unusually thick or dark bark) and contact information for people involved in breeding programs (Ostry et al. 1996; Ostry & Woeste 2004). This publication has resulted in the identification of a number of potentially canker-resistant trees that have been incorporated into breeding programs. These successes have occurred in spite of considerable practical difficulties in species-level identification. Butternut frequently hybridizes with non-native species, and in some cases species can be identified only through molecular techniques (Ross-Davis et al. 2008).

Dutch elm disease (DED) has been spreading throughout the United States since 1930 and has been described as the most virulent shade-tree disease in the United States (Karnosky 1979). Approximately 1 out of 100,000 American elms is DED-tolerant (Becker & Townsend 1996). Although the mechanism of tolerance is unknown, surviving trees are being propagated, their infection rates evaluated, and crossed to create commercially available cultivars (Becker & Townsend 1996; Schlarbaum et al. 1999). Although rooted cuttings have been used in elm propagation, researchers primarily use tissue culture because cuttings must be collected within a narrow time frame during the spring (Guries & Smalley 2000). Identification and propagation of DED-resistant individuals is also complicated because juvenile elms are uniformly resistant to DED and researchers can only assess host resistance in mature trees (Solla et al. 2005).

Potential Applications to Practice

The human intervention most likely to interfere with the identification and propagation of surviving individuals is preemptive logging. This type of logging seeks to reduce the economic impact of forest pests while protecting the public from falling trees killed by pests (Foster & Orwig 2006). Between 1996 and 2002, eastern states that had been recently invaded by HWA removed over 2400 ha of hemlock forests in an attempt to slow the spread of this pest and to maximize economic return on standing timber (Orwig et al. 2002). Removing large numbers of uninfected trees, however, can reduce population-level genetic variability and may inadvertently remove resistant individuals. Large preemptive salvage operations also disrupt ecosystem responses more than does uninterrupted postinvasion recovery (Foster & Orwig 2006; Jonasova & Matejkova 2007). The case of the American chestnut provides an example of some of the costs associated with widespread preemptive logging: the removal of trees at the leading edge of the blight greatly reduced the ability of later researchers to locate blight-resistant individuals.

Engaging citizen scientists and trained professionals in searches for resistant trees can enhance the success of breeding programs aimed at developing stock that are resistant to highly virulent forest pests. Although there are obstacles to identifying and propagating naturally resistant native individuals, the low cost and potentially high returns associated with incorporating volunteers into a research program may make such efforts worthy of consideration as a standard response to forest invasions.

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Supporting Information

The "Have You Seen This Hemlock?" brochure (Appendix S1) and "Assessing the Resistance to HWA on Surviving Eastern Hemlock Trees" (Appendix S2) are available as part of the online article. The authors are solely responsible for the content and functionality of these materials. Queries (other than absence of the material) should be directed to the corresponding author.

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