Diseases Caused by Oomycetes

First Report of the NA2 Clonal Lineage of the Sudden Oak Death Pathogen, *Phytophthora ramorum*, Infecting Tanoak in Oregon Forests

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Phytophthora ramorum Werres, de Cock & Man in't Veld, causal agent of sudden oak death (SOD) and ramorum leaf blight, is comprised of four clonal lineages in its invasive ranges of North America and Europe (Grünwald et al. 2012; Van Poucke et al. 2012). Of these, three – the NA1, NA2, and EU1 lineages – are found in U.S. nurseries, but only two, the NA1 and EU1 lineages, have been found infecting trees in North American forests (Grünwald et al. 2012, 2016). In the spring of 2021, tanoak (*Notholithocarpus densiflorus* Manos, Cannon & Oh) displaying symptoms consistent with SOD were detected north of Port Orford (Curry County, Oregon). Symptoms were canopy dieback and blackened petiole and stem lesions on tanoak sprouts. The pathogen isolated on PAR (corn meal agar plus 200 ml/liter of ampicillin, 10 mg/liter of rifamycin, 66.7 mg/liter of pentachloronitrobenzene) selective media was determined to be *P. ramorum* based on characteristic morphology of hyphae, sporangia, and chlamydospores (Werres et al. 2001). Positive identification as *P. ramorum* was obtained with a LAMP assay targeting a lineage-specific NA2 gene, indicating the presence of the NA2 lineage. NA2 was confirmed by sequencing a portion of the cellulose binding elicitor lectin (CBEL) gene using CBEL5U and CBEL6L primers (Gagnon et al. 2014).

Sequences (GenBank accessions MZ733981 and MZ733982) were aligned against reference sequences for all lineages (Gagnon et al. 2014), confirming the presence of NA2. Lineage determination as NA2 was further confirmed at 11 SSR loci (ILVOPrMS145, PrMS39, PrMS9C3, ILVOPrMS79, KI18, KI64, PrMS45, PrMS6, ILVOPrMS131, KI82ab, and PrMS43) using the methods of Kamvar et al. (2015). We completed Koch's postulates using potted tanoaks, wound-inoculated at the midpoint of 1-year old stems with either hyphal plugs or noncolonized agar ($n = 4$ per treatment). Tanoaks were maintained in a growth chamber (20°C-day/18°C-night temperatures) with regular watering and an 18-h photoperiod using F32T8 fluorescent bulbs (Phillips, Eindhoven, The Netherlands). After 7 days, brown to black lesions 1.2 to 2.9 cm in length were observed on the inoculated stems, from which *P. ramorum* was subsequently reisolated; no symptoms were observed on the controls, and no pathogens were recovered when plating the wound sites in PAR. This is the first detection of the NA2 lineage causing disease in forests worldwide. The outbreak was found on private and public lands in forests typical to the SOD outbreak in Oregon (mixed conifer and tanoak), and was 33 km north of the closest known *P. ramorum* infestation. Follow-up ground surveys on adjacent lands have identified over 100 *P. ramorum*-positive tanoak trees, from which additional NA2 isolates have been recovered from bole cankers. NA2 is thought to be more aggressive than the NA1 lineage (Elliott et al. 2011), which has been present in Curry County since the mid-1990s (Goheen et al. 2017). Eradication of the NA2 lineage is being pursued to slow its further spread and prevent overlap with existing NA1 and EU1 populations. The repeated introductions of novel lineages into the western United States native plant communities highlights the vulnerability of this region to *Phytophthora* establishment, justifying continued monitoring for *P. ramorum* in nurseries and forests.

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