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Early Detection and Rapid Response for Non-Native Bark and Ambrosia Beetles

Robert Rabaglia, Donald Duerr, Robert Acciavatti, and Iral Ragenovich On the cover: *Ips typographus*, the European spruce bark beetle. Image altered from a photo by Maja Jurc available at www.forestryimages.org as UGA2103068. Other images from forestryimages.org in this publication are credited by image number. The U.S. Department of Agriculture (USDA) prohibits discrimination in all its programs and activities on the basis of race, color, national origin, sex, religion, age, disability, political beliefs, sexual orientation, or marital or family status. (Not all prohibited bases apply to all programs.) Persons with disabilities who require alternative means for communication of program information (Braille, large print, audiotape, etc.) should contact USDA's TARGET Center at 202-720-2600 (voice and TDD). To file a complaint of discrimination, write USDA, Director, Office of Civil Rights, Room 326-W, Whitten Building, 1400 Independence Avenue, SW, Washington, D.C. 20250-9410 or call 202-720-5964 (voice and TDD). USDA is an equal opportunity provider and employer. The use of trade, firm, or corporation names in this publication is for information only and does not constitute an endorsement by the U.S. Department of Agriculture.

USDA FOREST SERVICE EARLY DETECTION AND RAPID RESPONSE PROJECT FOR NON-NATIVE BARK AND AMBROSIA BEETLES

SUMMARY OF THE 2001-2005 PILOT PROJECT

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Early Detection and Rapid Response to Non-Native Bark and Ambrosia Beetles	
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ACRONYMS

APHIS	Animal and Plant Health Inspection Service
CAPS	Cooperative Agriculture Pest Survey (APHIS)
CDFA	California Department of Food and Agriculture
EDRR	Early Detection and Rapid Response
FHP	Forest Health Protection (USDA Forest Service)
FHTET	Forest Health Technology Enterprise Team (USDA Forest Service)
NPAG	New Pest Advisory Group (APHIS)
PPQ	Plant Protection and Quarantine (APHIS)
USDA	United States Department of Agriculture

EXECUTIVE SUMMARY

The introduction and establishment of non-native invasive species is considered one of the greatest threats to the integrity and vitality of the nation's forests. The USDA Forest Service and Animal Plant Health Inspection Service (APHIS) are tracking several species that, unchecked, significantly degrade forests and cost millions of dollars to contain. The National Invasive Species Council identified early detection, diagnosis, and response as the most effective strategy for managing these invasive species.

In 2000, APHIS and the Forest Service agreed to coordinate detection efforts for exotic forest insects, diseases, and plants, and agreed to develop a framework for a national early detection and rapid-response program. An Exotic Pest Rapid Detection Team was assembled to develop objectives and protocols and to identify target species.

In 2001, the Forest Service initiated a pilot project for the early detection of non-native bark and ambrosia beetles. Ten target species were selected based upon frequency of past interceptions, ability of the species to establish in North America, and the severity of the threat they pose to forests. The target species were trapped using four specific chemical lures. From 2001-2005, protocols were tested and refined at more than 300 trap locations in 22 states.

In contrast to other surveys, all bark and ambrosia beetles collected in trap samples—not simply the target species—were identified. Although this added significant cost and time to the project, it was considered more important not to overlook other potentially damaging species. During the five years of the Pilot Project, five bark and ambrosia beetles new to North America were collected: *Hylurgops palliatus*, *Xyleborus similis*, *Xyleborus glabratus*, *Xyleborus seriatus*, and *Scolytus schevyrewi*. In addition, many new state records (i.e., first reported detections in a state) for bark and ambrosia beetles were found, nearly half of which were for established, non-native species.

The protocols developed in this Pilot Project are being used in the national implementation of the Early Detection and Rapid Response program for non-native bark beetles. The Team, which includes Forest Service, APHIS, and state representatives, sets national priorities, selects target species, and develops protocols for participation. Based upon available funding, approximately one-third of all states will be surveyed each year. States that participate in the program will be selected based on the risk of introduction and establishment of non-native bark beetles. These states will be responsible for site selection, trap placement, and data reporting. If new non-native species are found, the Team will coordinate with APHIS and states to rapidly assess, delimit, and respond to the incidents.

Introduction

Non-native species inadvertently introduced into North America through importation and travel represent one of the greatest threats to the integrity and vitality of North American forest ecosystems. While exclusion would be the ideal solution, some exotic insect pests inevitably escape detection and become established near ports-of-entry or inland importation sites. The USDA Forest Service and Animal and Plant Health Inspection Service (APHIS) are now addressing recent introductions of Asian longhorned beetle, emerald ash borer, and the European Sirex woodwasp, species that were most likely introduced in solid-wood packing material.

The National Invasive Species Council formulated the National Management Plan for early detection, diagnosis, and response to high-risk, exotic, and invasive insects, pathogens, and plants. The greatest opportunities for eradication or cost-effective control of non-native species are immediately after their introduction. Early detection, followed by rapid assessment and response, increases the likelihood of successful eradication or containment.

In the spring of 2000, the National Plant Board and National Association of State Foresters requested that APHIS and Forest Service coordinate their invasive species planning and budgeting procedures. A Forest Service and APHIS National Executive Team, established to implement this request, agreed to coordinate the early detection of non-native forest insects, diseases, and plants. In April 2001, an agreement between the Forest Service and APHIS was formalized through a Memorandum of Understanding that delineated responsibilities, identified activities, and established an Exotic Pest Rapid Detection Team to develop strategies for early detection and response to invasive, non-native species.

Later in 2001, the Exotic Pest Rapid Detection Team formulated a pilot project for the rapid detection of exotic bark and ambrosia beetles (Scolytidae). The goal of the Early Detection and Rapid Response (EDRR) Pilot Project was to develop a rapid detection system for these pests as the framework for a national exotic invasive species rapid-detection system and to determine cost, equipment, personnel, and other needs for a fully operational national program. During 2001, the effort focused on developing trapping protocols and enhancing detection and diagnostic capabilities.

Following the 2001 project and based on recommendations from the Team, the Forest Service continued the Pilot Project to refine protocols to meet project objectives. The Pilot Project continued from 2001 through 2006; this report summarizes protocols, accomplishments, and recommendations developed from 2001 through 2005.

Pilot Project Objectives

The principal objectives of the pilot project were to:

- 1. Identify potential exotic invasive species with a high risk of introduction, identify likely pathways for their introduction, and provide guidelines for their detection and subsequent response,
- 2. Detect and monitor populations of newly introduced exotic scolytids at selected high-risk sites,
- 3. Provide APHIS and Forest Service with current distribution information on newly introduced scolytids,
- 4. Identify gaps in detection protocols, taxonomic skills, and reference needs, and develop recommendations for addressing information and skill gaps, and
- 5. Use information from the Pilot Project to help Forest Service and APHIS-Plant Protection and Quarantine (PPQ) set priorities for research, development, and response activities, and modify protocols accordingly.

Target Species

Exotic Scolytidae were selected as a target group because bark and ambrosia beetle species have been among the most frequently intercepted groups of insects at U.S. ports-of-entry in recent years. In addition, species in this family have the potential to cause extensive tree mortality over large areas. Exotic Scolytidae are often transported into the U.S. within infested solid-wood packing material (SWPM). Their spread and establishment within the U.S. may be inadvertently fostered when the SWPM is off-loaded at ports, stored near warehouses, factories, and distribution centers, transported away from ports to be recycled, or moved by dock workers for use in construction or as fuel wood.

APHIS records indicated that nearly 7,000 interceptions of Scolytidae occurred between 1985 and 2000; about 5,000 of these were wood-inhabiting scolytids (other scolytids attack seeds and other plant material). Several species of true bark beetles were the most commonly intercepted species, and a good correlation exists between interception frequency and species establishment. As effective traps and lures were available for many of these species, the Project Team selected ten species as targets for the Pilot Project. However, there were several species of scolytids, especially ambrosia beetles (*Xyleborus* species), that were never intercepted but nevertheless became established in the U.S. and other countries. Consequently, the Project Team decided that all scolytids in trap samples would be identified so that non-target species of potential interest would not be missed.

The target species for this project were:

Orthotomicus erosus Pityogenes chalcographus

Ips sexdentatusIps typographusTomicus minorTomicus piniperdaHylurgops palliatusHylurgus ligniperdaTrypodendron domesticumXyleborus species

PROTOCOLS

Basic Protocols

2001. The Team identified three initial test protocols in 2001 for the national rapid detection system framework:

- Traps were to be placed very close to or within ports (Figure 1), airports, and other points of entry for international cargo.
- Four traps would be deployed at each trap site and the traps baited with one of the following lures: ethanol, three-component exotic bark beetle lure, ethanol plus alpha-pinene, and Chalcoprax®. The following table indicates the lures selected for specific target species.



Figure 1. Potential entry point for exotic insects.

Sleeve-type, vapor-permeable lures were used with Lindgren funnel traps (Figure 2). These lures and traps were chosen because they have been shown successfully to attract and capture bark beetles.

• Because of the difficulty of bark beetle identification, it was decided that all samples would be sent to one of three professional beetle taxonomists (E. Richard Hoebeke, Cornell University; Jim LaBonte, Oregon Department of Agriculture; and Robert Rabaglia, at the time, with the Maryland Department of Agriculture). While the decision to identify all species resulted in longer sample-processing times, it ensured that no exotic bark beetles of potential interest were overlooked. Lastly, the taxonomists identified (or arranged for identification of) non-bark beetle, wood-inhabiting species (e.g., woodwasps, flat-headed borers, and round-headed borers) that were unfamiliar or possibly non-native. This ensured that new exotic nonbark beetle specimens would still be identified. Voucher specimens of all species identified were kept by the taxonomists or deposited at the U.S. National Museum in Washington, DC.



Figure 2. Lingren funnel trap.

Target Beetle Species	Lure(s)
Ips typographus Ips sexdentatus Orthotomicus erosus Hylurgus ligniperda	methyl butenol (2-Methyl-3-buten-2-ol) cis-verbenol (cis-4,6,6-Trimethylbicyclo[3.1.1]hept-3-en-2-ol) ipsdienol (2-Methyl-6-methylene-2,7-octadien-4-ol)
Tomicus spp. Hylurgops palliatus Hylurgus ligniperda	ά-pinene (2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene) ethanol
Xyleborus spp. Trypodendron domesticum	ethanol
Pityogenes chalcographus	chalcogran (2-Ethyl-1,6-dioxaspiro[4.4]nonane)

The 2001 protocols worked well, and many new state records were generated. *Hylurgops palliatus* was detected for the first time in North America; *Arhopalus pinetorum*, a longhorned beetle, was detected for the first time in the U.S.; and *Xyleborinus alni*, a species native to Asia previously known in North America only from Oregon, Washington, and British Columbia, was found in New York and Pennsylvania. These discoveries demonstrated the appropriate placement of trap sites, the effectiveness of the lures and traps, and the accuracy of the taxonomists.

2002. The 2001 protocols remained virtually unchanged for 2002. Trap and lure use remained the same, and ports-of-entry were still the primary sites chosen. Additional trap sites were established in 2002 as a result of increasing interest and available funding.

2003. Several significant changes were made to the protocols in 2003. Chalcoprax® was dropped as one of the lures because of expense, the length of time it took to order it from Germany (the only source), and because it is an attractant to only one species on the target list, *Pityogenes chalcographus*. Therefore, under the 2003 protocols, there were three traps set per site, baited with ethanol, a three-component exotic bark beetle lure, or ethanol plus alpha-pinene.

In 2003, APHIS' Cooperative Agriculture Pest Survey (CAPS) program began an Exotic Wood Borer and Bark Beetle Survey targeted at ports-of-entry; to avoid duplication of effort, the Team decided that other types of sites should be targeted.

The Team, which then included the national program coordinator for the CAPS program, coordinated these two surveys to complement each other both on the national and state levels. At the national level, the CAPS Exotic Wood Borer and Bark Beetle Survey typically

places traps inside or very close to ports, airports, warehouses, and distribution centers, regardless whether the area was wooded or not. The Pilot Project began to focus on placing traps in wooded areas near a variety of high-risk solid-wood packing material "endpoints": landfills (Figure 3), wood recycling locations, dunnage piles, factories, and warehouses and distribution centers with SWPM stored outside that were not sites for CAPS. Traps were also set in urban and wildlandurban interface forests such as parks, arboreta, and fragmented forests near new developments (Figure 4).

The Team also encouraged state partners to coordinate with state CAPS committees to select trap sites for the two surveys that would complement each other. As a result of these two simultaneous programs, there is good survey coverage, with minimal duplication, for exotic bark beetles and wood borers at the national and state levels.



Figure 3. Endpoint for transportation of potentially infested packing materials.



Figure 4. Wildland-urban interface as potential establishment point for exotic species.

2004. No significant changes in protocols or objectives were made in the 2004 surveys, though the surveys were expanded. At the end of 2004, it became apparent that the number of samples were approaching the limit that the three professional taxonomists could process in a timely manner. Because there are very few expert scolytid taxonomists, simply recruiting new taxonomists to help process more samples was not an option. The training and use of pre-screeners was determined to be the simplest and most feasible method of expanding sample-processing capacity in the following year (see the following description of pre-screening).

2005. The main development in protocols in 2005 utilized pre-screeners to decrease the amount of time that the taxonomists were spending to sort out ubiquitous common species in the samples. For samples collected from 2001 to 2003, the taxonomists estimated that they were spending more than half of their time separating and counting common species that are frequently trapped in large numbers. A small number of species (8 to 10 species per region) constitute the vast majority of the specimens in the samples. Because identification of these common species can be taught to those unfamiliar with these beetles, it was decided to train pre-screeners to sort out the common species before sending the samples to the taxonomists. The latter would identify the unique and/or possibly exotic species. This would allow the taxonomists time to identify a larger number of samples in a given year, permitting more sites to be trapped.

Pre-screeners were employees of the state agency performing Pilot Project trapping, graduate students in forest entomology programs, Forest Service or APHIS employees, or other cooperators. Training for pre-screeners was given at Cornell University (Ithaca, New York) by the taxonomists prior to trapping. The pre-screening trainees were provided with dichotomous keys, specimens, images, and other identification aids.

As part of the pre-screening process, quality control measures were taken to ensure the accuracy of the pre-screeners' sorting and identifications. For the first three sample checks, pre-screeners identified and separated the common specimens and sent them to the tax-onomists along with the unidentified specimens. The taxonomists verified the accuracy of the pre-screened identifications, and thereafter, the pre-screener did not have to send the common specimens to the taxonomist.

Data from all aspects of the Pilot Project were kept on paper data sheets and then sent to the FHP office in Morgantown, West Virginia, where they were entered into a database. Site location, site type, dates, collector contacts, lure used, and species collected were recorded for each trap.

Delimitation Protocols (Rapid Response)

As new exotic bark beetles and/or ambrosia beetle species were found during the Pilot Project, the CAPS Exotic Wood Borer and Bark Beetle Survey, or other related surveys, the basic trapping protocols were adjusted to pursue delimitation trapping surveys for these new species. Survey protocols, sites, lures, and survey partners were selected to meet the needs of the specific delimitation survey being conducted. For example, *Xylosandrus mutilatus*, first found in Mississippi in 1999, was shown to respond well to high release rates of ethanol. Therefore, when the Pilot Project decided to pursue delimitation of this species' establishment in 2003 (in addition to the regular Pilot Project survey efforts), traps were baited with several pouches of ethanol. After the initial detection of *Scolytus schevyrewi*, discussions with APHIS and Forest Service and university researchers led to the selection of a lure for the delimiting survey. Also for this survey, the Team coordinated with APHIS' New Pest Advisory Group, state CAPS committees, and state forestry groups to set up and check traps regularly and send samples for identification to a Pilot Project taxonomist.

Sites

Throughout the duration of the Pilot Project, the selection of states to be assigned trap sites in a given year was based on the following criteria: relative risk of new introductions (based on interception frequency); length of time since a similar detection survey; state forestry/agriculture agency or APHIS personnel availability to select sites, deploy traps, and ship samples to identifiers; and the relative value of forest resources in the state.

Through 2005, traps were set at more than 300 sites (three traps per site) in 22 states (see the following table). Generally, as the Pilot Project funding increased, so did the number of sites being trapped. State participation in the Pilot Project depended upon interest, level of damage risk, and the taxonomists' work load. In 2004, several northeastern states increased the number of trap sites, with increased assistance from the Forest Service and APHIS and the deployment of additional state-supported field crews. At that point, it became clear that a limiting factor in the number of traps that could be set was the number of samples that the taxonomists could process in a timely manner.

Ctata	Number of Sites					
State	2001	2002	2003	2004	2005	Total
Alaska	0	3	3	0	0	6
Arkansas	0	0	0	0	3	3
Arizona	0	0	0	3	0	3
California	3	3	0	0	0	6
Colorado	0	0	3	0	11	14
Georgia	0	3	0	0	0	3
Kentucky	0	2	0	0	0	2
Louisiana	6	3	2	6	9	26
Massachusetts	0	0	0	3	15	18
Maine	0	0	0	5	0	5
Michigan	0	0	0	6	6	12
Montana	0	0	0	3	0	3
North Carolina	0	0	6	6	0	12
New Jersey	0	3	0	21	0	24
New York	3	0	6	6	0	15
Ohio	3	3	3	28	0	37
Oregon	3	0	0	3	5	11
Pennsylvania	3	3	20	21	18	65
South Carolina	0	0	3	7	9	19
Texas	2	3	0	0	6	11
Utah	0	0	3	0	0	3
Washington	3	3	3	3	0	12
TOTALS	26	29	52	121	82	310

RESULTS

In the five years of this Pilot Project, five species of scolytids new to North America were found:

2001 - Hylurgops palliatus near Erie, Pennsylvania

2002 – Xyleborus similis in Houston, Texas Xyleborus glabratus near Savannah, Georgia

2003 - Scolytus schevyrewi in Colorado and Utah

2005 - Xyleborus seriatus in Massachusetts

Following protocols developed by the Project Team, after each new find, APHIS and state regulatory officials were notified and delimiting surveys were conducted (either as part of this project or in cooperation with APHIS programs).

In 2001, the first year of the Pilot Project, a target species, *Hylurgops palliatus*, a species native to Europe, was found in traps near Erie, Pennsylvania. This was the first record of this species in North America.

In 2002 and 2003, delimiting surveys for *H. palliatus* (Figure 5) were conducted in north-western Pennsylvania, southwestern New York, and northeastern Ohio. Specimens of *H. palliatus* were found in each of these areas. Also in 2002, in partnership with local APHIS staff, a delimiting survey for *Xyleborus glabratus* (Figure 6) found in Pilot Project traps earlier in the year was conducted in Georgia; however, no additional *Xyleborus glabratus* were found in the Port Wentworth/Savannah, Georgia, area in 2002.

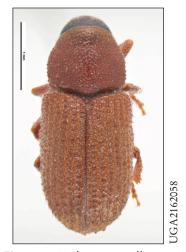


Figure 5. Hylurgops palliatus.

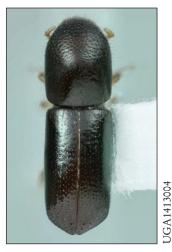


Figure 6. Xyleborus glabratus.

In 2003, after the find of *Scolytus schevyrewi* (Figure 7) in Colorado and Utah and, in coordination with APHIS and state departments of agriculture, an extensive survey for this species was conducted. With Forest Service and APHIS funding, traps were placed in Arizona, Colorado, Idaho, Kansas, Nebraska, New Mexico, Nevada, Montana, California, Oregon, Washington, Wyoming, Utah, and Texas. *Scolytus schevyrewi* were found in most of these states.



Figure 7. Scolytus schevyrewi.

In 2005, following the original detection of *Xyleborus seriatus* in Massachusetts, additional traps in the area found the species in three counties around Worcester.

For each of these finds, delimiting surveys found infestations over a large multi-county or multi-state area. Due to the large infested area, APHIS and the Forest Service decided that eradication, regulatory, or management actions in these areas were not feasible. If a potentially devastating species (e.g., *Ips typographus*) had been found, and found earlier, a management response would have been likely.

Delimiting surveys for three scolytids found in surveys not directly associated with the Pilot Project were also supported by the Team. A survey for *Xylosandrus mutilatus* (Figure 8) was conducted in 2003. This species of Asian ambrosia beetle was found in Mississippi in 1999 and in Florida in 2002. Detection traps were placed in Alabama, Florida, Louisiana, North Carolina, Tennessee, Texas, and Virginia. No *X. mutilatus* were found during the survey in 2003. Through Pilot Project funding, the Forest Service assisted Florida with a delimiting survey for *Euwallacea fornicatus* in south Florida once this species was found in host trees in the Miami area in 2002 and 2003. No additional *E. fornicatus* were found during the Florida trapping survey. In 2004, *Orthotomicus erosus* (Figure 9), a target species and a potentially important non-native pest, was collected in five counties in California by the California Department of Food and Agriculture (CDFA). In 2005, with financial and taxonomic support from the Team, 99 traps at 33 sites in 11 California counties were set up to delimit the infestation. During the survey, 3,314 *O. erosus* were collected, several in four counties previously not known to be infested.



Figure 8. Xylosandrus mutilans.



Figure 9. Orthotomicus erosus.

In addition to the five new North American records, there were more than 157 species of scolytids collected during this Pilot Project (specimens of *Hypothenemus* and *Pityophthorus* were identified to genus only, but several species in each genus were collected). Most (133) of these were native species; however, 47 of these were new state records for a species. Of the 24 non-native species collected that were previously known in the U.S., 19 were new state records. Several of these records represented significant species' range extensions. For example, *Xyleborus pfeili* was known in the eastern U.S. only from Maryland, but was found in Georgia and New Jersey during this project. The ambrosia beetle, *Xylosandrus mutilatus*, a relatively new find in Mississippi and Florida, was collected in Texas in 2005. All of the new state records from the Pilot Project are being prepared for publication in scientific journals by the Project taxonomists.

Although no management actions were taken as a result of these new state records, this information was useful to document the range of the non-native bark beetles already established in the country. These data also can be used as a baseline for native species and non-native species already established in North America.

More than 250,000 specimens were identified during the five years of the Pilot Project. The five most numerous species were all established non-native species. In each region of the country, there were consistent assemblages of 10 to 12 species that made up 80-90 percent of the regional samples. As discussed earlier, this information was used to develop a list of common species to train prescreeners. By having prescreeners sort out the common species, taxonomists were then able to handle more samples from more locations. This protocol was field tested in 2005 and 2006 and integrated into the protocol for national implementation.

NATIONAL IMPLEMENTATION

At the conclusion of the Pilot Project phase (2001-2005) of EDRR, protocols underwent additional refinement and implementation strategies were further developed. At the national level, several actions were taken in 2006: 1) the Forest Service-Washington Office appointed a project coordinator, 2) the Forest Service and APHIS further coordinated their respective roles, 3) the Forest Service-Forest Health Technology Enterprise Team (FHTET) began cooperative development of a project database, and 4) the Project Team developed a national strategic implementation plan. At the Regional level, the Team coordinated project efforts with Regional and Northeast Area FHP directors and with state partners. Also in 2006, the Project Team continued to refine field trapping protocols, pre-screener training, and taxonomist involvement.

Based on the experience and protocols developed in the Pilot Project, national implementation of the EDRR for non-native bark and ambrosia beetles began in 2007. A national EDRR Team has set national survey priorities, selected target species, and developed protocols for state participation. This Team includes state representatives, the National Survey Coordinator for APHIS PPQ, Forest Service R&D, FHP, and university representatives. The FHP National Director and WO-EDRR project coordinator approved Team decisions based upon available funding and national priorities. At current funding levels, state staffing levels, and taxonomist capabilities, approximately 15-18 states (one-third of the country) will be surveyed each year for exotic, non-native bark and ambrosia beetles.

States participating in the program will be responsible for selecting trapping sites in coordination with state agriculture (CAPS) survey committees. States will select approximately seven to nine sites (three traps per site) in high-risk forested areas or urban parks and forests. Traps samples will be collected every other week for 20 weeks. Samples can be prescreened by the states or sent to a regional prescreener. Screened samples then will be sent to an EDRR regional taxonomist for identification of all bark and ambrosia beetles. A new, national, Internet-based database will serve as the program's data entry, sample tracking, and reporting system. Data in this system will be compatible with APHIS' database, and information will be shared between the two programs.

The EDRR Pilot Project Team developed a protocol for notification of APHIS, state regulatory officials, and the Forest Service when a new, non-native species is found. The EDRR Team will notify all affected entities and discuss with APHIS a coordinated response. The APHIS New Pest Advisory Group (NPAG) will begin an assessment of the new find, and based upon the assessment, NPAG, APHIS and the EDRR Team will draft a response plan and coordinate response actions.

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CONCLUSION

The EDRR Pilot Project has identified an effective protocol for the rapid detection of new, non-native exotic scolytids and demonstrated that this protocol can be implemented nationally. Forest Service FHP, in cooperation with Forest Service R&D, APHIS, individual states, and universities, has begun to use this protocol to detect, assess, and respond to new introductions of non-native bark and ambrosia beetles in the U.S.