CALIFORNIA OAK MORTALITY TASK FORCE REPORT
AUGUST 2003

MONITORING/MANAGEMENT/REGULATIONS

On July 8, 2003, the Oregon Department of Agriculture (ODA) asked for the public's assistance in locating plants originating from a nursery in Stanislaus County, California that may have been infected with Sudden Oak Death. Anyone that purchased camellias after January 22, 2003 from one of Kasch's eight retail outlets in the Portland-Vancouver area was asked to contact ODA's Invasive Species Hotline at (866) INV-ADER. ODA staff has been responding to inquiries with onsite visits when necessary to determine if plants are symptomatic. If plants need to be collected, Kasch's is offering a refund or replacement plant.

So far, ODA has received almost 50 calls from Kasch's customers whom purchased 82 camellias in total. Nearly all of the 82 plant samples have been tested. To date, *Phytophthora ramorum* has only been found on one camellia plant, which has been destroyed. Kasch's Garden Centers and the ODA continue to ask for the public's assistance in locating these plants as it is estimated that as many as 300 camellias may have been sold.

First finding of *Phytophthora ramorum* on yew saplings - Containerized yew plants (*Taxus baccata*) were sampled for *Phytophthora ramorum* during a routine inspection of a medium-sized garden center in northwest England on May 8, 2003. The presence of *Phytophthora ramorum* was confirmed June 4th by the Department for Environment, Food & Rural Affairs' (DEFRA) Central Science Laboratory (CSL). Koch's postulates have been completed. Symptoms on the eighteen inch plants, in 2 liter containers, included leaf and stem dieback of top and side shoots.

REGULATIONS

APHIS has updated its list of *Phytophthora ramorum* host plants, categorizing them as either regulated or associated with the disease. Regulated plants are those that have been confirmed to be susceptible to *Phytophthora ramorum* through traditional Koch's postulates. Associated plants have only been confirmed using Polymerase Chain Reaction (PCR) or cultural isolation and are awaiting completion of Koch's postulates. Associated plants are not regulated and do not require certification under *Phytophthora ramorum* regulations, but they will be inspected in nurseries and Christmas tree plantations during inspections for other hosts, and if symptoms are observed, the plants will be held from sale pending testing.

An updated list of regulated and associated host species, as well as the federal regulations, will soon be posted to www.aphis.usda.gov/ppq/ispm/sod.

RESEARCH

The following is a draft Plant Disease Note provided by Everett Hansen, Oregon State University.

First report of A1 mating type of *Phytophthora ramorum* in North America. E.M. Hansen, P.W. Reeser, W. Sutton, and L. Winton; Dept. Botany and Plant Pathology; Oregon State University; Corvallis, OR 97331 and N. Osterbauer; Oregon Department of Agriculture; Salem, OR 97301.

Phytophthora ramorum is known from Europe and the West Coast of the United States (1). In Europe, it is found in nurseries and landscape plantings. In the United States, it has been confined to coastal forests and, in California, a few horticultural nurseries. All European isolates tested have been A1 mating type, while all North American isolates were A2 mating type (2). AFLP markers also indicated that the populations on the two continents are genetically distinct and that nearly all North American isolates are from a single clone (3). In June 2003, *Phytophthora ramorum* was isolated from diseased *Viburnum* and *Pieris* cultivars from a Clackamas County nursery and from a diseased *Camellia* cultivar from a Jackson County nursery. As part of the effort to determine the origin of these infestations, we tested the nursery isolates for mating type and compared their genotypes with those of known European and Oregon forest isolates using DNA microsatellite markers.

Mating type was determined by pairing seven Oregon nursery isolates, three Oregon forest isolates (representative of the predominant North American clone), and two European isolates. Agar plugs from 3-day-old colonies were placed in close proximity on carrot agar plates. Plates were examined for oogonia after three and 10 days (Brasier unpublished). Genotype was determined using four polymorphic microsatellite loci that distinguish *Phytophthora ramorum* isolates from Europe and North America (Winton, unpublished).

Oogonia and antheridia typical of *Phytophthora ramorum* formed when isolates from Clackamas County were paired with the Oregon forest isolates and when Jackson County isolates were paired with the European isolates. Sexual structures also formed in pairings between Oregon forest isolates and European isolates, but not in any other combinations. Microsatellite marker patterns of Clackamas County isolates were identical to the European isolates. Marker patterns of the Jackson County isolates were identical to the Oregon forest isolates.

These results indicate that the recent Oregon nursery infestations are of separate and distinct origins. The Clackamas County isolates are of A1 mating type and European genotype. According to shipping records, the nursery has received no host nursery stock directly from Europe. However, host nursery stock has been received from a Canadian nursery, suggesting this may be the source of this infestation. The Jackson County isolates are of A2 mating type and North American genotype. The latter result is consistent with the reported origin of these infested plants from a California nursery (CDFA, personal communication). The Oregon nursery infestations highlight the dangers of unregulated or under-regulated transport of host nursery stock from infested areas to non-infested areas. All host plants from infested nursery blocks at the affected

Oregon nurseries have been destroyed by incineration and a regular monitoring program implemented. Other host nursery stock on site has been taken off-sale pending verification of free-from disease status per USDA, Animal and Plant Health Inspection Service requirements.

References : (1) Davidson, J.M., et al. Plant Health Progress. (http://www.plantmanagementnetwork.org/pub/php/diagnosticguide/2003/sod/), 2003. (2) Werres, S. et al. Mycol. Res. 105:1155, 2001. (3) Ivors K, et al. Sudden Oak Death Science Symposium, 15-18 December 2002, Monterey, California. Online: (danr.ucop.edu/ihrmp/sodsymp/ paper/paper17.html), 2002.

***Phytophthora ramorum* from camellias in a nursery in Stanislaus County, CA was identified as the A2 mating type with AFLP and RFLP patterns identical to the U.S. clone genotype - by Kelly Ivors, Daniel Huberli, and Matteo Garbelotto; UC-Berkeley**. The isolate of *Phytophthora ramorum* from *Camellia sasanqua* 'Bonanza,' recovered from container plants at a wholesale nursery near Oakdale, was tested via restriction fragment length polymorphism (RFLP) and amplified fragment length polymorphism (AFLP). All molecular tests indicated this isolate was genetically similar to other California and Oregon isolates, with fragment patterns distinct from European isolates.

Huberli conducted mating tests for the Camellia isolate from Stanislaus County with known A1 and A2 isolates on carrot agar using the method described by Clive Brasier (unpublished). After two weeks, sexual spores (oospores) were observed in the cross with the A1 tester indicating that the Camellia isolate is mating type A2 (North America). An additional 12 isolates with eight different AFLP profiles from California were examined. Eight isolates were mating type A2, while 4 isolates did not produce oospores. The capacity to produce oospores of isolates varied with some producing very low to abundant (one isolate) numbers. The isolate producing abundant oospores was also one of the most pathogenic isolates and researchers fear may be a potential threat if it is able to pair with an A1 European isolate in the native ecosystem. Data of the 12 isolates will be presented in a paper to be submitted to Mycological Research titled "Phenotypic variation among eight AFLP genotypes of *Phytophthora ramorum* from California and Oregon." These tests indicate this Stanislaus isolate mostly likely originated from the established California population.

For an explanation of AFLP, RFLP and mating tests, see "Distinguishing mating and United States/European genetic types of *Phytophthora ramorum"* under the Learning Curve.

LEGISLATION

On July 15, 2003, Representative Lynn Woolsey (D-Petaluma) secured $3.2 million in the House of Representatives for Sudden Oak Death funding. The funds were approved as part of H.R. 2673, the Agricultural Appropriations bill for fiscal year 2004.

If the Bill is approved by the Senate and then signed, it would provide $1.1 million to USDA-Agricultural Research Service (ARS) for continued research into the possible causes of the disease, the pathogen's host range, disease biology, and modes of spread. Funding would also go to a new genomics research position ($250,000) at the UC Davis ARS laboratory to study the disease at a genetic level, identifying unique and common features of *Phytophthora ramorum*'s resistance mechanisms. In addition, USDA-APHIS would receive $2 million to continue regulatory monitoring efforts as well as inspecting plants to prevent the disease from spreading.

MANAGEMENT

A national Sudden Oak Death/*Phytophthora ramorum* strategic plan is being developed for Congress under the direction of Charles G. (Terry) Shaw, USDA-Forest Service, Research, Washington D.C. A group of federal officials representing the USDA, including the Forest Service, Agricultural Research Service (ARS), and Animal and Plant Health Inspection Service (APHIS), met July 21 - 22 to develop the plan. The report will articulate USDA Agency roles and responsibilities over the next 5 years for prevention, detection, monitoring, control, and restoration. A draft of the report will be available for review in late August. For more information, contact Terry Shaw at cgshaw@fs.fed.us.

WEB NEWS – WWW.SUDDENOAKDEATH.ORG

A new Sudden Oak Death general brochure is now available on the Task Force website under Publications and Resources, Task Force Publications. The brochure can be downloaded and printed out. Also available on the Publications page is the updated Symptoms Guide, a comprehensive resource of current *Phytophthora ramorum* hosts and symptoms.

A larger, more comprehensive bibliography of *Phytophthora ramorum* scientific literature is now available on the Task Force website under Publications and Resources, Science and Management Materials, Bibliography. Citations include journal articles, links to abstracts, agency reports, and technical publications.

THE LEARNING CURVE

Distinguishing mating and United States/European genetic types of *Phytophthora ramorum* - Regulators and plant pathologists inspecting for *Phytophthora ramorum* on nursery stock and other commodities are relying on new laboratory methods to determine genetic similarity of *Phytophthora ramorum* isolates. These visual and molecular tests have allowed scientists to identify pathways of migration and determine the potential origin of new *Phytophthora ramorum* finds. The next few paragraphs explain the potential significance of mating and genetic types of *Phytophthora ramorum* and the methods scientists are using to distinguish them.

In nature, *Phytophthora ramorum,* type A1, has been found only in European rhododendron, camellia, and other horticultural plants in nurseries and public gardens. It was recently intercepted in nurseries in British Columbia, Washington, and Oregon, but all infested plants have been destroyed. *Phytophthora ramorum*, type A2, is present only

in California and Oregon. Since these two mating types are geographically isolated from one another, it is assumed each population is reproducing asexually (via zoospores and chlamydospores), hence there is limited genetic recombination occurring within pathogen populations.

Plant pathologists are concerned that movement of *Phytophthora ramorum* on nursery stock will juxtapose the A1and A2 mating types, possibly allowing for sexual reproduction via oogonia and antheridia, creating new genetic types that are potentially more virulent or fungicide resistant and/or capable of exploiting new habitats and host species. Such traits may have deleterious impacts on forest and nursery plants. These concerns are based on observations of variation and race migration in other Phytophthoras, most notably *Phytophthora infestans,* cause of potato late blight. Potato late blight has been a problem for over 150 years, but recently the A2 race became widespread, causing earlier and more severe late blight attacks on potatoes. (Niederhauser 1997. See http://www.apsnet.org/online/lateblite/papers/lb014.htm.)

Although *Phytophthora ramorum* isolates recovered from Europe (A1) and isolates from California and Oregon (A2) are considered the same species since their DNA is very similar, there are molecular, morphological, and physiological differences between them. Molecular biologists theorize that the functional parts of the DNA (the genes that code for structural or physiological functions) are identical in all organisms of a particular species. For example, if a person didn't have the genes to code for globulin, they couldn't survive, so there is no surviving variation in the human globulin gene. But, many other human traits can vary, and individuals still survive. For instance, we have various genes that that code for hair, eye color, and gender. These areas of DNA, and others where the function is not known, vary among individuals within a species.

Phytophthora species also have regions of DNA that are non-essential and variable among individuals within a species. Variable DNA is found in different parts of the genome, has different characteristic patterns, and is analyzed in laboratories via various methods given different names (AFLP, RFLP, microsattelite). These variable DNA regions form the basis for molecular tests. Note: There are both mating type and genetic differences between the *Phytophthora ramorum* isolates recovered in Europe and North America.

How can scientists distinguish *Phytophthora ramorum's* mating and genetic types?

Mycologists and plant pathologists use visual differences, mating tests, and molecular analyses to distinguish between *Phytophthora ramorum* mating and genetic types. The first step in identifying *Phytophthora ramorum* is to isolate or culture the organism from a diseased plant. Scientists cut up small pieces of symptomatic tissue and embed them in growing media selective for the pathogen. After a few days, under proper incubation, temperatures, and light conditions, *Phytophthora ramorum* may grow out of the plant tissue onto the growth media. These methods were detailed recently in a publication by Davidson, J.M., and others in Plant Health Progress at:

http://www.plantmanagementnetwork.org/pub/php/diagnosticguide/2003/sod/.

Once cultures or isolates are obtained, they are analyzed with applied and molecular methods. Applied methods include comparing isolates' growth rates in petri dishes and examination of colonies under a microscope to compare the size, number, and color of various spore types (chlamydospores and sporangia containing zoospores).

In addition to these visual evaluations, mating type tests are done. *Phytophthora ramorum* is heterothallic**.** To form sexual reproductive structures, called oogonia (female structures) and antheridia (male structures), two opposite mating types must be present. Once the oogonium has been fertilized by the antheridum, oospore (sexual spore) development occurs. In nature, sexual structures of *Phytophthora ramorum* have not been found; oospores have only been observed in the laboratory as a result of scientists crossing the A1 isolates from Europe with A2 isolates from the United States. To determine the mating type of an isolate, a small piece of the isolate (called an agar plug) is placed on a petri dish near a small piece of an isolate of known mating type (called a tester). The colonies grow into one another and if they are different mating types, sexual recombination occurs and structures called oogonia and antheridia are produced after 3 to 14 days. These methods were first developed by Sabine Werres (Werres, S. et al. Mycol. Res. 105:1155, 2001) and refined by Clive Brasier (unpublished).

The genotype or isolates' DNA can be analyzed using molecular methods. Many different regions of *Phytophthora ramorum* DNA may be used; various laboratories have found variations in DNA (called polymorphisms) and used these different patterns to identify European vs. United States isolates. Everett Hansen and Lori Winton at Oregon State University compared portions of the DNA called microsatellites. Microsatellites are short strings of repeated nucleotides that are recognizable. These short strings vary consistently between *Phytophthora ramorum* geographic types, so they are useful genetic markers. For *Phytophthora ramorum*, four loci**,** or areas of DNA, were used to distinguish the European and North American genotypes.

Winton and Hansen developed polymerase chain reaction (PCR) techniques to identify and compare these microsattelite DNA markers. The DNA of the unknown isolate is placed in vials with probes made from the microsattelite DNA. The DNA from the unknown isolate binds with the DNA probe in a pattern diagnostic for each genetic type. The microsatellite DNA analysis and mating test results are compared to make sure that both tests provide the same results and indicate that the isolate is either European (A1) or North American (A2).

In addition to the microsatellite DNA comparison, other techniques or parts of *Phytophthora ramorum's* DNA or genome are used to distinguish European and United States genetic types. In Matteo Garbelotto's laboratory, UC Berkeley, Kelly Ivors and Daniel Huberli conducted a series of tests to determine mating and genetic types of *Phytophthora ramorum* isolated from the infected Camellias found in a nursery in

Stanislaus County, CA. They used restriction fragment length polymorphisms (RFLPs) and amplified fragment length polymorphisms (AFLPs) analyses. Basically, both methods use enzymes to cut up isolates' DNA into small pieces, then these DNA fragments can be analyzed and compared between samples. If the sample has a genetic profile similar to the European type, it will produce a pattern characteristic for the known European types; if the sample has a genetic profile similar to the United States type, it produces a different pattern, typical of U.S. isolates. They also did the mating test described above and looked for sexual spores, or oospores.

The molecular methods used to distinguish *Phytophthora ramorum's* mating type and those used to identify *Phytophthora ramorum* from other species of *Phytophthora* are continually being improved and refined. The Joint Genome Institute in Walnut Creek is sequencing the entire genome of *Phytophthora ramorum* which with further investigations will lead to an increased understanding of the molecular make-up of *Phytophthora ramorum* and improved diagnostic methods for detection of this newly described pathogen.

HOST OF THE MONTH

***Arbutus unedo* (strawberry tree) -** Strawberry tree is native to southern Europe and Ireland and is a common landscape tree in low-lying areas of the western United States that do not have severe winters. Since it is drought tolerant and attractive, it is commonly planted in the Bay Area. It is closely related to madrone (*Arbutus menziesii*), a native to the Pacific Coast of the United States and British Columbia, and also a host for *Phytophthora ramorum.*

Strawberry trees are attractive and compact, with smooth, shredding reddish bark, and grow to about 35 feet in height. It has clusters of small white urn-shaped flowers in the fall and winter, and round fruits turning from yellow to red that take a year to ripen. It is tolerant of a wide range of moisture conditions, wind, and pollution.

This spring, *Phytophthora ramorum* was isolated from strawberry trees and camellias in a garden center on the island of Majorca, Spain. *Phytophthora ramorum*-infected rhododendron and viburnum plants had been confirmed in this garden center prior to the new finds; consequently, it is believed that the camellia and strawberry trees were infected on-site. Symptoms of the disease on strawberry tree are similar to those on madrone, with leaf necrosis extending down the leaf, through the petiole and into the twig. Strawberry tree leaves have been found to be highly susceptible to *Phytophthora ramorum* in laboratory tests. (Moralejo and Hernandez, 2002. Inoculation Trials of *Phytophthora ramorum* on Detached Mediterranean Sclerophyll Leaves. Sudden Oak Death Science Symposium, http://danr.ucop.edu/ihrmp/sodsymp/paper/paper25.html.) The susceptibility of strawberry tree is not only of concern to the nursery industry and gardeners in Europe and North America, but for the natural ecosystem of the Mediterranean, since this species occurs extensively in this area (Moralejo and Descals, 2003. Risk analysis of *Phytophthora ramorum* establishment in the Mediterranean area,

Sudden Oak Death Online Symposium,
http://www.apsnet.org/online/sod/Papers/Moralejo_Descals/default.htm.)