***Large-scale Progeny Testing of Oregon Ash***

***A Foundation for Conservation and Breeding in Response to Emerald Ash Borer (EAB)***

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**Background**

Oregon ash (*Fraxinus latifolia*) and other North American ash species are highly susceptible to emerald ash borer (EAB, *Agrilus planipennis*), an introduced insect pest. The devastation caused by EAB in eastern North America has led to the listing of five ash species as being at risk of extinction by the International Union for Conservation of Nature (IUCN). EAB has killed millions of ash trees in the eastern U.S. However, there appears to be at least a low level of genetic resistance in species tested so far. Thus, there is promise for achieving useful levels of genetic resistance in Oregon ash via aggressive breeding. There is much concern about the future impacts of EAB on Oregon ash and associated ecosystems. In Oregon, EAB was first documented in the summer of 2022 in the Forest Grove area. By proactively developing a testing and breeding program, we may be able to maintain or restore Oregon ash using genetically resistant trees after most standing trees have been killed.

Because of concerns about the impact of EAB, gene conservation seed collections began in 2019, focusing on the Oregon portion of the species' range. Seed collections were expanded to California and Washington and will continue in fall 2023. These collections will serve as a foundation for gene conservation and breeding. Seed collections were made by the Oregon Department of Forestry (ODF), with a funding match from the USFS, USFS Dorena Genetic Resource Center (DGRC), and various cooperators. Two small genetic field tests (~27 and 26 open-pollinated seedlots, 26 in common) were recently established in Oregon (2020) and Washington (2021) at the DGRC and Washington State University Puyallup Research Center. Some of these materials were also sent to the USFS Northern Research Station for future testing of EAB resistance.

We propose to use existing and new seed collections to establish genetic test plantations throughout the current and future potential distribution of Oregon ash. These plantations could serve as the foundation for a breeding program aimed at conserving the species and as an archive of genetically characterized trees for future work on the fundamental aspects of resistance to EAB and other stressors. Given the scope of the threat and the importance of Oregon ash both environmentally and economically, the scale of the proposed effort is large; comparable to that of other large testing and breeding programs for forest tree species and horticultural tree crops. This large effort could be accomplished by organizing concerted efforts among interested individuals and organizations. USFS collaborators might include the National Forest system (including DGRC), USFS Research Stations, Forest Health Protection, and Western Wildland Environmental Threat Assessment Center. Other federal and state collaborators might include the Bureau of Land Management, Oregon Departments of Agriculture and Forestry, and Washington Department of Natural Resources. Finally scientific expertise is likely to be available from Oregon State University, Washington State University, and Penn State.

**Objectives**

This proposal focuses on the establishment of genetic test plantations of Oregon ash in the PNW. These test plantations will (1) act as sentinel plantings for genetic resistance, (2) give us a jump-start toward measuring genetic resistance to EAB in the field, (3) lead to the development of deployment zones for Oregon ash based on climate adaptation, and (4) serve as an open resource for other scientists studying EAB and the genetics of ash species. Ultimately, the tested parents and progeny will serve as the base breeding population for an Oregon ash breeding program for EAB resistance. We propose to form a

steering committee to guide the development of a long-term breeding plan for Oregon ash. This breeding plan will describe approaches for (1) collecting seedlots and creating a base breeding population, (2) propagating trees for outplanting, (3) establishing a series of progeny test plantations, and (4) evaluating genetic resistance to EAB and developing deployment zones.

**Steering committee**

We will form a steering committee to design a long-term breeding plan for Oregon ash and guide the establishment of a series of Oregon ash progeny test plantations. The steering committee will consist of tree breeders, professionals involved in collecting Oregon ash seedlots, individuals with professional experience in nursery/greenhouse production of tree seedlings, experts experienced in the establishment of genetic test plantations, and scientists engaged in fundamental research on EAB resistance.

**Collaborators and funding**

Collaborators are needed to provide sites for the establishment and maintenance of genetic test plantations. Collaborators may consist of state and federal agencies, tribal and municipal governments, private companies, and NGOs. Written agreements will be developed as needed to allow these collaborations to succeed. Many forest research and seed orchard collaborative agreements exist throughout the PNW that can serve as good models for these agreements. The steering committee will investigate sources of funding for this work. We expect that multiple sources of cash and in-kind funding will support different aspects of the project.

**Seed collections and base breeding population**

We propose that the base breeding population for Oregon ash should ultimately consist of many hundreds or thousands of genotypes (i.e., 'unrelated' trees). This number of genotypes is needed for two reasons. First, observations from eastern ash species suggest that genetic variation for insect resistance is very low. Second, because the intent is to help maintain or restore Oregon ash throughout its current and future potential distributions, the base breeding population should contain genotypes across the range of environments it currently occupies. To date, open-pollinated seedlots have been collected from trees in Oregon, California, and Washington (Appendices). Collections will need to continue in future years to complete the base breeding population. The final number of genotypes to be included in the base breeding program will be determined by the steering committee and described in the breeding plan.

**Plant propagation**

Initially, we expect to produce seedlings for outplanting under contract using a commercial or governmental nursery that has demonstrated experience in producing hardwood seedlings. Because clonal tests have large advantages, the breeding plan will explore the potential for clonally propagating ash trees via rooted cuttings.

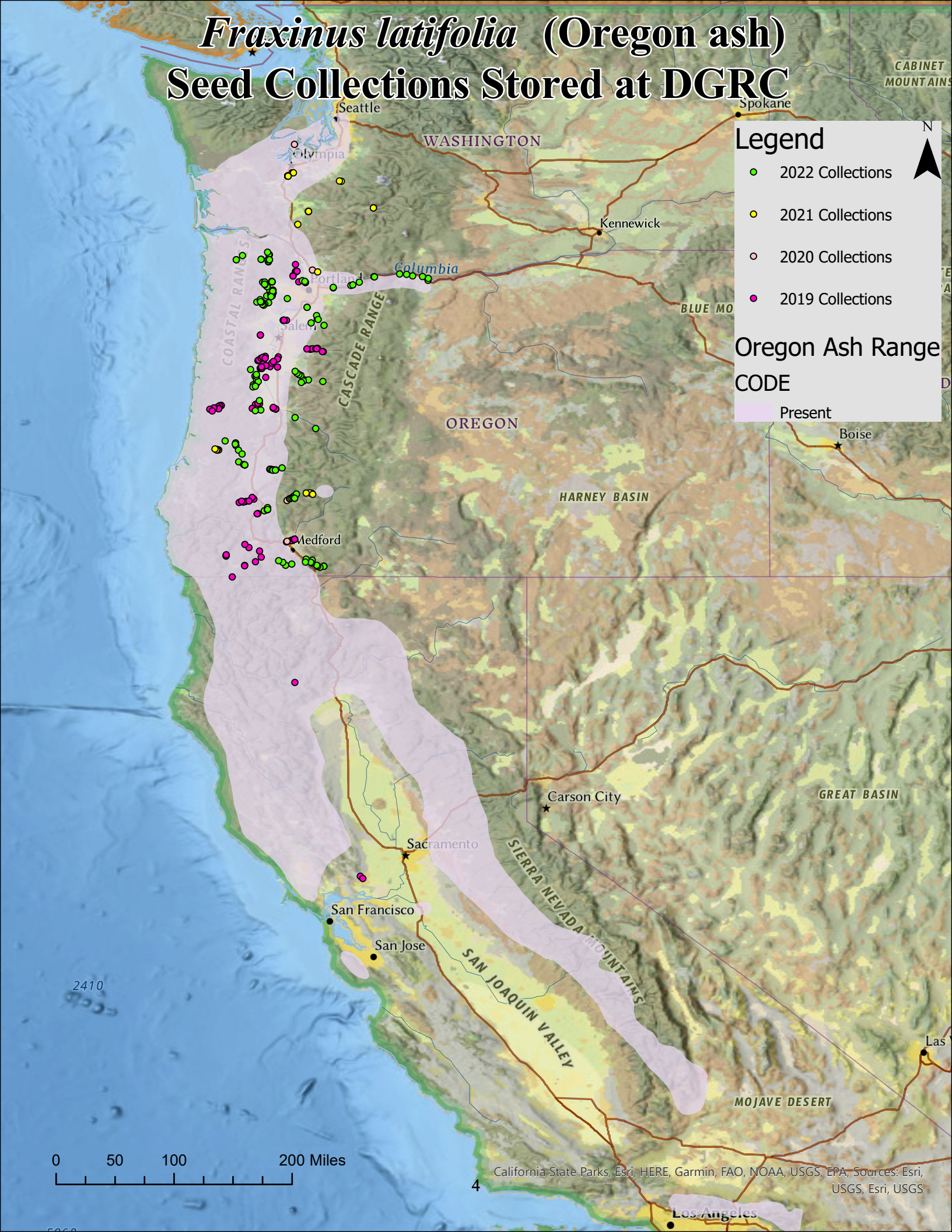
**Progeny test plantations**

We propose to establish progeny test plantations within the current and the future modeled distributions of Oregon ash. We propose to focus on field testing for three reasons. First, the number of genotypes we need to screen likely exceeds the capacity of indirect testing methods. Second, field tests are good for understanding the composite effects of various mechanisms of resistance (e.g., developmental, physiological, biochemical) and genotype by environmental interactions. Third, the field tests will serve as archives of known genetic materials that can be sampled to address fundamental research questions about insect resistance. To sample the range of Oregon ash and to accommodate thousands of genotypes, the target number of field tests is expected to be $\geq 30$, planted over a period of years (test series). Each test series will test a subset of the base breeding population (e.g., 300-500 open-pollinated families) with 'linking' families to allow comparisons to be made across test series. The structure of the progeny test plantations will be described in the breeding plan developed by the steering committee. This plan will describe the (1) target number of plantations in each series and in total, (2) geographical distribution of plantations, (3) test design (e.g., plot size and number and type of blocks, such as incomplete versus complete block designs), and (4) measurement schedule. To obtain early results on insect resistance, we

anticipate that some plantations will be specifically established near current and future sites of EAB establishment.

The progeny tests will be planted on sites owned by private and non-private cooperators. In addition to the criteria described above, we will choose sites based on the ability of the cooperator to maintain the site until the trees become well established. To make these plantations valuable, aggressive weed control is expected to be needed in the first 5 years or so via mowing and/or herbicide application.

# *Fraxinus latifolia* (Oregon ash) Seed Collections Stored at DGRC



# DGRC Stored Oregon Ash Seed Collections WA, OR, CA 2019-2022

| Oregon | |
|---|---|
| Counties | # of seedlots collected |
| Benton | 26 |
| Clackamas | 5 |
| Clark | 2 |
| Clatsop | 3 |
| Columbia | 21 |
| Douglas | 55 |
| Hood River | 4 |
| Jackson | 24 |
| Josephine | 8 |
| Lane | 36 |
| Linn | 17 |
| Marion | 16 |
| Multnomah | 12 |
| Polk | 2 |
| Wasco | 4 |
| Washington | 28 |
| Yamhill | 11 |
| Total | 274 |

| Washington | |
|---|---|
| Counties | # of seedlots collected |
| King* | 5 |
| Thurston* | 43 |
| Lewis* | 15 |
| Clark | 1 |
| Cowlitz | 1 |
| Mason | 1 |
| Pierce | 2 |
| Skamania | 1 |
| Total | 69 |

*includes Huntington 2022 collections not yet arrived at Dorena

| California | |
|---|---|
| Counties | # of seedlots collected |
| Sonoma* | 2 |
| Humboldt* | 2 |
| Siskiyou* | 1 |
| Solano | 2 |
| Del Norte | 1 |
| Trinity | 1 |
| Total | 9 |

*includes Huntington 2022 collections not yet arrived at Dorena

Efforts by various organizations, such as the Oregon Department of Forestry, The Huntington Library, USFS (United States Forest Service), and private cooperators, have been successful in collecting 352 separate Oregon Ash (*Fraxinus latifolia*) seedlots from Washington, Oregon, and California since 2019. However, the representation of seed collections varies across different counties within the range of Oregon ash.

In Washington, Oregon ash occurs within 11 different counties. Out of these 11 counties, 8 counties are represented in the seed collections, while Grays Harbor County, Pacific County, and Wahkiakum County are currently not represented.

In Oregon, Oregon ash occurs in 21 counties. Among these 21 counties, 17 counties are represented in the seed collections. However, the four coastal counties of Tillamook, Lincoln, Coos, and Curry do not have any representation in the seed collections.

In California, Oregon ash has a range that spans over 33 counties. Currently, only six counties are represented by seed collections, indicating a limited representation of Oregon ash seeds from California.