STREAM BAITING FOR SUDDEN OAK DEATH: FLUVIAL TRANSPORT AND ECOHYDROLOGY OF THE INVASIVE PLANT PATHOGEN *PHYTOPHTHORA RAMORUM* IN WESTERN WASHINGTON STATE.

by

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ABSTRACT

Stream baiting for sudden oak death: fluvial transport and ecohydrology of the invasive plant pathogen *Phytophthora ramorum* in western Washington State.

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Sudden oak death is the vernacular name given to a relatively newly discovered plant pathogen, *Phytophthora ramorum*. *P. ramorum* is transported in the nursery trade on plants such as rhododendron, camellia and viburnum. Many of Washington's native plants are potential hosts for the pathogen. *P. ramorum* has been found on plants and in soil, potting mix and surface waters in nurseries in Washington. This thesis looks at two years of detection efforts by the Washington State Department of Agriculture. WSDA baited six streams in 2006, and eight in 2007, for a total of 11 different streams, all but one of which are associated with infested nurseries. Of these streams, *P. ramorum* has been found in only three. Positive stream finds in all streams show a correlation with rising temperatures and decreasing precipitation in spring, particularly April. This thesis looks at factors that may explain why *P. ramorum* was found in these few streams, and not in others. Five nurseries are compared by soil and hydrologic factors. Of these five nurseries, only one has a stream that has become infested with *P. ramorum*, with multiple successful traps retrieved over the two year period. This nursery has distinct soil and hydrologic features, which may be diagnostic for sites conducive to the escape of *P. ramorum* from the nursery environment to establishment in the wider landscape - in particular, coarse, gravelly, shallow, sloped soil. While the nursery itself has apparently been cleared of *P. ramorum*, the stream remains infested. Potential pathways for the storage and transport of the pathogen in the watershed are explored.

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Introduction

Description of Sudden Oak Death and *Phytophthora ramorum*

Sudden oak death (SOD) is the vernacular name given to a disease caused by a newly discovered species of *Phytophthora*, a genus of fungi-like plant pathogens and soil microbes. In the mid 1990s, arborists and residents of Marin and Santa Cruz Counties, California, noticed that coast live oak *(Quercus agrifolia)* and tanoak *(Lithocarpus densiflorus)* were suddenly dying for no apparent reason. Tree crowns changed color from green to brown over the course of a few weeks, and trees developed bleeding cankers. At about the same time, workers in nurseries in Germany and the Netherlands noticed a new leaf blight/stem canker/tip dieback disease on their rhododendrons and viburnums. In 1993 it was determined that the causative agent of the nursery disease is a new species of *Phytophthora*. In 2000 the causative agent of sudden oak death was also determined to be a new species of *Phytophthora*. See Garbelotto (2004), CPHST (2004) and Goheen et al. (2006) for a history of the discovery of *P. ramorum*.

By the end of 2000 it was realized that the new *Phytophthoras* in European nurseries and California oak woodlands were the same species, despite producing very different diseases on different, unrelated hosts. See COMTF (2008) for a chronology of events. By January of 2001, this new disease was found on rhododendrons in a Santa Cruz County nursery. To prevent the spread of this deadly, mysterious, incurable disease, Oregon was the first to quarantine SOD host plants from California, followed within months by Canada. Despite the quarantine, by July of 2001, SOD had been found on tanoak in Curry County, Oregon. It had already spread beyond Marin and Santa Cruz Counties to be found in Mendocino, Monterey, Napa, San Mateo, Santa Clara, and Sonoma Counties and was soon found in Alameda and Solano Counties. Spread of the disease seemed uncontrollable, and more countries imposed quarantines on known host material from California and Oregon. Meanwhile the host list was growing: evergreen

huckleberry (*Vaccinium ovatum)* and Shreve's oak *(Quercus parvula var. shrevei)* were confirmed susceptible early in 2001, followed quickly by madrone (*Arbutus menziesii)* and bay laurel *(Umbellularia californica)*. More plants are added every few months; by April of 2002 the host list is 15 species long.[1]

The new *Phytophthora* species was officially named *Phytophthora ramorum* in April of 2001 (COMTF 2008). Most *Phytophthoras* are soil dwelling and saprophytic, and the pathogenic species are mainly root rotting agents. A few, such as *P. infestans*, the causative agent of potato late blight, will colonize leaves as well as roots, but strictly foliar *Phytophthoras* are few in number. The specific epithet *ramorum* refers to the foliar habitat of this species, although Parke (2007) has shown that root infections are possible and can lead to stem cankers and plant death in rhododendrons.

A plant disease is distinct from the pathogenic species that causes the disease. Disease does not develop automatically in the presence of the pathogen, and a single pathogenic species can cause more than one disease. In plant pathology, disease is considered to be a separate population from the causative agent, so three populations are involved in any epiphytotic: host plant, pathogen, and the disease itself (Hau and Kranz, 1990). Disease development requires all three legs of what is called the disease triangle (Strange 2003): presence of host, pathogen, *and* a conducive environment - thus the distinction between pathogen and disease. If the environment is wrong (i.e. too hot or too dry), no disease will develop regardless of how much pathogen is present on host material. And if no appropriate host is present, a conducive environment will not produce disease even in the presence of pathogen.

Phytophthora ramorum causes two distinct diseases: sudden oak death and *P. ramorum* leaf and stem blight (CPHST 2004). There are two kinds of hosts for *P.*

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[1]See http://nature.berkeley.edu/comtf/html/host_plant_lists.html for most current host list.

ramorum, reflecting the different diseases it causes: bark canker hosts (SOD) and foliar hosts (blight). Tanoak is unique among hosts in developing both kinds of diseases (Goheen et al. 2006). Bark canker hosts are those most likely to die of the disease, while foliar hosts rarely die. Foliar hosts propagate the disease through sporulation on leaf surfaces, while bark canker hosts do not support sporulation; bark canker hosts in the absence of foliar hosts do not develop the disease (Davidson et al. 2005). Bark canker hosts are something of a dead-end for *P. ramorum* (Parke and Lucas 2008). While each individual bark canker host presents a huge amount of biomass for the pathogen to feed on, it does not seem able to spread to other hosts from bark canker hosts (Davidson et al. 2005). Part of the environmental requirement for development of SOD is presence of foliar hosts that support copious sporulation, in particular California bay laurel, coast redwood, and tanoak (CPHST 2004, Goheen et al. 2006).

Damage to oaks

Tanoak *(Lithocarpus densiflorus)* is the sole North American representative of a mainly Asian genus, close relatives of oaks, and is the most susceptible of hosts of *P. ramorum* (Goheen et al. 2006). Tanoak displays the full range of symptoms of both diseases: bark and stem cankers, leaf lesions, tip dieback, leaf flagging, defoliation and shepherd's crooking of stems. All ages of tanoak are susceptible and death of even mature trees can occur within one year of infection (CPHST 2004).

The oak genus *(Quercus)* is divided into different groups by different authors. In general, there are white oaks and red oaks (Brockman 1986). Live oaks, the evergreen species, are in the red oak group. Various species of red oaks are attacked by *P. ramorum* (Garbelotto 2004, Goheen et al. 2006). Two species of white oak have been found susceptible in inoculation trials, but not elsewhere (COMTF 2008). The pathogen has not yet been reported on any of the white oaks native to the Pacific Coast states, including Washington's only native oak, *Quercus garryana*. The California coast live oak, *Quercus*

agrifolia, is the primary oak victim, second only to tanoak (CPHST 2004). Oaks develop bleeding cankers on the main trunk and large branches. So far these cankers have been restricted to above ground parts, unlike soil dwelling *Phytophthora* (Garbelotto 2004). Death may occur within a few weeks of first noticeable symptoms, or the tree may decline slowly over a period of a few years, and is caused by trunk girdling by the cankers. In either case the tree had been infected for some time, up to two years, before displaying symptoms of distress, at which point the tree is already dead (Garbelotto 2004). Leaf lesions and shoot symptoms are unknown so far in oaks. The disease appears to be restricted to mature trees in oaks, unlike tanoaks, which can be attacked as seedlings or saplings as well as mature trees (CPHST 2004).

The oak relatives *Fagus grandifolia* and *Castanea dentata,* American beech and chestnut, are also potential bark canker hosts. At this time the disease does not occur in their native range (CPHST 2004).

Other wildland hosts

Other hosts along with oak and tanoak are broadleaf evergreen foliar hosts that develop *P. ramorum* blight: bay laurel *(Umbellularia californica)*, madrone *(Arbutus menziesii)*, evergreen huckleberry *(Vaccinium ovatum)* and the native rhododendron *(Rhododendron macrophyllum)*. Coast redwood *(Sequoia sempervirens)* and tanoak both appear to be important spore-producing hosts as well (Goheen et al. 2006). Bay laurel develops leaf lesions but no dieback or mortality. Madrone and rhododendron develop leaf lesions and twig and stem dieback. Rhododendrons may die from stem dieback, but so far madrones do not. Evergreen huckleberries develop twig and stem cankers and dieback but not observable leaf lesions. Plants may die from stem dieback. These foliar hosts are the ones responsible for spread of *P. ramorum* through sporulation on leaf lesions (Garbelotto 2004). One of the primary factors in forest susceptibility to SOD is

presence of California bay laurel, necessary for spore production to infect oaks and tanoaks (Garbelotto 2004, Goheen et al. 2006).

Damage to nursery plants

The nursery hosts are primarily foliar hosts: rhododendron, viburnum, pieris, kalmias and camellia (Goheen et al. 2006). Rhododendrons may show small cankers on twigs, while viburnums and pieris can develop shoot and twig dieback as well as leaf lesions. Camellias so far show only leaf lesions and defoliation.

Death of nursery hosts is not typical, although rhododendrons and huckleberries can sometimes die from the disease. The real danger with foliar hosts is increased sporulation and long-distance transport of spores to other hosts in other locations. Pieris and viburnum support high levels of sporulation. Camellia, because of its propensity for defoliation, supports very little sporulation (CPHST 2004, Goheen et al. 2006).

Life History of *Phytophthora ramorum*

Phytophthora ramorum is a member of the water molds (Oomycota), which are similar in many ways to true fungi (Parke and Lucas 2008). The *Phytophthoras* used to be classified as primitive fungi, and are often still referred to as such. The primary differences between water molds and true fungi are: cell walls composed of cellulose in water molds and chitin in fungi; motile zoospores with two kinds of flagella in water molds but only one kind of flagella in fungi (most fungi have no flagella); diploid mycelium in water molds but haploid or dikaryotic in fungi; and production of oospores in water molds but not in fungi (Rossman and Palm 2006). See Strange (2003), chapter 1.3.5 and Erwin and Ribeiro (1996), chapter 1 for a discussion of plant pathogenic fungi and water molds.

As in fungi, the body of the organism is the mycelium, which is a collection of hyphae, or fungal threads (Strange 2003). Individual hyphae of the mycelium grow between cells of the host plant, or through soil pore spaces, absorbing and metabolizing food. As long as environmental conditions are favorable, the hyphae continue to grow. Should conditions become too dry or otherwise suboptimal, growth slows and sporangia are produced. Sporangia, which produce motile zoospores, detach from the mycelium and are spread through blowing or splashing rain, soil, leaf litter or surface water. When moisture returns, the sporangium releases its zoospores. Zoospores require free water in which to swim, either in soil pore spaces or as a film on plant surfaces. When zoospores hit a solid surface they encyst. Cysts may then either grow a germ tube with which to invade host tissue, or produce more zoospores. Chlamydospores are also produced by hyphae and are resting structures. Chlamydospores may produce new hyphae, or zoospore-producing sporangia. *P. ramorum* requires free moisture on plant surfaces for 48 hours to allow sporulation, and at least 9 hours to allow infection of hosts (CPHST 2004).

Phytophthora ramorum is a heterothallic species, meaning that it is not self fertile, but requires both mating types (A1 and A2) for sexual reproduction (Parke and Lucas 2008). Initially, only one mating type occurred in any one region of infestation, A1 in Europe and A2 in the USA, but both mating types have since been found on both continents, sometimes in the same site (COMTF 2008). Still, sexual reproduction of *P. ramorum* has not been seen. Sexual reproduction in water molds produces oospores, which are very resistant to unfavorable environmental conditions. The concern is that oospores would complicate quarantine and eradication efforts through their environmental resistance, and that sexual reproduction would lead to genetic adaptations (CPHST 2004). Single mating types of other heterothallic species of *Phytophthora* have been induced to produce oospores in the laboratory through chemical stimulation or physical wounding (Erwin and Ribeiro, 1996), so there are no guarantees that *P. ramorum* oospores will not appear in areas of single-mating-type infestation. And, *Phytophthoras*

are quite capable of genetic adaptation through relatively rapid mutations (generation time is measured in hours, not months or years [CPHST 2004]).

Longevity

Among *Phytophthora* in general, propagule survival in soil runs from short-lived mycelia to zoospores, then sporangia, then chlamydospores, with oospores usually the longest-lived propagules (Malajczuk 1983). No oospores have ever been seen for *P. ramorum*, and the role of chlamydospores is not yet understood (Garbelotto 2004). *P. ramorum* propagules collect in soil, shed from above-ground plant parts and carried by wind-blown rain (CPHST 2004). Chlamydospores of *P. ramorum* in damp sand have been shown to survive 7 days at 0°C and at 30°C, while spores in rhododendron leaf tissue also survived 7 days at -10°C (Tooley et al. 2008).

Sporangia readily survive the long dry California summer in soil (Fichtner et al. 2007), and in dried rhododendron leaves for up to three months (CPHST 2004). Chlamydospores have been shown to survive in soil for up to 8 months (CPHST 2004), and zoospores have been kept alive in distilled water for 6 months (Davidson et al. 2005).

Transport

Individual *Phytophthora* zoospores of other species are known to be able to swim several millimeters and to exhibit negative geotaxis, bringing zoospores to the soil surface where they can be picked up by wind and rain (Gregory 1983). *P. cryptogea* zoospores have been measured to swim 25-35 mm in surface water on flooded soil and through coarse soil at soil matrix potential (ψ) = -0.001 bar, and to move downwards as far as 40 mm in coarse soil (Weste 1983). Finer soils restrict zoospore mobility to about 5mm. *P. cinnamomi,* the causative agent of jarrah dieback (a disease of Australian

eucalyptus forests), has been found in groundwater as deep as 2 meters, infecting tree roots growing at that depth (Shearer 1985).

Sporangia are carried in flowing water and on wind-blown water droplets. These droplets can carry spores up to 1 meter in still air, and tens of meters by wind (Gregory 1983). *P. ramorum*, requiring water droplets for spore transport, will be greatly limited in dispersal distance due to the weight of water, compared to aerially dispersed *Phytophthoras*. (Moralejo et al. 2006). *P. infestans*, the causative agent of potato late blight, traveled 750 km from Belgium to Ireland in 1845 to start the Potato Famine (Schumann 1991). *P. ramorum,* in contrast, has been shown to travel only 15 m in wind-blown rain (Davidson et al. 2005), and 7 km in flowing water (Murphy et al. 2005).

Environmental conditions

Optimal conditions for *Phytophthora ramorum* include damp weather, whether rain, fog or heavy dew, and mild temperatures. Temperature extremes for *P. ramorum* growth run from 0-30°C. Optimal temperatures are 18-20°C (Garbelotto 2004). Lethal temperatures are not yet determined, although Swain et al. (2005) have shown that composting at 55°C for two weeks reduces *P. ramorum* to below-detectable levels.

Relative humidity affects survival of spores. Chlamydospores, the most environmentally resistant form of *P. ramorum,* are killed in only 30 minutes at 30% relative humidity at room temperatures (Fichtner et al. 2007). Sporulation is correlated with precipitation and fog, in addition to temperatures (Davidson et al. 2005, CPHST 2004).

But mere survival is not enough. To successfully invade new habitats, *P. ramorum* must be able to take advantage of dispersal agents (Bazzaz 1986). Plant pathogens that have successfully invaded new habitats have used dispersal agents such as

wind (chestnut blight), water (Port-Orford-cedar root rot), and insects (Dutch elm disease) (Owen and Lownsbery 1989, Anagnostakis 2000, Hansen et al. 2000). In forest settings, wind-blown rain poses a major dispersal agent for *P. ramorum*, picking up spores from tree tops (Goheen et al. 2006). But in Washington, *P. ramorum* is a pathogen of nursery settings, not forests; spores are produced on very small plants, less than 1m tall, and spores are dropped to the ground rather than picked up by wind and rain (Jennifer Falacy, WSDA, personal communication). *P. ramorum*'s ability to survive unfavorable weather in soil, and to be transported in water, suggests that in nursery settings, soil, soil water, and streams may be critical dispersal agents for this invasive pathogen.

Soil Microbiology

Soil is a habitat in which many microbial species, including plant pathogens, live or pass part of their lives . Soil microbes include fungi, bacteria, viruses, Protista, algae, nematodes, mites, springtails (*Collembola*), and the water molds such as *Phytophthora* (Paul and Clark, 1989). The smaller soil microbes live in the soil water filling the pore spaces, while the larger mesofauna such as the mites and springtails live in air-filled pores (Brady and Weil 2002). Soil inhabitants may be saprophytes, herbivores, detritivores, predators or a combination of these. Some foliar fungi simply fall to the soil with plant litter and may persist in the soil for some time without actively feeding or growing (Bruehl 1987). Inoculum of other foliar *Phytophthora* are washed from the foliage and into soil water through stemflow (Bruehl 1987). It appears that the same happens with *P. ramorum,* in addition to the introduction of spores to the soil surface through the shedding of infected leaves (Goheen et al. 2006).

Although *P. ramorum* does not live in the soil in the sense that other water molds and fungi do, with zoospores and hyphae traveling through soil pore spaces in search of food, its propagules do spend time in the soil environment. Shishkoff (2007) has shown

that *P. ramorum* can be recovered from moist sand or potting mix for up to one year from inoculation. It was also shown that roots of many plant species were capable of supporting *P. ramorum* for at least one month, and that chlamydospores germinated in the presence of roots, which subsequently became infected. Undoubtedly, spores of *P. ramorum,* while in soil, are subject to predation by soil microbes. Bacteria, amoebas and ciliated protozoa are all known to feed on fungal hyphae and sporangia (Malajczuk 1983).

Life forms in soil move by two methods. Larger forms such as plant roots, earthworms, insects, vertebrates and fungal hyphae are able to push their way between soil particles and aggregates. Smaller species such as nematodes, algae, bacteria, Protista and fungal spores (including those of *P. ramorum*) move through the soil in soil water (Brady and Weil 2002). Soil water movement is determined by soil texture and structure. Microbial species movement through soil is, therefore, determined by the size and distribution of soil pores, which is in turn determined by soil texture and structure.

Microbial movement in soil is generally considered to be negligible, due mainly to soil pores being very short and discontinuous. Measurable movement through soil seems to require the ability to push particles aside, which most creatures smaller than earthworms and insect larvae are not capable of doing. In loamy soils of mixed particle-sizes, pores tend to be very short, as their length is cut off by smaller particles settling in between larger particles, halting movement of microbial species (Paul and Clark 1989). Biopores, those created by life forms such as roots, earthworms and moles, tend to be both larger in diameter and much longer than typical soil pores (Brady and Weil 2002). Biopores form preferential flow channels in soil, as water prefers larger pathways. An old root channel, for instance, might measure as much as a few centimeters in diameter, rather than a few microns. Biopores can be responsible for much of the water movement in soil, including movement of contaminates in groundwater such as pesticides and microbes (Brady and Weil 2002).

Inoculum of soil-dwelling *Phytophthora* can be transported to new hosts through soil water. *P. cinnamomi,* a related species, has been found as deep as several meters in Western Australia (Shea et al. 1983, Kinal, Shearer and Fairman, 1993), and has been isolated from taproot lesions in the soil hardpan layer (Shea 1988). *P. lateralis* has been found to infect roots of Port-Orford-cedar through percolation of infested floodwaters through soil (Jules et al. 2002). *P. ramorum* has been found in streams draining infected sites (Murphy et al. 2005, Jennifer Falacy, WSDA, personal communication). It is possible that *P. ramorum* could be spread to new areas through first washing into the soil and then being carried through the soil water into streams. Once in streams, the inoculum could splash onto riparian vegetation, be deposited on streambanks through overbank flows, or be sprayed on vegetation as irrigation water pumped from infested streams and ponds. Tjosvold (University of California Extension, personal communication) reports the ability to produce ramorum blight on rhododendrons through irrigation with infested stream water.

Soil Science and Hydrology

For the purpose of this paper, soil will be defined as a combination of mineral and/or rock fragments, plant debris, roots, and microbial life. Soil is distinguished from rock in being loose enough to be dug with hand tools such as a shovel. Soil is also different from a simple deposition of rock and mineral particles in supporting rooted plant life, which requires the presence of organic material in the form of microbial life and organic debris.

Soil is the interface between the biological and the physical environments, and as such has a great influence on the life forms living on and in it. Plants are often viewed as the foundations of ecosystems, due to their photosynthetic activities that turn sunlight into food for herbivores, but plant growth depends on soil. Soil directs the movement of

water through a habitat and determines the availability of water and nutrients for plant growth.

The mineral portions of soil occur in three basic particle sizes, in descending order: sand, silt and clay (Brady and Weil 2002). Some soils contain larger particles, referred to as coarse fragments or cobbles, but they are not considered to be part of the soil. Organic soils contain large volumes of plant material in different stages of decomposition, but are still primarily mineral in content by weight (Brady and Weil 2002). The relative proportions of mineral particle size classes determines a soil's texture, and thus its hydrology. Soil with larger particles tends to have larger pores than soil with smaller particles. Soil particles are bound together by roots, hyphae, and organic compounds to form soil aggregates, which determine soil structure. Soil structure can be loose and friable, allowing easy passage of water and air, or it can be dense, impeding water passage and forming impermeable layers such as hardpans. The study sites described in this paper are associated with soils of a range of textures.

Water movement through a soil is determined by the pore sizes and the degree to which they are connected. Larger pores pass water more easily, while smaller pores tend to hold water through capillary action. Pores are also the living space for soil fungi and nematodes (Paul and Clark, 1989). Pore size, distribution and continuity are determined by both texture (micropores) and structure (macropores). Pore size and continuity are critical components of a soil's ability to disperse propagules such as zoospores and chlamydospores (Bruehl 1987).

Water in soil can be divided into three basic forms: gravitational, capillary and hygroscopic (Bruehl 1987). The form water takes is determined by what pore size it occupies (Brady and Weil 2002). Gravitational water is that portion that drains away through the pull of gravity, and tends to be associated with larger pores. Capillary water is held in smaller pores by capillary forces, against the pull of gravity. Hygroscopic water

is that remaining on the particle surfaces after soil is air-dried. Gravitational water is the primary form responsible for the transport of propagules (Bruehl 1987), while capillary water (a.k.a. available water) is the source of water and nutrients for plants (Dahlgren 2006).

Till versus Alluvium - Local Soil Formation Processes

The parent material for Puget Sound soils was largely derived from material transported by glaciers or meltwater (USDA 1973). Glacial till consists of mineral particles, varying in size from boulders to cobbles to clay, which were picked up, ground down and carried by moving glaciers, then deposited as the glacier melted. Some glacial till soils have a relatively shallow hardpan created by the weight of the glacier compacting soil particles tightly together. One study site is on glacial till soil.

Alluvial soils, deposited by rivers and streams, represent the main nonglacial soil formation factor in this area (USDA-SCS 1973). Outwash soils formed from the meltwater streams flowing off the glaciers, similar to alluvial soils along contemporary streams. Volcanic mudflow soils are limited in extent. Till soils are unsorted, meaning there is a mixture of particle sizes, from cobbles to clay. Outwash and alluvial soils are generally sorted to a smaller range of particle sizes by the movement of the water that created them. This sorting, and the lack thereof in till soils, determines the range of pore sizes available in a soil, for water retention and for microbial transport (Brady and Weil 2002). Many of the study sites are on alluvial soils.

Topography is a major factor in the development of alluvial soils (Brady and Weil 2002). Alluvial soils are those formed by the deposition of sediment carried by rivers and streams. As streams drain mountain slopes, they pick up and transport sediments (primarily sand, silt, and clays, even gravel in faster or steeper streams) and organic matter. This sediment load is deposited as current slows, either where the slope lessens

or the channel widens (Knighton 1998). When a stream or river overflows its banks, the sediment load is deposited as alluvium on the floodplain outside the channel and can now begin the process of soil formation (USDA-SCS 1973). Particle sizes are sorted on a gradient with water velocity, the finer particles deposited where water is slowest, in flat stretches and at the furthest reaches of flood waters (Knighton 1998). As a result, outwash and alluvial soils may be clay, silty or sandy, often with large amounts of organic matter from riparian vegetation, where till soils are generally gravelly sandy soils low in organic matter. The particle size of the alluvium depends on the sediment load carried by the stream and on the velocity of the floodwaters (Knighton 1998). Puget Sound alluvial soils form along the valleys of rivers such as the Green, Snoqualmie, Puyallup, and Skokomish (USDA-SCS 1973). These soils are often deep, level, fine-textured loams.

Transport

Soils vary tremendously in their ability to transport contaminants such as microbial pathogens in soil water (Brady and Weil 2002). Fine-textured soils, such as many alluvial soils, are poor transmission media, slowing movement of contaminants and increasing the time they are subjected to microbial decomposition and predation. Such soils act as filters, reducing transport of contaminants to surface and ground waters. Coarse-textured soils such as glacial till soils, on the other hand, are poor filters of contaminants, allowing even larger contaminants such as bacteria and fungal spores to pass from the soil surface into soil water and on to surface and ground waters (Brady and Weil 2002). Preferential flow channels, such as biopores and cracks between blocks of soil, can be responsible for much of the contamination of groundwater with the larger contaminants such as bacteria and fungal spores. Shallow soils can develop unconfined aquifers, where percolating rain water collects on top of a shallow hardpan. These unconfined aquifers are easily contaminated by pathogens such as *P. ramorum* when overlaid by coarse-textured soils or soils with preferential flow channels.

When rainfall is low, as it normally is in the summers in western Washington, contaminants (fertilizers, petroleum products, bacteria, metals, and perhaps *P. ramorum* spores) and debris accumulate on the soil surface until enough rain falls to wash them away (Brady and Weil 2002). For that reason, the first rains of autumn can carry very high concentrations of contaminants in stormwater runoff, because one precipitation event is carrying an accumulation that might be as high as several month's worth of contaminants.

Another phenomenon of the first storms of autumn is the reworking of stream sediments. Stream discharge diminishes over the dry season, depositing sediment loads as the water slows, then rises gradually with the return of the rain. Normally the dry soil soaks up a considerable amount of the first precipitation events, and there might not be any runoff into streams for the first couple of months, until the soil profile is saturated (Brady and Weil 2002, Mitsch and Gosselink 2002). When storms finally bring enough rain to overwhelm the soil's capacity to absorb it (infiltration), runoff can swell streams. Increased discharge will entrain sediments that were deposited the previous season, and carry them downstream to be redeposited elsewhere, in-channel or overbank (Knighton 1998).

If spores of *P. ramorum* have collected in the soil profile, it is possible that the draining stream would not receive any spores during periods of low runoff, until the soil becomes saturated or an intense storm creates high runoff. Similarly, if spores of *P. ramorum* have collected in channel sediment, either through entrapment of infested plant material or the deposition of chlamydospores or sporangia in alluvium, there might be no detectable spores in the water column until an increase in discharge reworks the accumulation of sediment and plant material.

Stream Channel Morphology

Fungal propagules, while living organisms, are not generally self-propelled, so can be thought of as part of the sediment load of a stream, similar to mineral particles (i.e. clay, silt, sand, pebbles) and plant debris (i.e. leaves, twigs, fruits). Spores of *P. ramorum* fall into the same size range as fine particulate organic matter (FPOM), and as silt particles. Zoospores of the water molds (including *Phytophthora*) are somewhat motile, but probably not to the point of being able to swim against a current, if for no other reason than their very small size (~0.001mm) (Weste 1983). To the extent that fungal propagules act as sediment load, understanding the behavior of sediment in streams can help to understand how streams transport pathogens such as *P. ramorum*. The nature and behavior of a stream's sediment load can often be inferred by simple observation of the morphological type of the channel bed (pool, riffle, braided, etc.).

Stream segments can be classified into three general sediment response types: sediment sources, sediment transport zones, and sediment deposition zones (WSFPB 1994). Although erosion, transport and deposition can occur in any stream segment, these general sediment response segments can be roughly differentiated by gradient. Source segments have gradients greater than 20%; transport, from 20-3%; and deposition zones, less than 3%. Sediment response type determines the particle sizes that form the channel bed, and thus the channel bed type.

The steepest stream segments, greater than 20%, are generally colluvial channels (WSFPB1994). The least steep, less than 1% gradient, tend to be pool-riffle beds. Gradients from 1-2% form pool-riffle and plane-bed channels. Gradients from 2-4% form plane-bed and forced pool-riffle (formed by large obstructions) channels. Gradients from 4-8% are generally step-pool channels, and those from 8-20% form cascades. Step-pool and cascade channels are therefore generally transport segments, where sediment load is carried along downstream rather than settling out, while pool-riffle and plane-bed

channels may be in either transport or deposition zones, with sediment settling out in some places, and being transported in others. Braided channels and forced pool-riffles are likely to be transport zones rather than deposition zones.

Sediment & Sedimentation

Moving water entrains (picks up) and then deposits particles (Knighton 1998). The speed at which the water moves determines the size and weight of the particles entrained and deposited. In general, accelerating water entrains particles, while slowing water deposits particles. As entrainment requires more energy than transport, entrainment and deposition are not symmetrical. Water velocity tends to be greater at entrainment than at deposition; once in motion, particles tend to stay in motion. Water velocity is determined by channel form, slope, and water discharge (Q) (Knighton 1998, Mitsch and Gosselink 2000).

In general, transport of larger and heavier particles requires a higher water velocity than smaller and lighter particles, so clay and silt particles are more easily moved along than gravel or cobble. Most fungal spores, including *P. ramorum,* are in the same size range (although with a much lower density) as silt particles. Table 1 provides two versions of mineral particle-size classes. Figure 1 shows the relative sizes of *P. ramorum* spores, the smaller soil particles, and fine particulate organic matter (FPOM).

Table 1: Particle-size classes.

Particle Class	Size (USDA 1973)	Size (Knighton 1998)
boulder	> 254 mm	> 256 mm
cobble	254-76 mm	256-64 mm
gravel (pebble)	76-2 mm	64-2 mm
sand	2-0.05 mm	2.0-0.062 mm
silt	0.05-0.002 mm	0.062-0.004 mm
clay	< 0.002 mm	< 0.004mm

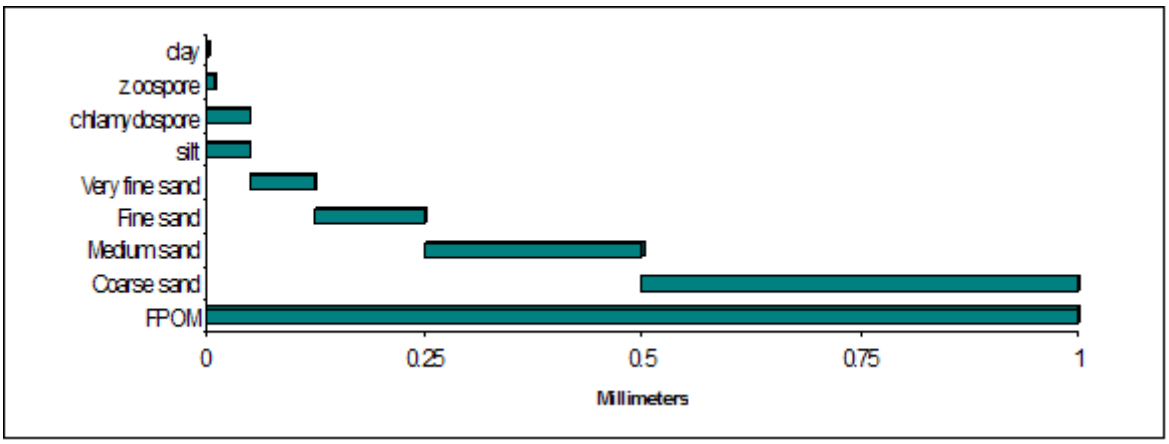

Figure 1: Relative sizes of spores of *P. ramorum*, mineral particles, and fine particulate organic matter.

Sediment Types

Sediments are divided into three categories, depending on how the stream transports the material: bed-load, wash-load and dissolved-load (Knighton 1998). Bed-load is composed of the larger particle-size classes that tend to bounce or roll along the bottom of the stream - generally, boulder, cobble and gravel. Bed-load usually consists of the same particle-sizes that make up the channel bed. Wash-load sediment is carried in

the water column, above the channel bed, and is usually composed of the smaller particle-size classes - silt, clay, and organic materials. Wash-load consists of smaller particle-sizes than those making up the channel bed. Dissolved-load consists of ions and molecules dissolved in the water. It is important to note that it is how the stream carries the sediment that determines the type of load, not the particular particle-size. Particles that constitute bed-load in a small, slow stretch of stream may be wash-load in a faster, more powerful stream stretch. Fungal spores are not only very small, but also very light. Being composed largely of water as are most living cells, they are very near neutral buoyancy, and would fall into the wash-load sediment type in just about any water body as long as they are floating free, rather than adsorbed onto mineral sediment particles or contained in plant material.

Settling velocity (U) is the rate at which a particle drops. For very small particles, less than 50 microns, settling velocity can be estimated by Stokes Law (Gregory 2005):

$U = \frac{gd^2}{18\mu}(\rho_s - \rho_l)$ where U = velocity of the particle's vertical drop, g = gravity, d = diameter of the particle, ρ = density of the particle and of the liquid (water), and μ = viscosity of water, which varies inversely with temperature. For a 50 micron- sized particle, about the size of a chlamydospore of *P. ramorum*, and of density = 1.1 g/cm^3, about the average density of a living cell, the settling velocity U ≈0.1 mm/sec at a water temperature of 20°C (Gregory 2005). At that U, these particles would take 13 minutes to settle to the bottom of an 80 cm deep stream, such as the smallest stream in this study (R Stream).[2] Of course during those 13 minutes, the stream is carrying the particles farther downstream, so they will settle out about 500 m downstream at 0.72 m/sec, a typical speed for R Stream at the downstream nursery property line. And, turbulence in the water

[2]Place names, except where public knowledge, have been abbreviated to maintain confidentiality as required by USDA-APHIS' Confidential Business Information Management protocols.

column will kick these small particles up and down as they travel, potentially extending downstream transport distance (Knighton 1998, Vogel 1994).

As water temperature cools, U will decrease, and particles will be carried farther as they settle more slowly. In western Washington, streams and ponds are generally much cooler than 20°C, often in the 10°C range. R Stream had a water temperature of 6°C when the first traps were picked up in January 2006. Knighton (1998), discussing mineral particles, which are much more dense,[3] states that particles less than 0.062 mm are carried at very near the velocity of the water flow; and that the flow velocity must be very low for particles in this size range to settle out. This suggests that a particle such as a fungal spore (<< 0.062 mm) can be carried in the current of even a slow stream for a very long distance before settling to the bottom, and that settling might not occur until flow stops, in a lake, pond, or wetland, or in overbank flow. In fact, spores of aquatic fungi have been shown to travel 3-4 km in streams (Barlocher 1992). These particular spores possess long arms and irregular shapes that limit downstream drift (Barlocher 1992), although Vogel (1994) contends that long arms and irregular shapes increase lift and thus extend drift. Spores of *P. ramorum* do not possess these shapes, but are more or less spherical (Goheen et al. 2004), offering less resistance to the water, but decreasing lift compared to spores of aquatic fungi (Vogel 1994).

Drift

Small aquatic creatures can drift in the water column, traveling downstream in running water (Giller and Malmqvist 1998). Drift distances are affected by lift and drag, which are determined by the density and shape of the drifting object, and on the viscosity (which depends on temperature) and speed of the water (Vogel 1994). Nonmotile creatures, such as aquatic fungi, depend on drift to escape unfavorable habitats and to

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[3]approx. 2.6 g/cm^3 (Childs 1969).

access new food sources (Barlocher 1992, Giller and Malmqvist 1998). Drift is similar, but not identical, to fluvial transport of sediments. Drift distances given for aquatic creatures do not match transport distances of mineral sediments. For instance, Giller and Malmqvist (1998) state that algae drift only short distances, while Knighton (1998) states that similarly-sized mineral particles will not settle out of moving water, but remain suspended until flow stops. Both cite turbulence as affecting transport distances.

Turbulence

In most natural streams, water flow does not follow uniform, parallel flow lines, but is irregular, i.e. turbulent (Knighton 1998). Eddies, backwashes, and vortices flow in all directions, including upstream, even as the current as a whole proceeds in one direction (Vogel 1994, Wohl 2000). Turbulence can have a considerable effect on transport and deposition of small particles, including the size ranges of silt and particulate organic matter (POM). Turbulence contributes to lift, increasing drift distances (Vogel 1994). Turbulence is also responsible for pushing small particles through the laminar sublayer at the bottom of stream channels, resulting in deposition, and then pulling them back through again in resuspension. Small, gravel-bed streams, such as R Stream, can have near-bed flows dominated by vortices created by bed materials (Wohl 2000). Turbulence creates flow dead zones at flow obstructions, carries FPOM into them, and then traps them there until a change in turbulence (such as vortex detachment, or a change in discharge) carries the particles back into the current (Vogel 1994, Wohl 2000).

Stream Ecology

Woodland streams such as R Stream receive large amounts of plant debris shed by riparian vegetation, either directly or in stormwater runoff (Mathews 1988, Giller and Malmqvist 1998, Richardson 2005). Microbes colonizing the leaves and twigs enter the

stream along with their substrate (CPHST 2004). In areas where infested plants grow near a stream, leaves and twigs containing inoculum of *P. ramorum* could enter the water as well.

While spores that enter the water directly can be carried long distances in a short time, those inside leaves can be retained in the stream for extended periods. Leaves in the water can collect in leaf packs and debris jams (Giller and Malmqvist 1998, Webster et al. 1999). Leaves in leaf packs and debris jams are colonized by aquatic fungi and microbes, beginning the process of decomposition. Chlamydospores of *P. ramorum*, produced inside host plant leaves, would be released into the stream in the process of leaf decomposition (Jennifer Falacy, WSDA, personal communication).

Freshly-fallen leaves float, but decomposing leaves sink (Smith and Smith 1998, Hoover et al. 2006). Leaves in water are first leached of soluble organic compounds, then colonized by aquatic fungi to begin the decomposition process and become coarse particulate organic matter (CPOM), or particles larger than 1mm. Leaves that have been leached and colonized are referred to as conditioned. CPOM collects in flow obstructions, flow refugia, and depositional zones, such as debris jams, weed beds, side pockets and pools, bed roughness elements, the channel boundary layer, and floodplains. POM on the channel bed may enter the hyporheos, the portion of the water column that flows beneath the surface of the channel bed through spaces between sediment particles (Stanford and Ward 1993). The hyporheos is another example of a flow refugium (Olsen and Townsend 2005). Once held in these areas, CPOM can be further broken down into fine particulate organic matter (FPOM), particles ranging from 0.0005mm-1mm.

Retention time describes how long POM remains in the stream, and thus available to aquatic life as a food source. Retention time is highly variable, depending on the presence of flow obstructions, the timing of leaf fall in riparian vegetation, and the resistance to decay of the vegetative input (Giller and Malmqvist 1998). The efficacy of

flow obstructions will vary with discharge and with the lifespan of organic obstructions such as large woody debris (LWD). High discharges can flush a stream reach of POM and remove flow obstructions. Woody material forming debris jams will itself eventually decay, releasing the collected POM to the current.

Figures given for leaf travel in streams vary widely, from 20m (Webster et al. 1999) to 200m (Barlocher 1992). Part of the difference may be due to stream order; Webster et al. (1999) were specifically looking at 1st and 2nd order streams only. Part of the difference may also be due to discharge, which of course varies greatly both between streams and over time. Leaf travel time varies with stream size, channel depth and Q in particular. FPOM travels shorter distances than leaves and CPOM, averaging less than 10 m/yr (Webster et al. 1999). This is probably due at least in part to the greater specific gravity of FPOM compared to leaves. In small streams, 1st and 2nd order, all leaves entering pools collected at the bottom at baseflow conditions, but were readily remobilized by elevated flows (Hoover et al. 2006).

Leaves and POM move in the water column in suspension, saltating along the channel bottom (Webster et al. 1999, Vogel 1994). Channel bottoms of small streams are relatively rough, composed primarily of coarse particles that occupy a significant portion of the water depth (Nowell and Jumars 1984, Knighton 1998, Richardson et al. 2005, Hoover et al. 2006). This hydraulic roughness creates turbulent flow - backwashes, eddies, vortices. Leaves and larger CPOM can be trapped by the current on larger, protruding stones (Hoover et al. 2006). FPOM is small enough to be entrapped by the backwashes, eddies and current dead zones that form on the upstream and downstream sides of the same larger, protruding stones (Nowell and Jumars 1984, Wohl 2000). Once trapped, POM is generally decomposed in place, unless released by physical disturbance such as collapse of debris jams or increasing discharges (Webster et al. 1999).

Spores, in contrast to POM, travel long distances in suspension as wash load, not in contact with the channel bottom (Barlocher 1992). Spores of aquatic fungi range in size from 50-100µm, and can travel 3-4 km in small headwater streams. These spores are small enough that the foam that develops on the water surface downstream of waterfalls can serve as a retention device. They are removed from the water column by filter feeders, and by catching on flow obstructions and plant material. Spores of *P. ramorum* have been found 7 km downstream of the nearest known inoculum source (Murphy et al. 2005)

In small streams, CPOM collects primarily on LWD, in pools, and in riffles on protruding rocks (Richardson et al. 2005, Hoover et al. 2006). Larger streams, due to their greater channel dimensions, have fewer flow obstructions than 1st and 2nd order streams - less channel-spanning LWD, fewer channel-blocking stones, finer bed sediments with fewer protruding stones and lower hydraulic roughness. Larger streams also tend to have faster currents, which keep POM in suspension rather than settling or entangling on flow obstructions.

While there is significant autumnal leaf drop in western Washington from a few deciduous species, the majority of the biomass here is evergreen coniferous, and so POM in streams will be largely coniferous needle foliage that is both shed year round and resistant to decay (Mathews 1988, Richardson et al. 2005). The plants most often infected with *P. ramorum* in Washington are broadleaf evergreens: rhododendron, camellia, kalmia, evergreen viburnums, and pieris. These will be somewhat resistant to decay. Debris jams tend to be made of branches, boles and rootwads from western red-cedar, which is highly resistant to decay, or from Douglas-fir, which is fairly resistant (Mathews 1988, Larry Dominguez, DNR, personal communication). Red alder forms numerous smaller, shorter-lived debris jams. Retention times of decay-resistant foliage on decay-resistant LWD jams should be relatively long. Long retention times could provide a long-term source of inoculum, if the foliage is infested with *P. ramorum*.

Time necessary for decomposition from CPOM to FPOM is variable, depending on the aquatic microbial community, water temperatures, and resistance to decay of the leaf substrate. Initial colonization by aquatic fungi can take a couple of weeks (Smith and Smith 1998). Rhododendron leaves used as baits by WSDA are still intact after two weeks in the water in spring, although they often have black lesions on them. Leaves are similarly intact after two months in the water in winter. Leaves from a trap that broke loose during a winter storm, and were buried in alluvium for four months, were still intact when found. Leaves that have spent three winter months in the Green River have been intact enough to remove from their sleeves in one piece. It would appear that rhododendron leaves are relatively resistant to decay, and can provide a long-term source of inoculum of *P. ramorum* when submerged in water or sediment, lasting over the winter if not flushed by high discharges.

FPOM can collect in smaller spaces than that required by CPOM (Hoover et al. 2006). FPOM is both small and relatively dense, from 1.2-1.7 g/cm^3 (compared to 1.06g cm^3 for green algae [Giller and Malmqvist 1998]) and moves along the channel bottom by saltation, similar to bedload (Webster et al. 1999, Vogel 1994). Relatively large, upright obstructions in the channel create current dead zones both upstream and downstream (Knighton 1998, Vogel 1994, Wohl 2000). FPOM can be trapped in these dead zones, for instance collecting in the lee of cobbles on the channel bed in riffles (Cushing and Allan 2001, Giller and Malmqvist 1998). Hydraulically rough stream channel beds, such as the gravel-bed R Stream, contain numerous pockets and troughs capable of entrapping particles of the FPOM size range (Vogel 1994). The troughs between ripples, and the pockets around cobbles and LWD, create flow vortices that both trap and resuspend mineral and organic sediments.

FPOM could enter the hyporheos as coarse sediment settles in the same small-scale flow dead zones. FPOM also enters the hyporheos when it is buried by sediment during floods (Olsen and Townsend 2005). FPOM on the channel bottom could also be

incorporated into the hyporheos by infiltration with the current, and perhaps by hyporheic inhabitants (fungi, stonefly larvae) (Stanford et al. 1994). FPOM from the stream channel is a major food source for a surprisingly large and varied community of hyporheic inhabitants, thus spores could continually be released by decomposition in the hyporheic zone. Hyporheic flow could then bring inoculum of *P. ramorum* back to the stream channel or into a riparian aquifer (Wondzell and Swanson 1996), or scouring of the channel bed in high discharges could release the incorporated FPOM (Giller and Malmqvist 1998).

Stream bait traps themselves act as flow obstructions in a small stream, often taking up half or more of the channel width of the smallest stream in this study, and most of the depth. CPOM and mineral particles collect in the traps - twigs, decomposing leaves, silt, sand, and small pebbles. It is probably safe to presume that FPOM also collects with the silt, but is simply too small to see. Traps could be functioning as debris jams, collecting *P. ramorum* inoculum in the form of infested POM, rather than (or in addition to) attracting spores floating free in the water column. Traps also affect current speed and turbulence, which in turn will affect deposition and transport of FPOM, at the trap itself and for some distance both up- and down- stream (Nowell and Jumars 1984, Vogel 1994).

Floodplains play a significant role in retention of both mineral sediments and POM (Knighton 1998, Giller and Malmqvist 1998). Overbank flows spread onto floodplains, carrying sediments and POM with them. Receding floodwaters deposit sediments and POM as current slows and particles settle out. Deposited sediments are stored in the floodplain until a subsequent overflow remobilizes them. Chlamydospores of *P. ramorum* are capable of surviving over a year in damp soil in laboratory conditions (Linderman and Davis 2006), and, when buried to a depth of 6cm, for the duration of summer in field studies (Fichtner et al. 2007). Floodplain storage of infested POM or contaminated sediments could be a significant long-term source of inoculum. In

additional, many host plants grow naturally in floodplain areas: willows, salmonberry, cascara, Oregon ash, bigleaf and vine maples, and baldhip rose.

Distribution of *P. ramorum* in Washington

P. ramorum occurs in forest settings in coastal northern California and extreme southwestern Oregon (Garbelotto 2004). It is also found regularly in the nursery trade in California, Oregon, Washington and British Columbia, and occasionally in other states (COMTF 2008). *P. ramorum* occurs in nurseries and gardens throughout the European Union, but particularly in the Netherlands and the United Kingdom. At the time of this writing, the known distribution of *P. ramorum* in Washington state is limited to nurseries that import host plant material from out of state, and in three streams, two of which are associated with infested nurseries. No infested plants have been found along these streams. Only once has *P. ramorum* been found in Washington in plants outside of a nursery. This was in a newly-installed landscape, on plants recently purchased from an infested nursery, and the eradication of *P. ramorum* at this site appears to have been successful (Jennifer Falacy, WSDA, personal communication).

There are five nurseries and one commercial landscape site of particular interest to this study: they have infested soil, and they have been repeatedly stream baited or water sampled during the study period 2006-07. Place names, except where public knowledge, have been abbreviated to maintain confidentiality as required by USDA-APHIS' Confidential Business Information Management protocols. The different nurseries will be referred to by their soil series names. All soil series information comes from the USDA Natural Resource Conservation Service, both the older Soil Survey print publications (King, Snohomish, Pierce and Thurston counties) and the new Web Soil Survey, at http://www.soils.usda.gov. It should be kept in mind that, while soil series are

often named after towns, the nurseries in this study are not in the towns that their soil series are named after.

Of the nurseries and one landscape site in western Washington that have developed *P. ramorum* -infested soil during this study period, only one is definitely associated with infested surface water.[4] Five border directly on a stream or river, and three have retention ponds on site (one has both a stream and a pond) that could potentially have become infested. If we knew what factor(s) control the movement of *P. ramorum* from soil to surface water, it would be possible to better target monitoring and survey efforts, and to potentially prevent infestation of surface waters and subsequent spread of the pathogen.

One possibility, of course, is that the pathogen has infested some or all of these waters, but has gone undetected. The potential for false negatives cannot be discounted, and should be kept in mind, although it is not a topic for this paper. See Bulluck et al. (2006) for a discussion of detection techniques for *P. ramorum*. See also the COMTF newsletter from May 2008 (http://suddenoakdeath.org/html/newsletter_archive.html) for a brief discussion of different results obtained by different organizations analyzing replicate samples from the same nursery in Mississippi. Another possibility is that the pathogen has gone undetected by chance. Populations of newly introduced species are often low, and survey methods do not do a good job of detecting low numbers (Perkins 1989).

The first nursery sits on Harstine gravelly sandy loam, 6-15% slope. Figure 2 shows Nursery Harstine and R Stream. The nursery itself is terraced, but the stream has

[4]A second nursery is associated with a stream positive, but has not been definitively linked by DNA comparisons of *P. ramorum* on the nursery plants and that from the stream.

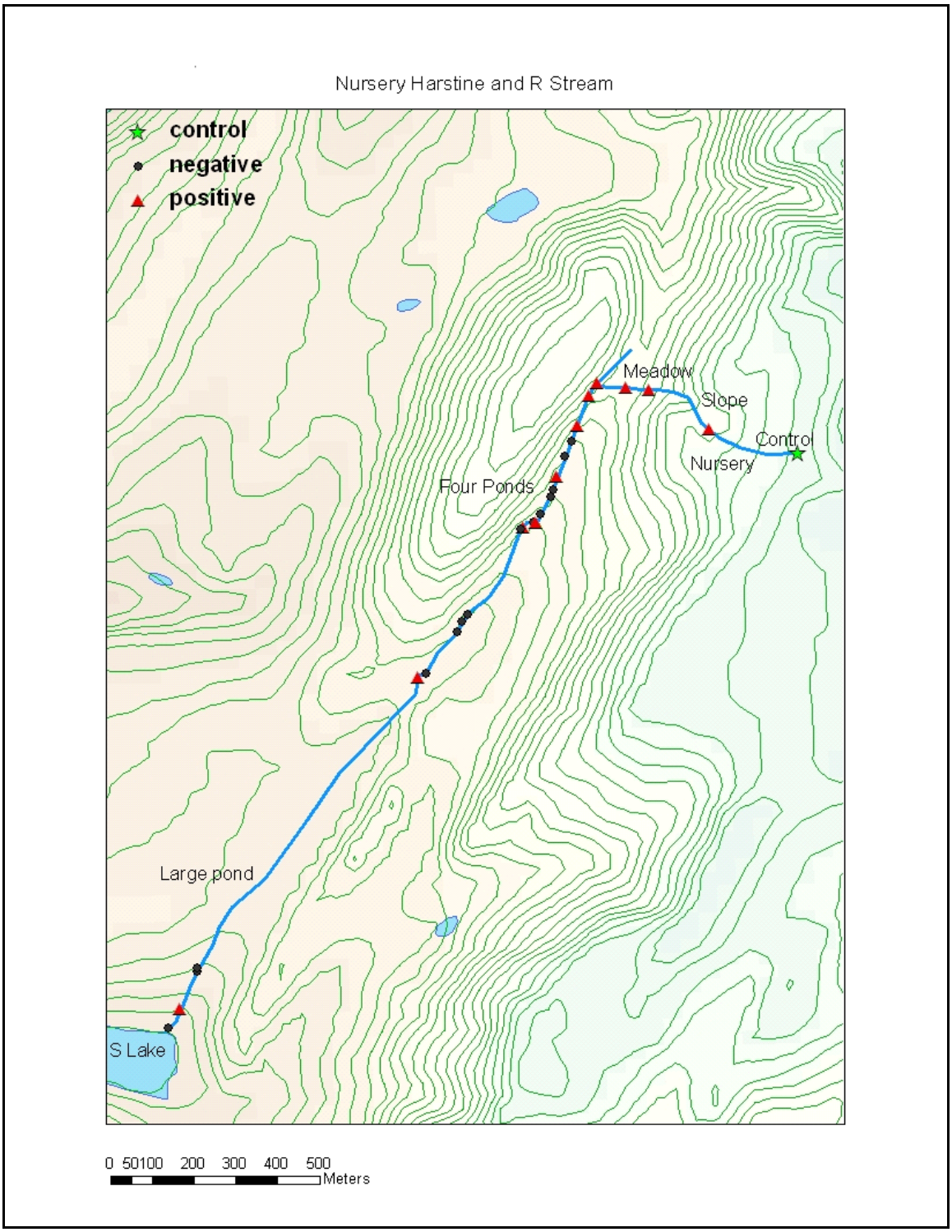

Figure 2: R Stream.

about 10m of drop from the top of the nursery to the bottom, a distance of about 150 m. It is located in a rural residential area of the Kitsap Peninsula, surrounded by woodland vegetation, gardens and pastures. The typical profile for Harstine gravelly sandy loam is described as possessing a cemented, compacted hardpan at 80 cm (USDA -SCS 1979). A seasonal water table perches on top of the hardpan and flows laterally, seeping out at slope bottoms. The soil is 5-15% gravel and cobble.[5] Harstine soil formed from sandy glacial till, and is classified as moderately well drained, meaning water moves easily through the soil until it reaches the hardpan. Permeability is moderate, with water moving 1.5-5 cm/hr through the soil. Given these numbers, water would move from the surface to the hardpan in anywhere from 16 to 55 hours, given sufficiently prolonged precipitation. It is not uncommon in this area to have more or less continual rain for 16-48 hours in the winter (author's personal observation). Once on the hardpan, soil water could flow downhill relatively quickly, to emerge at the bottom of the slope, or in the stream channel (Knighton 1998).

The second nursery sits on Woodinville silt loam, which occurs in stream valleys, and is formed from alluvium. The typical profile is described as possessing silt loam, silty clay loam, and peat lenses. The soil is described as having formed from grasses and sedges in alluvium, so organic content is high. There is no hardpan layer, and the water table is at the surface in winter. Drainage is poor, indicating that the soil is wet for long periods, and permeability is moderately slow, meaning water moves only 0.5-1.6 cm/hr through the soil. Even in September, when soil is normally driest, the nursery has standing water in the lowest areas and water within 30 cm of the surface in higher areas. Percolating water merges with the water table rather than collecting on and flowing over a hardpan, as at Nursery Harstine. Cattails grow wild in Nursery Woodinville, and duckweed and algae are common groundcovers.

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[5]Particles measuring from 2.0 mm to 76 mm.

The third nursery sits on a peninsula of the Green River, which is made of Nooksack silt loam on the upstream side, and Newberg very fine sandy loam on the downstream side. Both soils are alluvial. There is no hardpan, no slope, no erosion, and no coarse fractions. While not as small as silt, very fine sand particles are only just larger than silt, and capable of filtering out nearly 100% of microbial contaminants from water (WHO 1974). Permeability is high to moderately high, and while the soil may flood, it does not pond. Seasonal winter water table is 1m or more below the surface, and there is no erosion on this flat surface. The nursery has few drains, and only one very small retention pond, yet irrigation runoff does not puddle anywhere on the nursery, but infiltrates into the sandy alluvial soil and percolates away. No drains or channels, artificial or natural, direct runoff into the Green River.

The fourth nursery (which has not had positive soil but is interesting for other reasons) sits on Tukwila muck, just a few meters from the bank of the Sammamish River. Again, there is no runoff, no erosion, no coarse fractions, and no hardpan. There is some slope along the side of the nursery farthest from the river, but the majority of the nursery property is on level, mucky soil. Muck soils have a very high percentage of partially decomposed organic matter in them (40-90% by volume), where Woodinville soil, a mineral soil high in organic matter, is only 5-10% organic material by volume. Tukwila soil formed from sedges growing in depressions on stream terraces, with layers of diatomaceous earth. Water ponds to up to 30cm over the surface, and the water table is within 1.5m of the surface year-round. Permeability is moderate.

Tukwila Nursery's grounds are built up with gravel and sawdust beds to raise the plants above the wet soil. It is the sawdust in the raised bed that tested positive for *P. ramorum*, rather than the soil itself. A large retention pond in the center of the nursery collects irrigation runoff. A drainage ditch runs along the south border of the nursery, turns north after leaving the nursery property, runs through a culvert under a gravel road, drops into a plunge pool, enters another culvert to go under the Sammamish River Trail,

and then flows into the river. The overflow from the retention pond flows into the drainage ditch, along with some direct runoff from the south side of the nursery, including the infested area.

The fifth nursery sits on a Terric Medisaprist muck soil, with raised sawdust beds for the plants, and a drainage ditch flowing along the east border of the nursery. Again, it is the sawdust that tested positive for *P. ramorum* rather than the soil itself. This soil has moderate permeability, very poor drainage, no slope, no hardpan, no coarse fractions, and no erosion. It ponds rather than floods, and the water table is at or near the surface in winter. Runoff from the positive area flows into the drainage ditch, which is part of the municipal stormwater system, and eventually flows into a tributary of the Sammamish River. There is likely to be some sort of sediment trap before the ditch flows into a natural stream.

The commercial landscape site is a street-side landscape for a new subdivision. The landscaping company brought in planting mix, which was laid on top of the native Yelm soil to a depth of about 15cm. The division between the two can be clearly seen in the planting holes left empty after the *P. ramorum* - infested plants were removed. Water stands in these holes even in the dry season, and samples of this standing water were taken for culturing (Nathan Chambers, WSDA, personal communication). No streams or ditches drain this site, so no stream baiting could be done. Surface runoff enters the municipal stormwater system. Yelm soil is a deep, fine, sandy loam, formed from outwash. There is no hardpan, no coarse fractions, no slope; moderately rapid permeability and moderate drainage. Yelm is classified as a prime agricultural soil (USDA-NRCS 1990), but the addition of imported topsoil in a distinct layer creates a water-impermeable barrier that causes drainage problems for the newly-installed landscape (Brady and Weil 2002). Under normal circumstances, Yelm soil would not have standing water, but would allow water to infiltrate and percolate away.

Stream Baiting

USDA's Confirmed Nursery Protocol (USDA-APHIS 2004) calls for sampling of soil and water whenever a nursery is found to have *P. ramorum* - positive plant material. No soil or water samples are taken until plant tissue samples have been confirmed positive. Soil is taken from just below the surface, at scattered locations within the plant destruction area. Water collecting in or draining from the destruction area is collected in 250 ml bottles for baiting in the lab. If water draining from the destruction area enters any kind of surface water, such as a retention pond or a stream, that is both large enough and long-lasting enough to submerge a stream bait trap, then that is baited in place.

The US Forest Service has developed a standardized methodology for baiting streams for *P. ramorum* (USFS 2006). Mesh bags measuring approximately 40cm x 30cm are constructed from window screen material. The mesh material is folded to form a pouch, and sewn or stapled into sleeves. The Forest Service calls for 4 sleeves, but WSDA uses 5 sleeves, as their sampling protocol calls for a minimum of 5 leaves per sample (Jennifer Falacy, WSDA, personal communication). A wooden dowel or a short length of PVC pipe is used at the top to hold the material flat. The mesh material is folded over the dowel and fastened to form a flap covering the sleeve openings. Each sleeve is filled with one rhododendron leaf *(Rhododendron macrophyllum* is the standard on the West Coast) and paired bait bags, tied together by about 1m of rope, are submerged in the stream for two weeks. Two-pound lead fishing weights are used to anchor the traps in place. Plates 1 and 2 show stream bait traps as used by WSDA.

The Streams

R Stream, the primary focus of this study, is a very small, low-gradient, low-order stream draining a rural residential area of Pierce County. The lower end of the channel barely qualifies as a first order stream, as the channel might carry only a trickle at the end of the dry season. Total stream length is approximately 2km, and the summer channel rarely over 1m wide and 60cm deep. The soil in this area is primarily glacial till, of the Harstine series: poorly sorted gravelly sandy loam, acid, many cobbles, and with a shallow glacial hardpan. The streambed is primarily sand, pebbles and small cobbles, with deep silt and clay in depositional areas, and patches that appear to be exposed hardpan. The highest elevation in the watershed is well under 100m. Figure 2 shows the trap locations on R Stream. Figure 3 shows a closeup of the upper reach. Plate 3 shows the soil profile for Harstine soil, in the wooded area just downstream of the infested area. Plate 4 shows R Stream flowing through the meadow below the nursery.

The infested area of the nursery borders directly on the stream, from which point the stream runs through a relatively steep (6-15%) wooded slope. At the base of the slope, the stream enters the meadow. The very first positive trap was located in a plunge pool where the gradient changes from the slope (>6%) to the mostly level meadow (~1%). At the far side of the meadow, R Stream joins another small intermittent stream. This confluence has produced multiple positive traps throughout the study period, and continues to do so in 2008.

Along its length, R Stream's gradient varies from 1-4%. It flows through wooded areas of western red-cedar (*Thuja plicata*) and red alder (*Alnus rubra*), salmonberry (*Rubus spectabilis*) and Himalayan blackberry (*Rubus armeniacus*). Floodplains support skunk-cabbage (*Lysichiton americanus*) and slough sedge (*Carex obnupta*). Pastures,

meadows, lawns and gardens border the stream in some sections. Numerous culverts carry the stream under roads and driveways.

At its mouth, R Stream flows into an artificial lake (S Lake). A sediment trap filters the stream before it flows into the lake. The furthest-downstream positive trap site is in a small LWD step-pool just upstream of the dammed pool formed by the culvert.

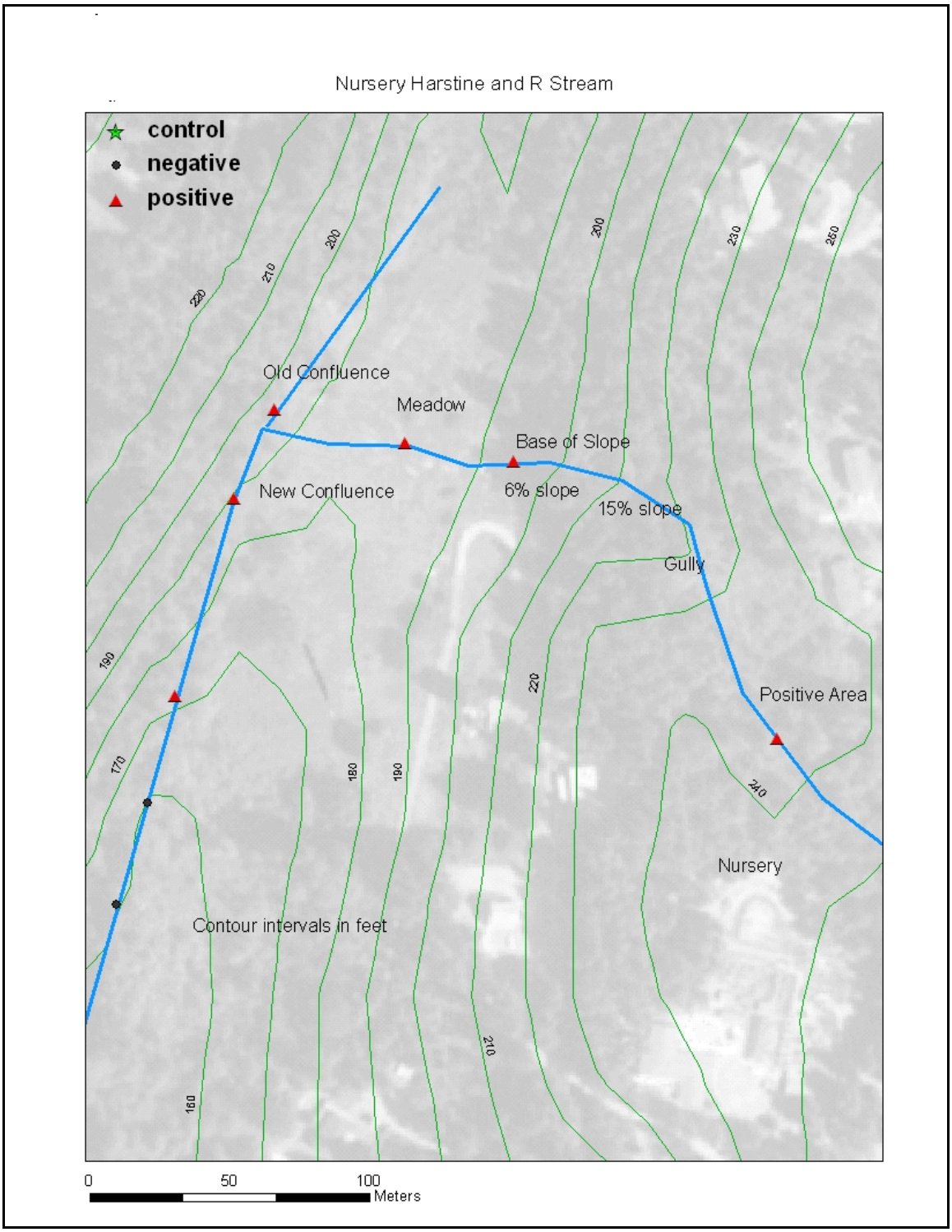

Figure 3: Nursery Harstine and R Stream.

R Stream presents a variety of channel morphology types: riffles, pools, ponds, steps, braided channels, deltas, gullies, culverts, and undifferentiated channel. Assignment of trap locations to a channel type in the data analysis is somewhat subjective, and in some cases is based on third-party descriptions. Channel morphology type is not included in stream baiting protocols, and is not used in either APHIS or FS *P. ramorum* survey work. Sites that were not personally inspected by the author (for instance prior to major morphology changes in November 2006) have been classified as 'undifferentiated channel.' To reduce subjectivity, the Washington State Forest Practices Board (WSFPB) (1994) definitions have been used as much as possible.

P. ramorum has been detected in two other streams in Washington state. The Sammamish River has produced positive results at the same site two years in a row, but the *P. ramorum* recovered does not match the genotype of any infestation found in the Sammamish watershed. (Jennifer Falacy, WSDA, personal communication) Its source is a mystery. A small drainage ditch draining into the Sammamish River has also produced a positive result, but its genotype does not match the Sammamish River sample, and has not been definitively matched to the infested nursery directly upstream of the ditch (Jennifer Falacy, WSDA, personal communication). Figure 4 shows the vicinity of the positive trap site on the Sammamish River.

No analysis of soil type can be made for the Sammamish River find, since its source is unknown. The vicinity of the find is urban, with a network of drainage ditches, storm drains and stormwater ponds. Runoff in these situations is high, and is primarily from impervious surfaces, not soil. The first find was in April of 2007. The trap site is on the outside of a gentle bend in the river, just downstream of a major tributary, and a second, much smaller tributary draining the residential area just uphill. The site does collect some sediment, but is not a major depositional area. The water is shallow here as

the channel bed forms a shelf, with cobbles plainly visible through a thin, discontinuous layer of fine sediment.

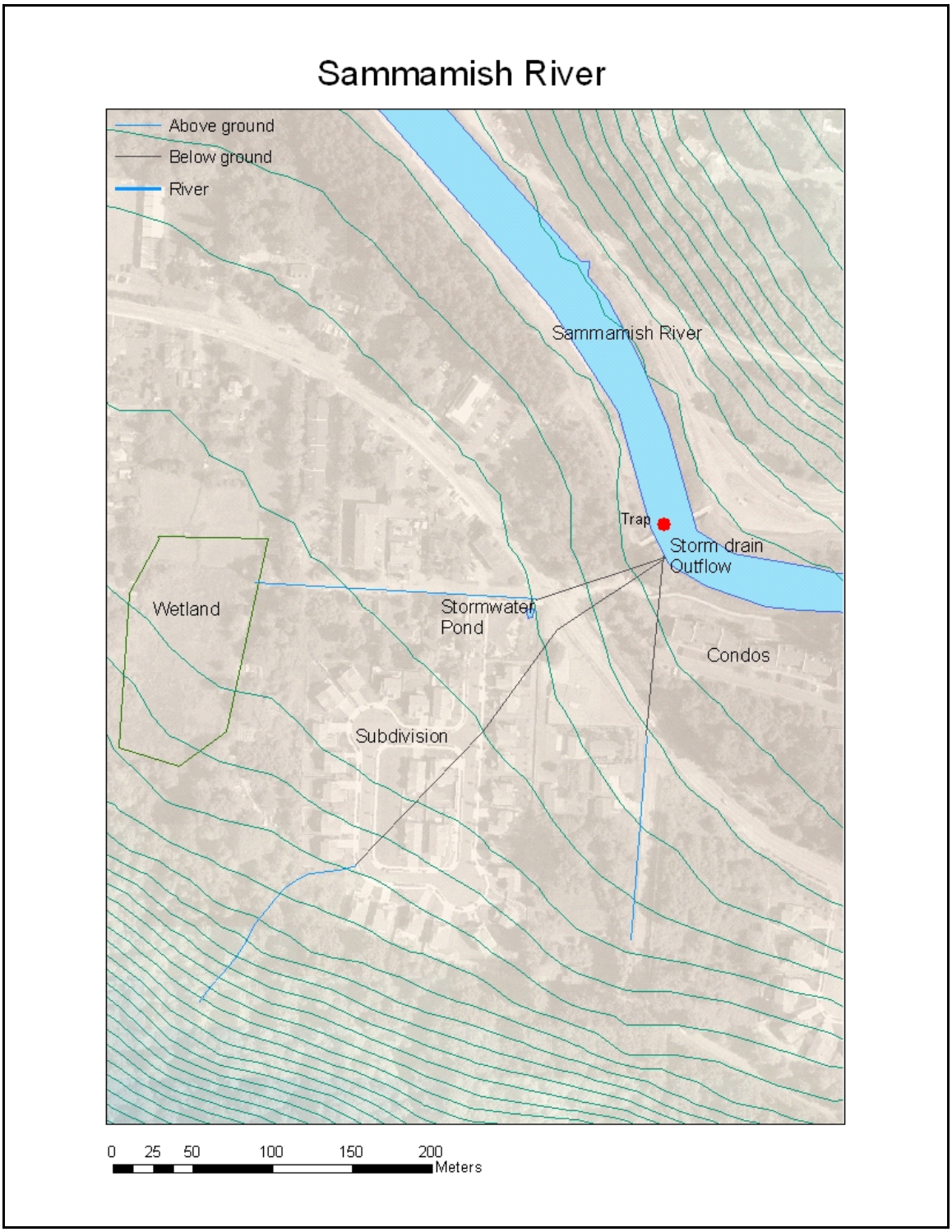

Figure 4: Sammamish River positive trap site.

In January 2008, just beyond this study period, the same location in the river tested positive again. At the time of retrieval, the trap was reported to be lightly buried in sediment, much more so than at previous retrievals (Matt Densley, WSDA, personal communication). This trap was in place during the extreme storms and flooding of December 2007. Seattle received 10cm in the 24 hr period on December 3rd, measured at the Atmospheric Sciences building on the UW campus, across Lake Washington from the mouth of the Sammamish River, with a total for the December 1-4 storm of about 15cm (UW 2008). There is no active stream gauge on the Sammamish, so there is no way of knowing the discharge through this reach at that time. The sediment accumulation, combined with flood debris seen in trees and alongside roadside ditches and stormwater ponds, indicates that while the Sammamish did not overflow, it did receive an unusually large influx of runoff and vegetative debris from its tributaries.

The drainage ditch associated with Nursery Tukwila was baited in numerous places. The trap that returned positive for *P. ramorum* was in a plunge pool carved by converging culverts, one for the ditch from the nursery, and a second culvert for a drainage ditch flowing off a neighboring construction site. Nursery Tukwila has an artificial hardpan, created by building raised beds of composted sawdust on top of muck soil. The infested plants were sitting on this composted sawdust, on the border of the nursery immediately adjacent to the drainage ditch. This area did not drain into the nursery's central retention pond, but was graded towards the drainage ditch. A trap was placed in the ditch immediately adjacent to the positive area, but did not come up positive. Other traps were placed in the ditch between the nursery and the plunge pool, as well as in the retention pond, but only the trap in the plunge pool became positive. Unlike R Stream and Nursery Harstine, there is no slope beyond artificial grading for drainage, and the 'soil' has a high available water capacity and no coarse fractions. The only similarities are the restrictive layer blocking drainage and directing it laterally, and the plunge pool downstream of a culvert.

Nursery Woodinville is another nursery with *P. ramorum* - infested soil, but no positive stream baits. Plate 5 shows a profile of Woodinville soil. M Creek runs along one side of the nursery, and drains into the Green River 1 km downstream. Plate 6 shows traps in M Creek. The nursery is very flat and poorly drained. Water ponds on the surface, and is pumped into a retention pond (referred to as the Duck Pond due to its duck blind) and into M Creek. Plate 7 shows flooding at the nursery. Stream bait traps were placed in flooded fields, in the Duck Pond, and in both M Creek and the Green River, 7 locations in all, starting in October 2006. All stream baits were removed in February 2007. Two-hundred-fifty ml water samples were also taken from the hoophouse with the infested soil. No traps or samples returned positive for *P. ramorum*. Although M Creek borders directly on a portion of the nursery, the positive areas are about 100 m from the creek and from the flooded field that is pumped into the creek.

Nursery Newberg/Nooksack sits on a peninsula of the Green River, just downstream of Nursery Woodinville. Figure 5 shows the locations of Nurseries Woodinville and Newberg/Nooksack, along with the trap sites. The soil on this peninsula is of two series: Nooksack silty loam on the upstream side, and Newburg fine sandy loam on the downstream side. Permeability is moderate, runoff slight, the seasonal water table is 1 m or more below the surface, and there is no ponding, erosion, or slope. There are few drains on the nursery, and only one very small retention pond. Irrigation runoff infiltrates into the sandy alluvial soil, and percolates away. No drains or channels, artificial or natural, direct runoff into the Green River, which at this point is about 30m wide across the active channel. The infested area is approximately 10-15 m from the river. 21 traps have been placed directly downstream of the nursery, for a distance of approximately 0.5 km, but no traps have tested positive. The current of the Green River is quite fast, and the water column is heavily loaded with sediment. Traps are held flat against the steep sandy banks by the current, and quickly buried in sediment. They take up essentially no portion of the wetted perimeter, and are armored against the water column.

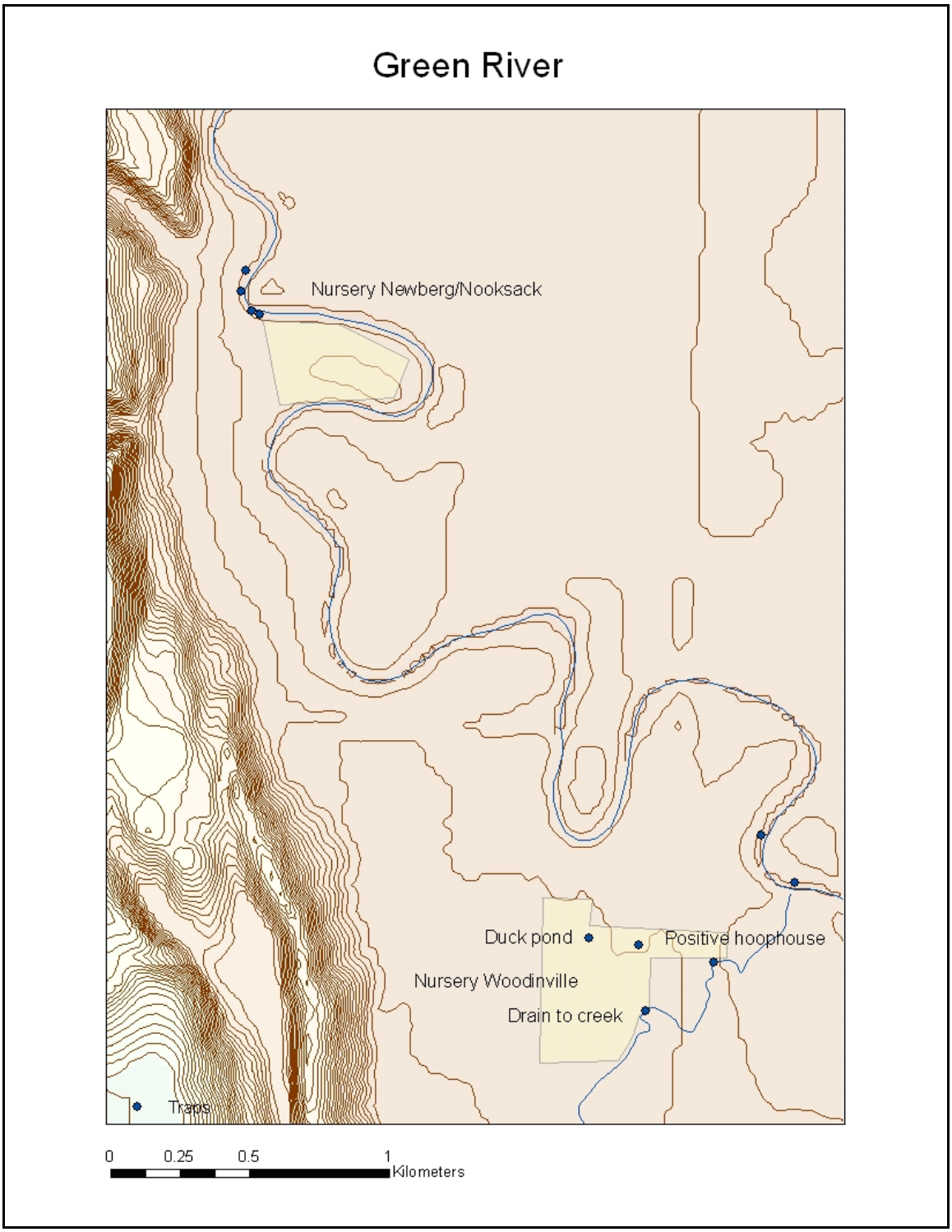

Figure 5: Nurseries Woodinville and Newberg/Nooksack.

Other Sites

Nursery Terric Medisaprist, like Nursery Tukwila, has a restrictive layer resulting from raised beds of composted sawdust constructed on muck soil. And, also like Nursery Tukwila, *P. ramorum* - infested plants were found on one of the raised beds, and the composted sawdust itself was also infested. Unlike Nursery Tukwila, no water or stream positives were found here. Water runs from the raised bed, across a gravel drive, and into a roadside drainage ditch, a distance of perhaps a dozen meters. Two-hundred-fifty ml water samples were taken from puddles in the gravel drive and from water collected in the drainage ditch. None of the water samples produced a positive result. The drainage ditch was baited with 10 traps, from late April to early June of 2007. No traps produced a positive result.

Landscape Yelm contains another restrictive layer. Here, in a street-side landscape for a new subdivision, the landscaping company brought in planting mix, which was laid on top of the native Yelm soil to a depth of about 15cm. The division between the two can be clearly seen in the planting holes left empty after the *P. ramorum* - infested plants were removed. The imported planting mix was also infested with *P. ramorum*. The native Yelm soil was not tested separately from the imported planting mix. No streams or ditches drain this site, so no stream baiting could be done. Surface runoff enters the municipal stormwater system. The empty planting holes had several centimeters of standing water in them after the plants were removed, and two 250 ml water samples were taken of this water. Neither gave a positive result.

Nursery Newberg, in southwestern Washington, sits on an alluvial terrace, some hundreds of meters from the nearest stream. There is no stream running through or along the nursery property. The nursery site is flat, and the sandy loam soil does not allow water to stand. Two, 250 ml water samples were taken in March 2007. Neither produced a positive result.

Other than these streams, the only stream in the US (during this study period) to have been infested with *P. ramorum* associated with an infested nursery is a small creek outside of Jackson, Mississippi (Mark Batchelor, USDA-APHIS, personal communication). The nursery was found positive in June 2006 (COMTF 2008). A grassy drainage ditch and adjacent creek have produced repeated positive water samples and stream baits, from December 2006 through April 2007, and again from December 2007 through April 2008.

Mississippi, of course, has a significantly different climate and soil than western Washington. Precipitation comes year round, without the pronounced wet and dry seasons typical of the West Coast. Winters are warm, and summers hot. Mean annual temperature is about 21°C, and annual rainfall 1780 mm (USDA-NRCS 2008). Figures for western Washington are 10°C and 1300 mm in Olympia (Western Regional Climate Center 2008). It is interesting to note the seasonality associated with the data from Mississippi, compared to Washington and California. In Mississippi, water and stream positives appear from December through April, rather than April and May in Washington, and March through May in California. May in Mississippi is described as having weather unconducive to pathogen recovery (COMTF 2008), where May in Washington is considered ideal (Jennifer Falacy, WSDA, personal communication).

The area of the Mississippi nursery is composed of various alluvial silt loams with no slope, coarse fractions, or hardpans. The nursery is on a floodplain, and the area is subject to occasional flooding. Soils are deep, and permeability is high. The water table ranges from 45-75cm, available water capacity is high, and water does not pond on the soil. The nursery was paved with a thin, brittle, water-permeable concrete outwash,[6] which may not serve to intercept and direct runoff. Positive soil samples were taken from underneath the concrete outwash (Jeff Head, USDA-APHIS, personal communication).

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[6]Very watery concrete, the result of washing out a concrete mixer.

The fear, of course, with *P. ramorum* in streams, is escape from nurseries into the greater environment - wildlands and gardens. While one infected *Salix* has been found alongside the infested ditch in Mississippi, no infected plants have been found alongside any of the infested streams in Washington. Both R Stream and the Sammamish River have been extensively surveyed and sampled for possible infection of riparian vegetation, both official host species and nonhosts, with no positives. The one positive find in a landscape setting was in newly installed plants, and there is no evidence that *P. ramorum* is still present on site, or has escaped. But as shall be shown, the potential for escape through soil water and streams is real and should not be ruled out.

Other invasive pathogens have exerted enormous effects on forest ecosystems. Conversations with regulators and the regulated community often bring up comparisons to Dutch elm disease and chestnut blight. Both diseases have greatly changed the landscape of eastern North America by essentially eliminating the forest dominants American elm (*Ulmus americana*) and American chestnut (*Castanea dentata*) (Owen and Lownsbery 1989, American Chestnut Foundation 2006). There are fears that SOD is doing the same to California oak woodlands by eliminating tanoak and greatly reducing coast live oak, and could do the same to red oak in eastern oak woodlands (CPHST 2004).

Ecosystem-altering Invasive Forest Pathogens

If it is true that those who cannot remember the past are condemned to repeat it, then review of other invasive forest pathogens could provide useful insights for those working to limit the spread of *P. ramorum*. An understanding of how other pathogens spread and established themselves could indicate fields to be investigated for SOD. Accordingly, a review of selected forest pathogens is presented here: Dutch elm disease, chestnut blight, Port-Orford-cedar root rot, and jarrah dieback. Each has informative similarities (and differences) to the present situation with *P. ramorum*.

Dutch Elm Disease and Chestnut Blight

Dutch Elm disease (DED) is caused by the fungi *Ophiostoma* (formerly *Ceratocystis) ulmi* and *O. novo-ulmi*, which spread through two primary insect vectors in this country: the European elm bark beetle *Scolytus multistriatus*, itself an invasive species; and the native elm bark beetle *Hylurgopinus rufipes.* The American elm, *Ulmus americana,* native to the eastern portion of the country, is the most susceptible host, followed by the introduced European elms (Owen and Lownsbery 1989). Asian elms, in particular the Chinese elm *(Ulmus parvifolia)* widely planted in California, are resistant. The origin of DED has still not been determined (Scheffer 1997). *O. ulmi* was introduced into this country from Europe, where it may also be invasive, some time in the 1920s through infested elm logs imported for veneer. Importation of live elms had been prohibited in 1919 due to concerns over DED but the quarantine did not include timber, due to incomplete knowledge of the pathogen. *O. novo-ulmi* appeared, and largely replaced, *O. ulmi* some time in the mid 20th century (D'Arcy 2005).

Chestnut blight is caused by the fungus *Cryphonectria parasitica*, an Asian species introduced to this country in the late 19th century on imported Asian chestnut trees (Anagnostakis 1997). Chinese (*Castanea mollissima)* and Japanese *(C. crenata)* chestnuts are resistant to the disease but American *(C. dentata)* and European *(C. sativa)* species are very susceptible. Chestnut blight is spread by wind-borne spores, by birds and insects, and by infected nuts and plants (Anagnostakis 2000). It has spread throughout the native ranges of both the American and the European chestnuts, but does not appear to have spread to the western US. Similar to SOD, chestnut blight causes bark cankers that girdle and kill the tree.

Prior to introduction of *O. ulmi/novo-ulmi* and *C. parasitica*, elms and chestnuts were both forest dominants and widely planted in parks, landscapes, woodlots and orchards. The original infestation area for *O. ulmi* (New York, New Jersey and

Connecticut) supported anywhere from 5-10 million susceptible American elm trees, plus other, more resistant *Ulmus* species that would harbor and propagate the fungus without displaying symptoms of the disease (Owen and Lownsbery 1989). Scheffer (1997) estimates a billion wild elms in North America plus a half billion cultivated elms at peak population levels. Elms have not been eliminated by the disease, however (D'Arcy 2005). Most elms live long enough to set seed, so elms continue to persist, and the disease can be managed in landscape settings (D'Arcy 2005).

Chestnut populations prior to chestnut blight are estimated at 4 billion individuals (ACF 2006). Chestnut trees comprised roughly 1/4 of the population of hardwood trees in its native range and up to 50% on suitable sites, could easily grow to 30m tall with a dbh of 2m (Paillet 2002). American chestnut as a species, however, is not extinct. Many trees survive as shrubby sprouts from living roots (Paillet 2002). A few survive long enough to set seed, and a very few scattered mature trees still persist in the native range (ACF 2008). American chestnuts are still planted in orchard settings, where the disease can be somewhat managed, especially in the Pacific Coast states of California, Oregon and Washington, where chestnut blight has not yet invaded (NNGA 2008).

In contrast to *P. ramorum*, *O. ulmi/novo-ulmi* are spread by insects and root grafts, not by weather conditions or natural drainage patterns. Both transmission routes, however, are amorphous and difficult to control. Insect populations and root grafts are somewhat controllable, compared to wind-blown rain and surface water runoff, although insecticide use has been largely discontinued for control of DED due to lack of efficacy (D'Arcy 2005). Similar to *P. ramorum*, *O. ulmi/novo-ulmi* attack a widespread and numerous population of hosts, thus spread will not be limited by a lack of suitable hosts. And, both pathogens can be maintained and propagated by asymptomatic hosts, acting as long-term reservoirs of inoculum in the absence of susceptible hosts. From looking at the DED epiphytotic, it would appear that SOD is comparable in potential for widespread transmission and environmental damage.

While the loss of elms and mature chestnuts is regrettable, neither species has gone extinct, the eastern hardwood forest community has adapted, and few people miss what they never had. Paillet (2002) suggests that canopy dominance of American chestnut was a relatively recent phenomenon, dating back only 2500 years, and that chestnut blight is simply the most recent of a series of climactic and anthropogenic forest disturbances that brought the chestnut first to canopy dominance, then to understory shrubbiness.

Similar to chestnut blight, SOD attacks only live oaks old enough to produce bark. Seedlings and saplings are unaffected. Unlike chestnut blight, SOD does not kill every oak victim. Where mature chestnuts have been all but eliminated by blight (ACF 2006), and tanoaks can suffer nearly 100% mortality from SOD (CPHST 2004), SOD-induced coast live oak mortality is approximately 50% (CPHST 2004) and some individual coast live oaks survive for years with *P. ramorum* infections, without developing bark cankers (Garbelotto 2004). Still, analogies between chestnut blight and SOD are not unreasonable, where tanoaks are concerned. Mortality rates for SOD on eastern red oaks, Mediterranean live oaks, and beeches are unknown, but may be very high (Garbelotto 2004).

Both *P. ramorum* and *C. parasitica*, are spread by wind (wind-driven rain in the case of *P. ramorum*), while *O. ulmi/novo-ulmi* use the highly-motile elm bark beetles, and *C. parasitica* can also use insect vectors. From looking at the DED and chestnut blight epiphytotics, it would appear that *P. ramorum* suffers a slight disadvantage in natural dispersal, due to distance limitations on spread through wind-driven rain, compared to wind-borne or flying insect-borne spores. Spread by wind-driven rain is limited in dispersal distance, compared to airborne spores, by the weight of water drops (Moralejo et al. 2006). On the other hand, *P. ramorum* has many more species of potential hosts (the foliar hosts) that can produce large amounts of inoculum over large areas of forests and gardens (CPHST 2004), where *C. parasitica* and *O. ulmi*/*novo-ulmi* appear to be limited

to a single genus each (Anagnostakis 2000, D'Arcy 2005). All three pathogens (*P. ramorum, C. parasitica* and *O. ulmi/novo-ulmi*) are spread long-distance in the nursery trade by infected plants and plant parts.

Neither *C. parasitica* nor *O. ulmi/novo-ulmi* are water-borne, so do not spread in stream water. There does not appear to be any evidence yet that stream water contaminated by *P. ramorum* has spread SOD in natural settings, but other *Phytophthoras* are known to invade new areas through stream water. Analogies to chestnut blight and Dutch elm disease may be common in conversations due to general familiarity with these diseases, but there are other, less-familiar invasive forest pathogens that offer more appropriate lessons for *P. ramorum* and SOD; in particular, *P. lateralis* and *P. cinnamomi*.

Port-Orford-cedar Root Rot

Port-Orford-cedar root rot (POCRR) is caused by *Phytophthora lateralis*. The main, and for most purposes only, host of *P. lateralis* is the Port-Orford-cedar (*Chamaecyparis lawsoniana*) (Erwin and Ribeiro 1996), a timber tree native to coastal southwestern Oregon and northwestern California, which can grow over 50m tall and over 1m dbh. One particularly old specimen is dated at about 900 years old (Jules et al. 2002). There are numerous horticultural varieties of *C. lawsoniana*, all also susceptible to POCRR, but other species of *Chamaecyparis* show little or no susceptibility. The native range of *P. lateralis* has not been determined (Roth et al. 1987). Because of the resistance of Asian species of *Chamaecyparis*, it is assumed that the native range of *P. lateralis* may be in Asia, although the species has not been found there (Roth et al. 1987). As the Alaska yellow-cedar, *C. nootkatensis*, is also resistant to POCRR, it is also possible that the pathogen is native to the Alaska and British Columbia coasts.

Pacific yew (*Taxus brevifolia*) is also susceptible to POCRR, but at much lower rates than Port-Orford-cedar (Erwin and Ribeiro 1996). For instance, inoculation studies showed an average 72% infection rate for Port-Orford-cedar seedlings but only 4% for Pacific yew; and a field survey showed 46% of streamside Port-Orford-cedars dead of the disease, but only 10% of the yews (Hansen et al. 2000).

Port-Orford-cedar grows over a wide variety of environments in a very small range (Hansen et al. 2000). Port-Orford-cedar ranges along the Pacific coast and into the Coast Range and Siskiyou Mountains, from Coos Bay, Oregon to Eureka, California, and east as far as Grants Pass, Oregon. It occupies the northernmost range of the coast redwood (*Sequoia sempervirens*), and extends just a bit farther north; and is just south of the range of the western red cedar, *Thuja plicata*. Port-Orford-cedar requires year round moisture and so is primarily a riparian or wetland species over the inland and southern portions of its range (Hansen et al. 2000). It is often the only large riparian tree in its range (Hansen et al. 2000), filling the ecological role that western red cedar fills in western Washington. Port-Orford-cedar is a critical component of both old-growth forests and riparian habitat, providing stream shading and structure, bank stabilization, and old-growth habitat (Hansen et al. 2000).

POCRR was first seen on imported nursery stock of *C. lawsoniana* in Seattle, considerably north of the native range of Port-Orford-cedar, in 1923; but the causal agent was not discovered until 1941 (Erwin and Ribeiro 1996). POCRR was discovered in 1952, in southwestern Oregon, the native range of *C. lawsoniana* and the location of commercial timber stands (Roth et al. 1987). The disease was probably introduced in ornamental plantings as it first appeared in the area's towns and cities, and only later appeared in rural and forested areas, following roads, surface waters and livestock trails (Roth et al. 1987).[7] The spores are carried in surface waters and overland flows,

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[7]Hansen et al. (2000) believe the introduction of *P. lateralis* to southwestern Oregon was in the potting soil of rhododendrons imported from an infested nursery.

percolating into the soil and infecting roots through infiltration; also on the feet of cattle, and on earth-moving machinery. Long distance spread is generally by transport of contaminated soil or infected plants. Spores are not airborne (Roth et al. 1987).

The distribution of POCRR along streams and drainage patterns clearly indicates waterborne transmission (Roth et al. 1987), and the preference of Port-Orford-cedar for riparian habitats in inland areas serves to expose a large percentage of the population to the pathogen. Infected cedars are found along stream banks, generally with their roots either in the stream channel or within easy reach of overbank flows, and downstream of roads (Jules et al. 2002), as well as downslope of infected soils in drainage patterns (Hansen et al. 2000). The spores are carried from stream to stream in mud on machinery, feet and hooves, and then downstream within a stream channel. There is a direct relationship between proximity to roads and disease incidence (Hansen et al. 2000).

Spore dispersal in streams appears to be limited to about 200m (Jules et al. 2002). As trees within 200m of the inoculum source become infected, they themselves produce and release more spores into the stream channel, moving the disease downstream over time. Upstream dispersal occurs through root grafts between trees and through infected mud on tires and feet, and of course is much more limited than downstream dispersal.

Cedar roots become infected when the water-borne spores are washed onto roots, whether through stream current in the case of roots growing into the channel, or percolation of infested floodwater into soil outside the channel (Hansen et al. 2000). Jules et al. (2002) theorize that water-borne spore dispersal may be limited by spores settling out, or by spores being physically damaged by stress factors in the current. We know that spores of *P. ramorum* have been carried, and are still infective, a distance of 7 km from the nearest inoculum source (Murphy et al. 2005). von Broembsen (1984) reports that entire rivers in South Africa are infested with *P. cinnamomi* from headwaters to mainstem over a distance of 50 km and more, with much higher concentrations in headwaters than

in lower reaches. Gregory (2005) states that particles in suspension do not interact with other particles except at very high concentrations. Nearly all interactions will be with water molecules rather than with other particles, which suggests that physical damage of fungal spores by other particles, such as sand, is unlikely.

I would suggest that there may be other factors besides settling or physical damage limiting downstream dispersal distances of *P. lateralis,* such as simple dilution of spores below a critical threshold with increased channel volume, as suggested by von Broembsen; or water chemistry or aquatic microbial predation affecting mortality or infectivity of spores (Barlocher 1992). Both Murphy et al. (2005) and von Broembsen (1984) were recovering infective material in their surveys; uninfective spores would not have been detected due to limitations inherent in sampling for *Phytophthora.*[8] And, we know that particles of the size range of fungal spores do not settle out in moving water (Knighton 1998). Spores of *Phytophthora* are known to settle out in ponds (von Broembsen.2007), but flowing streams would keep particles as small as spores (~50 μm) in motion at even very slow currents.

The production of horticultural varieties of *C. lawsoniana* has essentially disappeared in Washington and Oregon due to POCRR (Roth et al. 1987). While over 200 cultivars are known, only one local production nursery propagates any (Nils Sundquist, Sundquist Nursery, personal communication). A few mature *C. lawsonianas* can be seen in gardens and parks around western Washington but new trees are rarely planted. Conversely, cultivars of Asian species of *Chamaecyparis* are numerous, widespread and very popular.

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[8]Murphy's study involved suspending rhododendron leaves in streams and then culturing out any spores that infected them. von Broembsen poured collected river water through filters and then cultured the filter material. Only live, infective spores would be detected by either method.

Commercial timber production continues, but is becoming restricted to low-susceptibility sites (Roth et al. 1987). While not endangered by the disease, *C. lawsoniana* grows over a very restricted range and has never been a forest dominant, preferring mixed coniferous forests. Even so, *C. lawsoniana* timber is so valuable that loss of a single tree can be financially significant (Roth et al. 1987). The US Forest Service recommends locating *C. lawsoniana* stands on hilltops and ridgecrests, where there will be no surface runoff from diseased sites; in areas not traversed by cattle or elk; and where human activity can be controlled. In essence, the habitat of *C. lawsoniana* is shifting uphill and away from riparian areas, in response to the presence of *P. lateralis*.

Because of the restricted host range of the pathogen, the extreme value of *C. lawsoniana*, and the restricted movement of the pathogen, POCRR has not completely altered the forest it attacks, although a combination of logging and disease has shifted the age structure of the population to smaller, younger trees growing farther apart and farther from roads and stream channels; and many miles of riparian and wetland habitat have been all but stripped of trees (Hansen et al. 2000). Careful management of soil and water can limit the spread of the disease, and the financial motivation to do so is high. The fact that the pathogen cannot persist in the absence of the single species of host,[9] and that spores cannot disperse by air, makes eradication and exclusion feasible; while a pathogen with many potential hosts and aerial transmission of spores, such as *P. ramorum,* would not be controlled in this manner.

No eradication program was attempted against POCRR, but disease management is ongoing. Both the Forest Service and the Bureau of Land Management engage in a number of techniques to minimize spread of the disease, in particular road closures, road management, tree thinning, and vehicle sanitation (Hansen et al. 2000). One technique used to minimize disease spread is sanitation, or removal of Port-Orford-cedar trees

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[9]The disease does not propagate well at all on Pacific yew; only yews in close proximity to infected cedars become infected themselves.

growing along roadsides. Port-Orford-cedar seeds heavily, and sprouts profusely in the disturbed soil alongside forest roads, a site also favored by *Phytophthora lateralis* (Hansen et al. 2000). Since the pathogen cannot survive in the absence of Port-Orford-cedar, theoretically it should be possible to eradicate the pathogen from an area through removal of all Port-Orford-cedar trees and removal of all seedlings as they sprout.

P. lateralis is similar to *P. ramorum* in many ways, not surprising, since both are *Phytophthoras*. While POCRR is a root rot and SOD is an aerial disease, transmission of *P. lateralis* through stream water and subsurface drainage, and findings of *P. ramorum* in streams, suggests that *P. ramorum* may also be capable of spreading through similar pathways.

Jarrah Dieback

Jarrah is the common name for *Eucalyptus marginata,* native to Western Australia, a forest dominant and valued timber tree. Jarrah dieback is the common name for a disease of jarrah trees and other members of the jarrah forest (in particular species of *Banksia*) caused by *Phytophthora cinnamomi.* *P. cinnamomi* is believed to be native to Southeast Asia, possibly Taiwan, but was spread globally so early, before the discovery of fungi and water molds as disease causal agents, that its native range cannot be determined (Erwin and Ribeiro 1996). For instance, the water mold is believed to have been introduced into what would become the US in the mid 18th century on imported plant material (Zentmyer 1983), but the discovery of fungi as plant pathogenic agents did not occur until 1872 and this species was not described until 1922 (Erwin and Ribeiro 1996).

P. cinnamomi is believed to be the most widely distributed species of *Phytophthora*, with over 1000 hosts (Zentmyer 1983, Erwin and Ribeiro 1996). It is a tropical species, needing warmth and dampness for growth, but infects many nontropical

hosts. In the US it is a major pest of tree fruits, particularly avocado (*Persea americana*) and stone fruits *(Prunus spp.)*, and woody ornamentals such as rhododendron (Erwin and Ribeiro 1996). *P. cinnamomi* was introduced into Western Australia's jarrah forests some time prior to 1922 but was not identified as the causal agent of jarrah dieback until 1965 (Shearer and Bailey 1989). Meanwhile the pathogen was spread throughout southwestern Australia through road building gravel (Gregory 1983). The pathogen is believed to be spread primarily through contaminated soil on machinery and vehicles, road building gravel, and through downslope drainage from contaminated soil, as well as wind-driven splash. Long-distance dispersal occurs on asymptomatic plant material in the nursery trade (Erwin and Ribeiro 1996).

When jarrah dieback first made its most dramatic appearance, after WW II, large areas of jarrah forest were subjected to rapid dieback, particularly in the 1950s (Shea 1988). Yet it was not until the mid 1960s that the cause of dieback was determined to be *P. cinnamomi.* Even after the discovery of *P. cinnamomi* as the causative agent, its transmission mechanism remained a mystery for some time. *P. cinnamomi* was known to spread through surface flows, but surface flows rarely occur in this area in the absence of major disturbance, such as from logging or mining (Shea et al. 1983). Soil conditions in the forest were considered inhospitable to spore survival (too hot and dry), and few spores could be isolated from soils in diseased areas.

Lack of knowledge led to fears that the entire forest would be destroyed (Shea et al. 1984). Shea, working on dieback in 1966, noticed that the distribution and intensity of the disease varied greatly, leading him to investigate a correlation between soil environment and disease intensity (Shea 1988). Soil in water-gaining sites tended to have *P. cinnamomi* in the soil, but the plants on these soils were not susceptible to dieback. Soil on well-drained upland sites, where the jarrah grows, had little or no *P. cinnamomi* in the soil, yet these were often the sites subjected to dramatic dieback.

A breakthrough was achieved in 1983, when an intense jarrah dieback episode occurred following abnormally heavy summer rains (Shea 1988). Soil sampling in the rapidly dying forest still revealed little disease agent; entire jarrah root systems were excavated by mining equipment borrowed from Alcoa, leading to the discovery of lesioned taproots in the hardpan layer (Shea et al. 1984, Shearer and Bailey 1989). Previous to this, *P. cinnamomi* was believed to mainly infest fine feeder roots growing near the surface, and to never infest large diameter, deep taproots (Shea 1988). The discovery of lesioned taproots allowed forest managers to realize that jarrah dieback was not capable of destroying the entire forest, but could only attack those sites with the conducive soil profile (Shea et al. 1984) - another example of the disease triangle concept of host-pathogen-environment. Even in the presence of suitable host and pathogen, no disease develops in areas of inhospitable environment, in this case a deep, well-drained soil.

In southwestern Australia, *P. cinnamomi* is not well adapted to the hot, dry climate, but has methods to compensate. Mycelia utilize what amounts to a 'freeway' through the hot dry soils - the laterally spreading root systems of bull banksia (*Banksia grandis)* (Shearer and Bailey 1989). Bull banksia is widely distributed through the jarrah forest and is highly susceptible to *P. cinnamomi*, which spreads as much as 1cm per day through bull banksia roots even when the soil is too hot and dry to support the water mold. Additionally, the chlamydospores of *P. cinnamomi* travel downwards through the soil in soil water to the hardpan, where soil water collects and flows laterally (Shea et al. 1983). Jarrah, a phreatophyte, sends its roots as deep as 10 meters to tap into this and other aquifers. Zoospores of *P. cinnamomi* collect on top of the hardpan and infest the jarrah taproots where they pass through. Zoospores can also be found as deep as 2 meters below the hardpan, on top of an impervious clay layer (Kinal, Shearer and Fairman, 1993). Where there is no hardpan layer within a meter of the surface, there is no dieback in the jarrah trees (Shea et al. 1983). Spores flow along the hardpan layer, traveling

downslope as far as 2 meters in one study (Shea et al. 1983), and potentially as far as 120 meters (Kinal et al. 1993).

No eradication program was attempted against jarrah dieback. None was possible. The water mold was well established and widely spread in the soil long before its discovery. Australian land managers manage the pathogen through a variety of means, including road closures during wet weather, eliminating bull banksia with controlled burns, modifying forest floor drainage, establishing vehicle washing stations in infected areas, and development of resistant jarrah varieties for reforestation (Shea et al. 1984).

There are two critical lessons for SOD from jarrah dieback. First, that lack of knowledge can lead to unnecessary panic (or, conversely, unwarranted complacency). Large-scale episodes of dieback led to fears that the entire jarrah ecosystem would be destroyed, because the critical element of the conducive environment was unknown - in this case, the requirement of a shallow hardpan. Susceptible species may be removed from areas with a shallow hardpan, but these areas represent only a small part of the jarrah forest. Jarrah on deep soils are unaffected by dieback. The second lesson is that hydrology and soil type, texture and profile are critical elements in the environment of a water-borne pathogen, and should be investigated thoroughly to properly understand disease spread and risk.

Dire predictions for forests invaded by *P. ramorum* may also turn out to be overstated. In particular, forests lacking in tanoaks and California bay laurels may be at lower risks than initially anticipated (CPHST 2004). While damage is already severe in parts of California, forests in Washington and Appalachia will not necessarily suffer anything like the same fate. And since *P. ramorum* is neither soil dwelling nor a root-rotting pathogen, it might be assumed that soil type, texture and profile are irrelevant to its spread, in contrast to *P. cinnamomi*. But then we are unable to explain why in some infested sites, *P. ramorum* is found in soil and draining surface waters, while in other

infested sites it is not; and why it persists in R Stream, in the absence of symptomatic host material.

Methods

P. ramorum is known to survive, and be transported, in soil and water (CPHST 2004). USDA-APHIS Confirmed Nursery Protocol (2006) requires that nurseries with *P. ramorum*-infested plants be tested for soil and water infestations. Soil samples are taken from beneath the infested plants, water samples are taken from puddles and rivulets, and larger water bodies (ponds and streams) are baited.

Of the nine nurseries and one landscape site in western Washington that have developed *P. ramorum* -infested soil, only one is definitely associated with infested surface water.[10] Five border directly on a stream or river, and three have ponds or pools on site (one has both a stream and a pond) that could potentially have become infested. If we knew what factor(s) control the movement of *P. ramorum* from soil to surface water, it would be possible to better target monitoring and survey efforts, and to potentially prevent infestation of surface waters and subsequent spread of the pathogen.

One possibility, of course, is that the pathogen has infested some or all of these waters, but has gone undetected. The potential for false negatives cannot be discounted, and should be kept in mind, although it is not a topic for this paper. See Bulluck et al. (2006) for a discussion of detection techniques for *P. ramorum*. See also the COMTF newsletter from May 2008 (http://suddenoakdeath.org/html/newsletter_archive.html) for a brief discussion of different results obtained by different organizations analyzing replicate

[10]A second nursery is associated with a stream positive, but DNA fingerprinting has not definitively linked the *P. ramorum* in the stream with that in the nursery.

samples from the same nursery in Mississippi. Another possibility is that the pathogen has gone undetected by chance. Populations of newly introduced species are often low, and survey methods do not do a good job of detecting low numbers (Perkins 1989).

Review of the epiphytotics POCRR and jarrah dieback give some indication of factors that might control movement of *P. ramorum* from nurseries into streams. In particular, the strong correlations between soil type and jarrah dieback (Shea et al. 1984), and between root/stream water contact and POCRR (Hansen et al. 2000), are suggestive of possible transmission pathways. For these reasons, this study focuses on soil types and surface water drainages.

Study Sites

The five nurseries and one landscape site selected for this study were chosen for the following reasons: they have infested soil or soil-like substrate, and they have been repeatedly stream baited or water sampled during the study period 2006-07. Place names, except where public knowledge, have been abbreviated to maintain confidentiality as required by USDA. I will refer to the different nurseries by their soil series names. All soil series information comes from the Natural Resource Conservation Service's Web Soil Survey, at http://www.soils.usda.gov.

Nursery Harstine is the focus of this paper, as its stream (R Stream) has been baited considerably longer than any other site: essentially continuously from January 2006 to the present. The stream has typically had 8 bait stations scattered from the nursery property to S Lake, a distance of about 2km. In spring 2007, there were as many as 28 trap sites in this stretch. One trap was always placed upstream of the nursery to act as a control. Two traps were placed downstream of S Lake, which is drained by a perennial, first order stream.

Location of Traps

The initial placement of the traps in R Stream was based on two factors: proximity to the positive area of Nursery Harstine, and accessibility by the field personnel. The stream itself tends to move the traps to similar locations within the channel. Most traps get dragged downstream to either a pool, an in-channel obstruction, or the limit of the tie rope. In the latter case, the traps tend to be held flat against the bank by the current. Figure 2 shows locations of trap sites on R Stream. The first traps were placed in the stream where it runs through the nursery property and the adjoining meadow. Later traps, when baiting was extended downstream, tended to be placed at road crossings for ease of access. Road crossings, of course, mean culverts. The first classifications for trap locations lumped all culvert locations together, but it quickly became apparent that the upstream ends differ dramatically from the downstream ends. Both ends tend to form pools, but pools vary greatly (Church [1996] describes 7 different kinds of pools). The upstream pools differ markedly in current velocity and sedimentation patterns from the downstream pools. And, traps at the upstream end of a culvert tended to be dragged into the culvert, and subjected to currents as much as an order of magnitude greater than the traps at the downstream end of the same culvert.

Starting in 2007, WSDA was denied access to the nursery itself, to continue the stream baiting required by APHIS. For that reason, later trapping does not include the reaches running through the nursery and the meadow, which had recently been purchased by the nursery. Figure 3 shows the vicinity of the nursery and meadow.

There are a few factors affecting selection of stream bait trap locations. In no particular order, they are property ownership, bank steepness, riparian vegetation, floodplains, vandalism, and parking issues. R Stream flows entirely through private property, and WSDA had to contact landowners for permission before placing stream baits on private property. Some landowners were understandably reluctant to allow a state

regulatory agency to enter their property, or to allow monitoring for an organism that, if found, would result in the destruction of the landowner's vegetation.[11] Along some reaches, the channel is sufficiently entrenched that field personnel could not reach the water safely, or without damaging the banks. Dominant riparian vegetation in this area tends to be very thorny and dense, sometimes impassably so: primarily salmonberry and Himalayan blackberry. Streams attract children and racoons, both of whom will pull out strange objects such as stream bait traps. Traps that were easily visible sometimes disappeared, so less-visible sites were selected over more-visible. Roads in this area tend to be narrow rural roads, with no shoulder sufficiently wide and stable enough to safely park a truck on. Time limitations precluded walking long distances from safe parking areas to traps. A few reaches, while accessible and free from all the other issues, would have required wading through dangerously deep, soft, alluvial sediments deposited in a wide floodplain, in order to reach the active channel.

Similar considerations dictated locations of trap sites on other streams. Entrenchment might have been a more serious factor in site selection on the Green and Sammamish Rivers than on R Stream, as these two urban rivers are very deeply entrenched, with soft sandy banks that collapse easily underfoot. Impenetrable thickets of blackberries and reed canarygrass are common, as well. Private property issues were less of a concern on these rivers, as there are numerous parks alongside the rivers, linked by public walking trails that hug the bank along most of their lengths. Vandalism was a more serious issue on the larger rivers, due to the public recreation facilities, a sizeable homeless population on the Green River, and anglers and boaters on both rivers. Maps 3 and 4 show trap locations on the Sammamish and Green rivers.

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[11]Road crossings generally fall into the public right of way, negating the need to contact landowners.

Stream Baiting

The US Forest Service has developed a standardized methodology for baiting streams for *P. ramorum* (USFS 2006). WSDA followed this protocol with slight variations. Mesh bags measuring approximately 40cm x 30cm were constructed from window screen material. The mesh material was folded to form a pouch, and stapled or sewn with fishing line into sleeves. The Forest Service calls for 4 sleeves, but WSDA used 5 sleeves, as our sampling protocol calls for a minimum of 5 leaves per sample (Jennifer Falacy, WSDA, personal communication). A wooden dowel or a short length of PVC pipe was used at the top to hold the material flat, and to anchor the tie ropes. The mesh material was folded over the dowel and fastened to form a flap covering the sleeve openings. Each sleeve was filled with one rhododendron leaf *(Rhododendron macrophyllum* is the standard on the West Coast). Leaves are first folded to break the midrib, as wounds attract zoospores of *Phytophthoras* (Nathan Lubliner, WSDA, personal communication). Paired bait bags, connected by about 1m of rope, are submerged in the stream. Two-pound lead fishing weights are used to anchor the traps in place. Plates 1 and 2 show stream bait traps as used by WSDA.

Stream baits are left in the water for 2-4 weeks depending on season: 2 weeks in warm weather, longer in winter. When the leaves in the traps are collected, the traps are rebaited at the same time, and put back into the water. There is no lag time between collecting and rebaiting, unless baiting is being suspended for the season. Once collected, the 5 leaves from each bag are pooled into one sample for laboratory analysis. See USDA-APHIS (2004) for a description of stream baiting and sample analysis protocol. Because the bait bags are deployed in pairs, there are 2 baits, and 2 samples of 5 leaves each, per trap. A positive result can occur from only 1 infected leaf out of the 10 in the paired bait bags.

Stream Morphology

The Forest Practices Board (1994) defines 8 general channel types. R Stream, despite being only about 2 km in length, displays 6 of them: colluvial, braided, pool-riffle, plane-bed, step-pool, and bedrock. Traps were placed in a variety of channel types. Table 2 lists these, plus undifferentiated channel, and gives a general description of the characteristics used to assign trap sites to morphologies.

Table 2: Channel morphologies as used in this study.

Type	Description	Response	Bedload
Braid	multiple longitudinal gravel bars split channel	transport	coarse
Culvert	manufactured pipe constricting flow	transport	none
Delta	submerged bar where current drops sediment	deposition	fine
Gully	very deep and narrow, current very fast	transport, source	cohesive
Pond	wider and deeper than average channel, very slow current	deposition	fine
Pool	deeper than average but not much wider, very slow current	deposition	fine
Pool DC	plunge pool at downstream end of culvert	transport and deposition	coarse grading to fine
Riffle	shallow, rapid; water surface dimpled by bedload	transport	coarse
Undifferentiated	plane-bed, or undescribed; not a pond or gully		medium to coarse

Data Collection and Analysis

Each trap had the following information recorded: collecting personnel, location in stream (by general description, not by GPS point), date placed, and date retrieved. The very first traps placed also had water temperature recorded, but since stream water temperatures do not vary much in this area, that was discontinued. The information was entered into the WSDA database, along with the results of the lab analyses - ELISA, PARP, PCR, and the final determination, positive or negative. See USDA-APHIS (http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/) for information on laboratory analysis for *P. ramorum*.

For this study, the WSDA database was queried for all stream and water baits placed in 2006-07, and the results exported to an Excel spreadsheet. The location descriptions were then standardized, after review of every record. Location codes were assigned to each trap site, starting at the most downstream point, and then morphology types were assigned to each trap site. The majority of trap locations maintained the same general morphology throughout the two year period, but the reach of R Stream from the nursery to the confluence shifted dramatically after the November 2006 storm and major earthmoving work by the nursery in April 2007. These locations are assigned to two different morphologies, - one for traps collected in 2006, and another for traps collected in 2007. Final determinations of infestation by *P. ramorum* were then compared with season, length of time in water, morphology, and location.

GPS readings for locations were taken with a Garmin eTrex Vista GPS receiver (Garmin Intl. Inc.; Olathe, KS; www.garmin.com). Maps were generated using ArcGIS 9.2, (ESRI, Redlands, CA; www.esri.com). Public-access GIS datasets were obtained from the Washington State Department of Natural Resources. Stream discharge was calculated from current velocity measurements made with a Swoffer 2100 current velocity meter (Swoffer Instruments, Inc.; Seattle, WA; www.swoffer.com). The slope of the stream through the nursery was estimated with a Suunto PM-5 clinometer (Suunto Oy, Finland; www.suunto.com).

Current Velocity and Flow Lines

Particles in water cannot be assumed to travel at the same speed as the water (Knighton 1998, Gregory 2005). Friction from the bank causes variation in current speed throughout the channel profile, while particles have their own frictional losses. Particles also drop at a settling velocity determined by size, shape, and specific gravity, and this vertical drop, along with directional variations caused by turbulence, will reduce

horizontal velocity. Travel time and pathways through a short reach of R Stream were observed by dropping paper discs, collected from a paper hole punch, into the water, and timing and observing their travels downstream. Paper discs are, of course, much larger than *P. ramorum* spores, and of a different mass and specific gravity, but they are similar to particulate organic matter, such as decomposing leaves.

Streams as a whole flow in one direction, but turbulence within the water column creates multiple flow lines, or pathways, for small particles in suspension. Approximately 25 paper discs were used to allow for illustration of different flow lines.

Four other streams were baited, but did not produce any positive traps. For these the number of traps, duration of trapping, season, and soil types were compared with Nursery Harstine and R Stream. Soil data were obtained from the Natural Resource Conservation Service (NRCS), through printed soil surveys and the website www.soils.usda.gov.

Results

WSDA baited six streams in 2006, and eight in 2007, for a total of 11 different streams, all of which are associated with infested nurseries. Of these streams, *P. ramorum* has been found in only three. This paper looks at factors that may explain why *P. ramorum* was found in these few streams, and not in others. It should be kept in mind that with so few positive finds, no firm conclusions can be made with these data. Some correlations can be seen, which bear further investigation. The three streams examined are R Stream (182 traps), the Green River and one tributary (56 traps), and the Sammamish River with multiple tributaries (220 traps).

Precipitation and Temperature

Figure 6 shows a comparison of monthly average temperatures in degrees F, and precipitation totals in inches, for the vicinity of Nursery Harstine, compared to number of positive traps collected from R Stream.[12] Storms brought extreme rainfall in January 2006, November 2006, and December 2007. From this limited dataset, however, it is impossible to say whether the storms are correlated with the positive stream baits. Winter normally brings periods of heavy rains, and the positive stream baits in spring may be an unrelated seasonal phenomenon. It can be seen that the late winter positive stream finds in 2006 are coincident with elevated temperatures along with heavy rain - what is referred to locally as a Pineapple Express storm (heavy rains and unseasonably warm temperatures, blowing in from the general direction of Hawai'i). The November 2006 and December 2007 storms were not accompanied by either elevated temperatures or positive stream finds. Temperatures and precipitation for the Green and Sammamish River sites

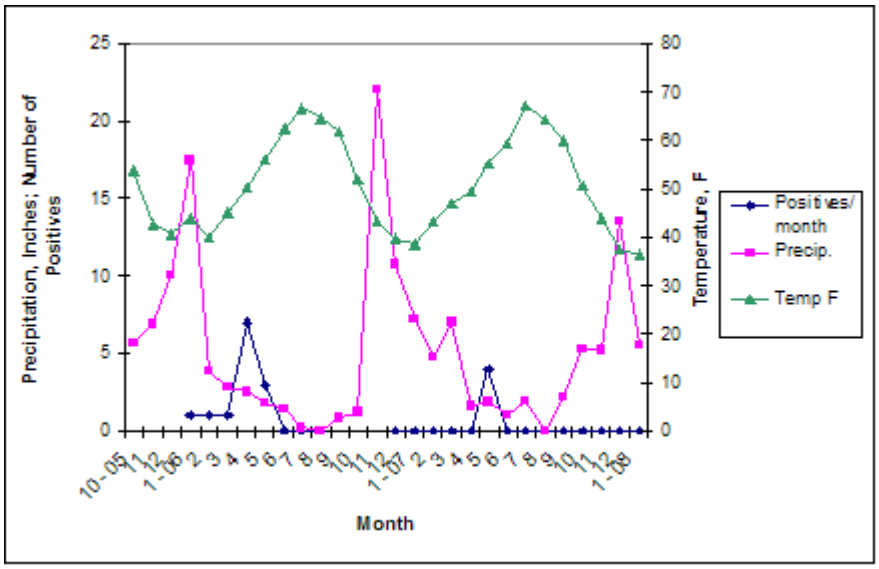

Figure 6: Precipitation, temperature, and positive traps for R Stream area. Climate data from Western Regional Climate Center, http://www.wrcc.dri.edu.

[12]To maintain confidentiality of nurseries as required by the USDA, weather stations are not named.

are similar in pattern, if not exact values, to R Stream (Figure 7).

P. ramorum is known to prefer periods of rain and moderate temperatures for sporulation (Garbelotto 2004). Also, prolonged precipitation is generally necessary to transport spores of aerial *Phytophthora* into or over soil (Bruehl 1987), thus it seems reasonable to expect that positive stream finds would be associated with spring precipitation events.

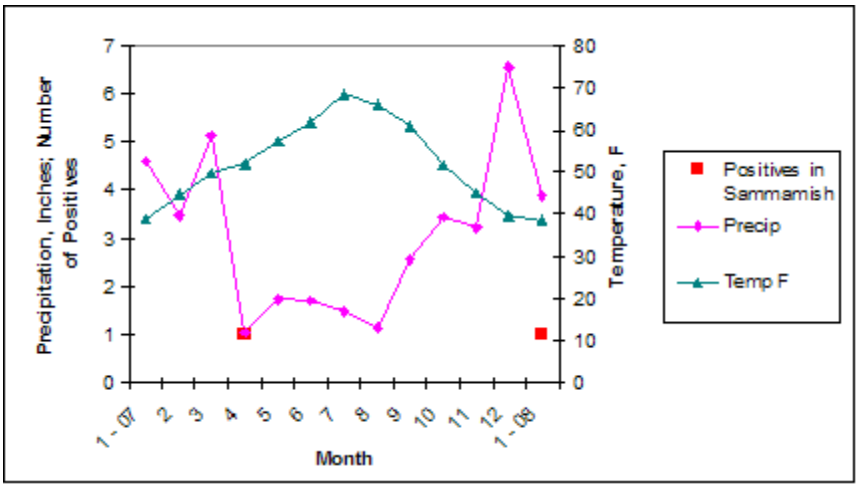

Figure 7: Precipitation, temperature, and positive traps for the Green and Sammamish River areas. Climate data from Western Regional Climate Center, http://www.wrcc.dri.edu.

Soil Physical Characteristics and Water Features

Of 7 sites with *P. ramorum* infested soil, only Nursery Harstine is definitively associated with an infested stream. Nursery Tukwila is circumstantially associated with an infested drainage ditch.[13] Each of the 7 sites is located on a different soil series. Table 3 lists these soils.

Table 3: Soil series of study sites.

Soil Series Name	General type	Slope, %	Permeability	Hardpan	AWC	Hydrologic group	Coarse fractions	Drainage	Soil erosion (K factor)
Harstine	gravelly sandy loam, glacial till	6	moderate	yes	low	C	15-25%	mod. well	mod. low
Newberg	very fine sandy loam, alluvial	0	moderate	no	very high	B	0	well	moderate
Nooksack	silt loam, alluvial	0	moderate	no	high	C	0	well	moderate
Terric Medisaprist (under composted shavings)	muck	0	moderate	artificial, at soil/shavings interface	moderate	ponded	0	very poor	n/a
Tukwila (under composted shavings)	muck	0	moderate	artificial, at soil/shavings interface	high	D (ponded)	0	very poor	low
Woodinville	silty clay loam, alluvial	0	mod. slow	no	high	D (flooded)	0	poor	moderate
Yelm	fine sandy loam, outwash	0	mod. rapid	Artificial, at soil/planting mix interface	high	C	0	mod. well	moderate

Permeability refers to the ease of movement of water through the soil. Hardpan is the presence of a layer within the top 150cm that impedes downward percolation of water. AWC is the available water capacity, or the portion of soil water that is available to plants. Hydrologic group is a categorization of runoff production, used in calculating stormwater runoff. Coarse fractions are particles greater than 2mm. Drainage class is the ability of water to percolate downward. Soil erosion (K factor) is the susceptibility of bare, cultivated soil to particle detachment and transport by rainfall.

[13]DNA comparisons between the *P. ramorum* cultured from the nursery plants and that from the water were inconclusive.

As can be seen from this chart, Harstine soil is very different from the other soils. It is the only soil with a natural hardpan, a significant slope, significant coarse fractions (rocks and gravel), and low available water capacity. It is also the only glacial till soil in this group. The other soil series are all variations of alluvial soils. Other characteristics, namely permeability, class, hydrologic group, and soil erosion potential, vary among all the soils. A larger set of soil series may have allowed for statistical analysis of relationships between characteristics of soils producing stream positives and soils not producing stream positives, but this sample set is too small.

Coarse, shallow soils are associated with transport of soil microbes (Brady and Weil 2002). From these data, it does appear that the single coarse, shallow soil type is associated with transport of *P. ramorum* spores into the stream.

While there may be seasonal differences associated with soil sampling as there appears to be for stream sampling, WSDA does not conduct soil sampling on a continual, long-term basis as with stream sampling. Soil samples are generally taken all at once; if none come up positive, no other soil samples are taken. Positive soil samples have been taken in August, and in January.

Slope

R Stream and Nursery Harstine have significant slopes, from 6% to 15%, according to the NRCS (2008). Slope of R Stream where it exits the nursery and enters the meadow was estimated at about 6% by clinometer. Figure 8 shows a profile of R Stream from the control trap above the nursery to the lowest traps near tidewater. There is a considerable drop in elevation through the entire profile, but particularly from the nursery to D Lane, with a drop of 40m over 625m (6.4%). This is also the section that produced all but 2 of the positive traps.

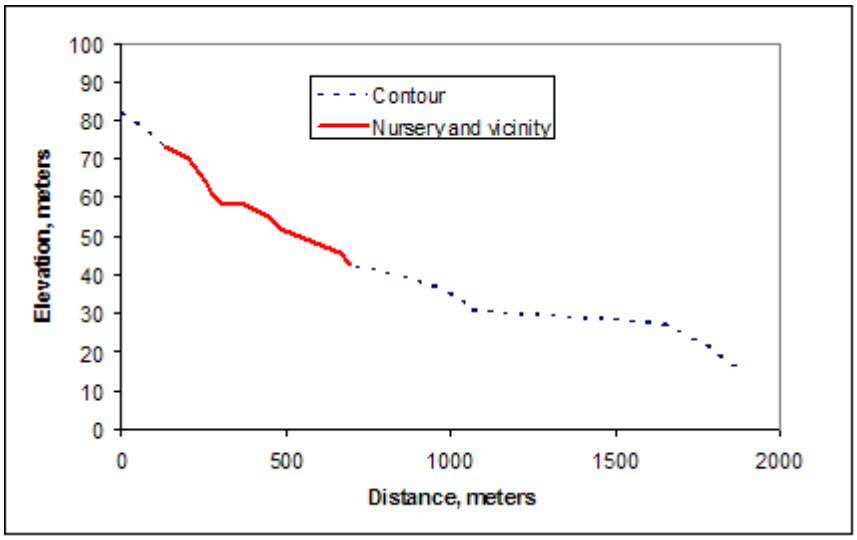

Figure 8: Profile of R Stream.

Both the Green and Sammamish Rivers have essentially flat profiles in the baited sections. Sloped soils produce more runoff and deliver more sediment and debris into stream channels than flat soils (Brady and Weil 2002, Knighton 1998). It would seem reasonable that the single sloped site would have more stream contamination than the other sites, which are all flat, if spores are being washed into the stream from the soil.

Soil Disturbance and Erosion

Soil disturbance is not a prerequisite for stream contamination, as the initial stream positives in R Stream appeared before any significant disturbance of the soil in the positive area of the nursery, through vegetation removal and soil fumigation. But, once infested plants were removed as a source of inoculum, there may have been a correlation between positive stream samples, and soil disturbance or erosion of infested soil. In 2006, stream positives were found in R Stream from January to May, but in 2007 no stream positives were found until early May, after the nursery began landscape work on the meadow involving major earth moving in April. Soil disturbance and erosion dislodge

soil particles and organic matter, allowing them to fall or be washed into streams (Brady and Weil 2002). The microbial community, including spores of *P. ramorum*, could be expected to travel with the soil particles and organic matter.

No significant soil disturbance or erosion occurred in the Green River area. In the Sammamish River area, while the source of the infestation is unknown, the second positive find occurred after extreme stormwater runoff in the December 3, 2007 storm carried unusually high amounts of debris from gardens and streets into ditches and the river.

Stream Channel Morphology

R Stream presents a variety of channel morphology types: riffles, pools, ponds, steps, braided channels, deltas, gullies, culverts, and plane-bed. Baited traps were placed in a variety of channel types. Figure 9 shows the distribution of total traps, and of positives, by channel type. Other streams were not baited to sufficient extent to allow an analysis of traps by morphology, and the Green and Sammamish Rivers did not present any clear variation in morphology at trap sites.

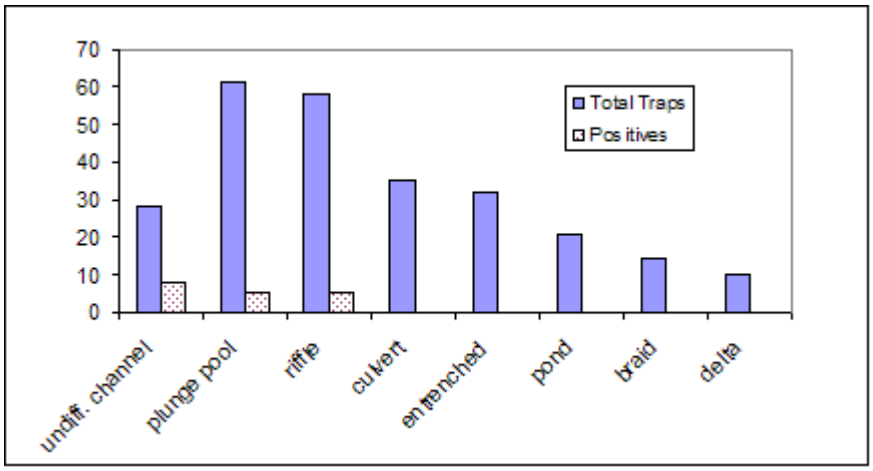

Figure 9: R Stream channel types baited, number of traps total per channel type, and number of positives per channel type.

Forty percent of all positive traps were located in undifferentiated channel, 30% in the plunge pool downstream of a culvert, and 30% in a riffle. No positives were found in any of the other channel morphology types: the upstream ends of culverts (often the same culvert that produced a positive trap at the downstream end), ponds, braided channels, a delta, and the entrenched gully that developed after the storm of November 2006. Interestingly, before the formation of the gully, this reach produced 3 positives, included in the undifferentiated channel category, but none after the change in morphology.

There are three instances of culverts baited at both ends, where the downstream end came up positive while the upstream end did not. Traps in each instance were placed at the same time, and retrieved at the same time, yet only the downstream trap produced a positive result.

Sediment Transport

Traps collect sediment and debris of various types while in the water. The screen material used for the traps has openings large enough to allow particles as large as sand grains to enter, and the openings at the top often allow debris and organisms to enter. Traps in transport zones frequently contained sand and pebbles, earthworms, leeches, freshwater snails, aquatic insect larvae, newt eggs, twigs, and bits of decomposing leaves. Traps in deposition zones were generally covered in fine sediment.

Downstream Travel

Initially it might appear that the spores of *P. ramorum* have been slowly working their way down R Stream, as the first positives are in or very near the nursery, and only later appear downstream. This might, however, be simply an artifact of initially limited trap placements. Figure 10 shows the spatial and temporal locations of traps when positives were found. The first round of baiting did not extend downstream very far at all, less than 200m. With trapping extended downstream, positive traps do appear to migrate downstream over time.

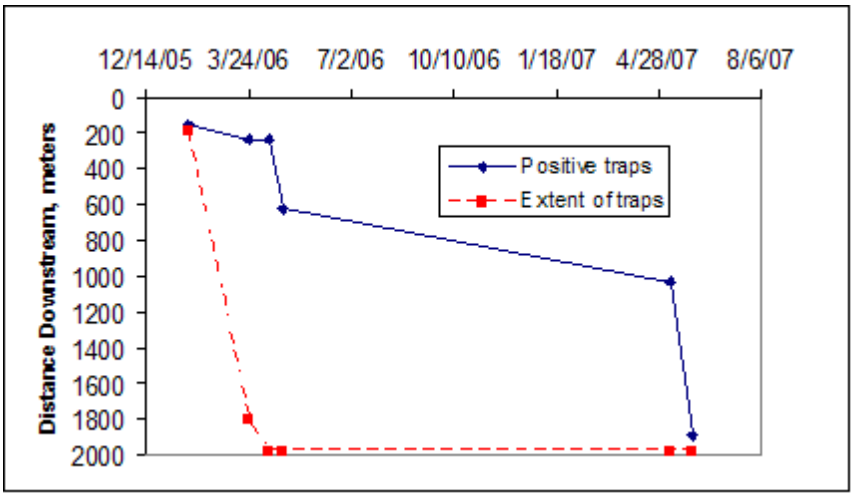

Figure 10: Downstream migration of positive traps over time in R Stream, compared to downstream extent of trapping.

Particulate Organic Matter

Baited traps act as flow obstructions in a stream as small as R Stream, often taking up half or more of the channel width, and most of the depth. Coarse particulate organic matter and mineral particles collect in the traps - twigs, decomposing leaves, silt, sand, and small pebbles. It is probably safe to presume that fine particulate organic matter also collects with the silt, but is simply too small to see. Traps in R Stream function as debris jams, collecting leaves in various stages of decomposition.

To get an estimate of travel time and flow lines for particulate organic matter in the stream, paper discs from a hole punch were dropped into the stream, and their travels observed and timed. Even this small stream provides multiple flow lines to small particles in suspension. Approximately 25 paper discs were put into the water at the base of the nursery slope, and it appeared that every one traveled a different path, at a different speed. This stretch included a 3% slope, a 90-degree turn, two constructed waterfalls with pools, and two short culverts. Paper discs were observed for approximately 40m. The first disc appeared at the end of the stretch in approximately 2 minutes. More discs were still appearing at 6 minutes, when the observation was ended. The fastest discs traveled at a slow walking speed, roughly 1 km/hr. At that speed, bits of leaves containing spores of *P. ramorum* could potentially reach the first ponds on R Stream, a distance of about 530m, in less than an hour, passing over 11 trap sites along the way.

The slower discs presented information on potential pathways for downstream contamination of riparian areas by stream-borne leaves containing spores of *P. ramorum*. Many discs washed up onto the bank of the stream, or lodged on emergent or overhanging vegetation, or collected on bed cobbles. Some were quickly remobilized by the current, while others remained where they landed.

Stream Discharge

Stream discharge (Q) is a measure of how much water is flowing in a channel per unit time, commonly cubic meters (cms) or cubic feet (cfs) per second. Stream discharge for the Green and Sammamish Rivers was taken from the USGS National Water Information System (http://waterdata.usgs.gov/wa/nwis/). Stream discharge for R Stream was obtained by direct measurements with a Swofford current velocity meter. R Stream (17 positives) has a Q on the order of 0.01cms at the nursery in March, while the Sammamish River (2 positives) at its positive site might have Q of about 13cms, and the

Green River (0 positives) might have Q in March at Nursery Woodinville of about 30cms - a 3000 times difference in water volume between R Stream and the Green River.

Stream discharge is directly related to stream channel dimensions (Q = width * depth * velocity). R Stream has channel widths ranging from 0.25 - 1m in confined reaches, up to 3m and more in unconfined reaches in winter floods. WSDA stream bait traps measure 0.4m wide, so they take up a significant percentage of the channel width, easily 50% or more in many sites. Trap sites on most other streams that were baited, however, are much wider than the traps. The Green and Sammamish Rivers measure approximately 30m and 15m, respectively, at trap sites, and the traps took up 1.33% and 2.66% of the channel width. On M Creek, the one tributary of the Green River that was trapped, the traps occupied less than one quarter of the channel width. Dimensions of the Nursery Tukwila drainage ditch are similar to those of R Stream. While it is obviously possible to trap *P. ramorum* in large streams, percent channel width occupied by traps could be correlated with probability of intercepting infested leaves or spores in the water column. Figure 11 shows number of positive traps in the first year of trapping, plotted against percentage of occupied channel width on a logarithmic scale, for each stream. There are, of course, confounding variables in these data. M Creek, for instance, was not trapped during the April-June prime trapping season. And no attempt has been made to separate out number of total traps, density of trapping, or duration of trapping for each stream.

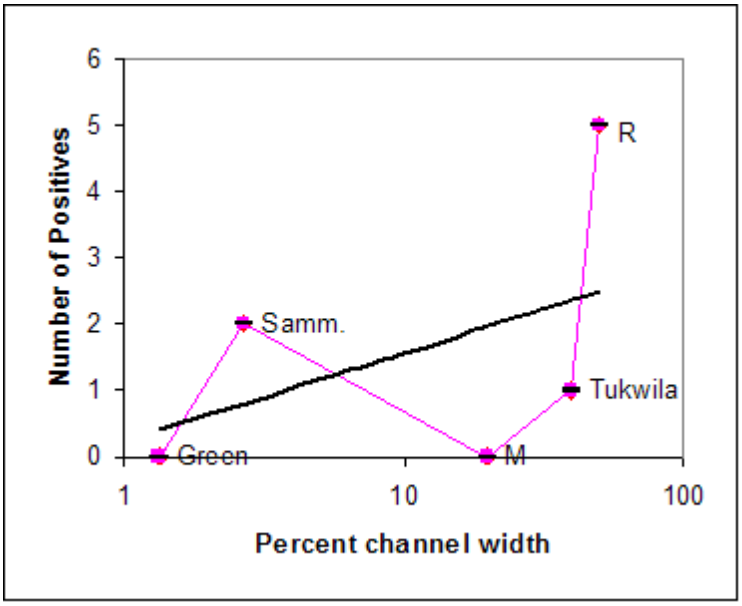

Figure 11: Number of positive traps by percent of channel width occupied by traps.

Figure 12 shows an estimation of the volume, in liters, of water flowing over traps in R Stream, using 40cm as the width of the 'channel', and 1cm as the depth.[14]

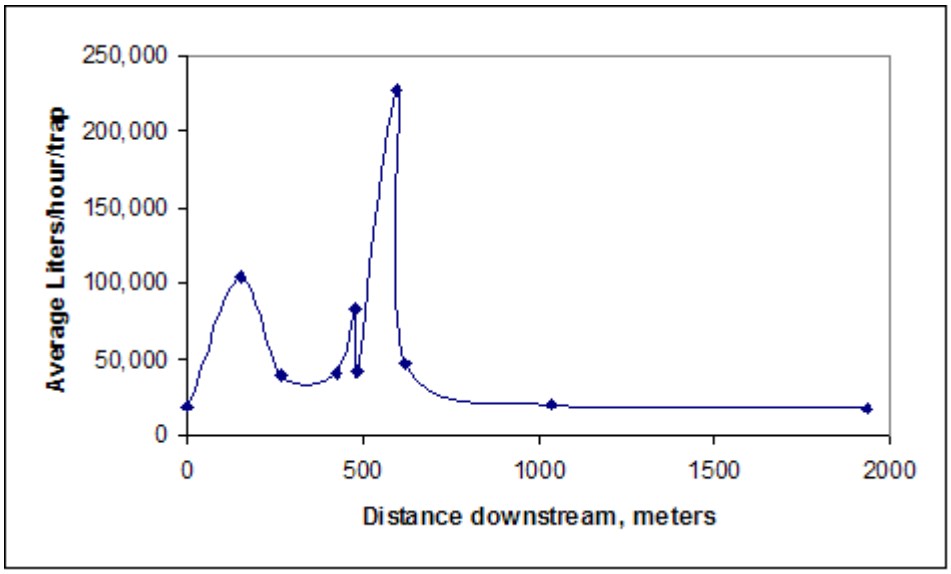

Figure 12: Estimation of liters per hour flowing over traps (w = 40 cm) in R Stream, to a depth of 1cm.

Measurements were taken over a period of 4 weeks in late March and early April, 2008, at previously positive sites and the upstream control. Streams defy definitive measurements, and the smaller the stream, the higher the percentage of error. In particular, the current velocity meter used, while designed for small streams, warns of errors in measurements at current velocities less than 0.5 m/sec. In R Stream, most sites had current velocities much less than 0.5 m/sec. These numbers, therefore, should be taken as rough estimates.

[14]A conservative estimate of how far a zoospore of *P. ramorum* could potentially swim, based on findings with other species of *Phytophthora* (Weste 1983).

Density of Baiting

The number of traps deployed varied considerably over the course of this study. Initially, 2 traps were deployed in R Stream, in the first round of baiting in January of 2006. In spring of 2007, as many as 28 traps were in the stream at one time. Of the 2 traps installed in January 2006, one came up positive. In contrast, of the 27 in the water in April 2007, only one came up positive (4%). In May of 2006, of 6 traps in the water, 3 came up positive, while in May of 2007, with 28 traps in the water (a nearly five-fold increase), only 4 came up positive (14%). Figure 13 shows the number of traps in place for each trapping period that produced a positive, along with the number of those traps that were positive.

For the two year period 2006-07, Figure 14 shows the percent of all traps in place by month compared to the percent of total positive traps. It can be seen that the majority of the traps are in place in R Stream from January through June, and that there are very few traps in the stream from July through December. This is due to a lack of water in the stream. Low stream flows start being problematic for stream baiting in R Stream as soon as precipitation drops off in April.

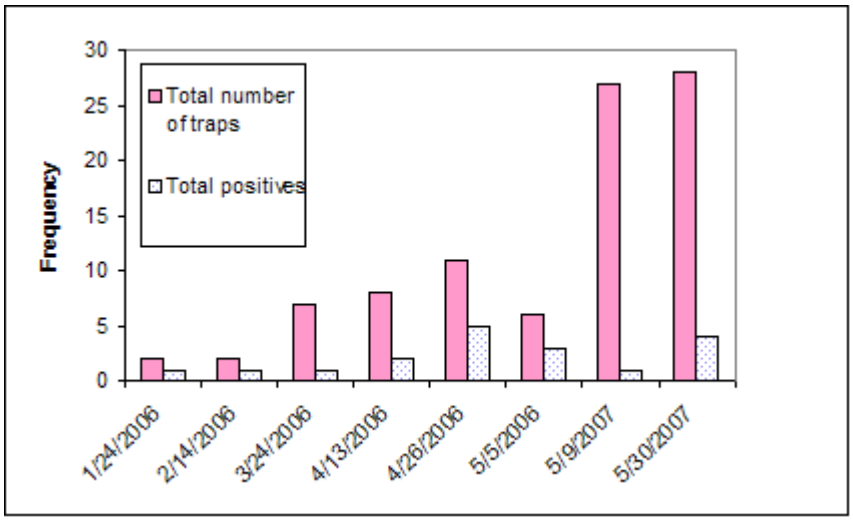

Figure 13: Total number of traps deployed per positive result in R Stream, with total number of positives, by month.

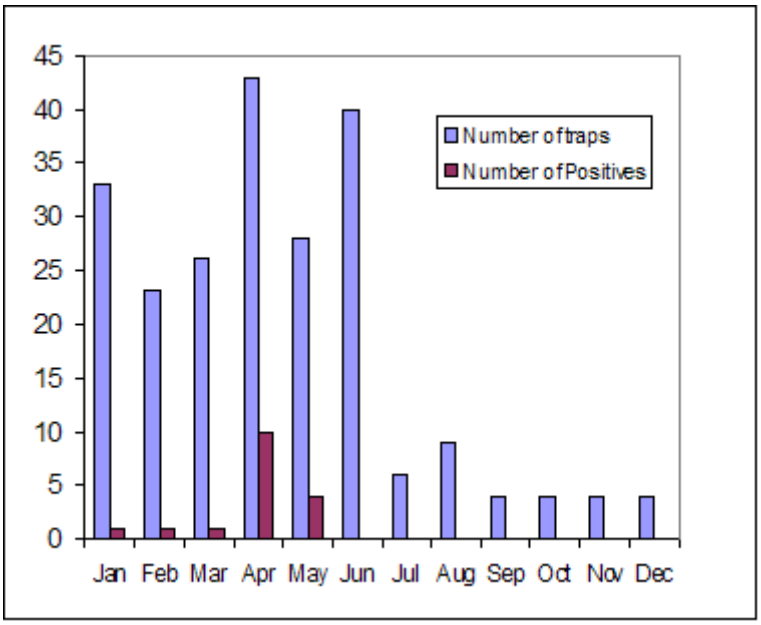

Figure 14: Comparison by month of traps on R Stream, 2006-07.

The seasonal distribution of traps appears to be more or less even from January through June, but the distribution of positive finds is not at all evenly distributed, having a distinct peak in April. Figure 15 compares distributions of positive traps by month. While it is true that more traps were in the stream in April, the majority of all positive traps were retrieved in this month. Less than 25% of traps in May were positive, for instance, and only 6% of traps in March.

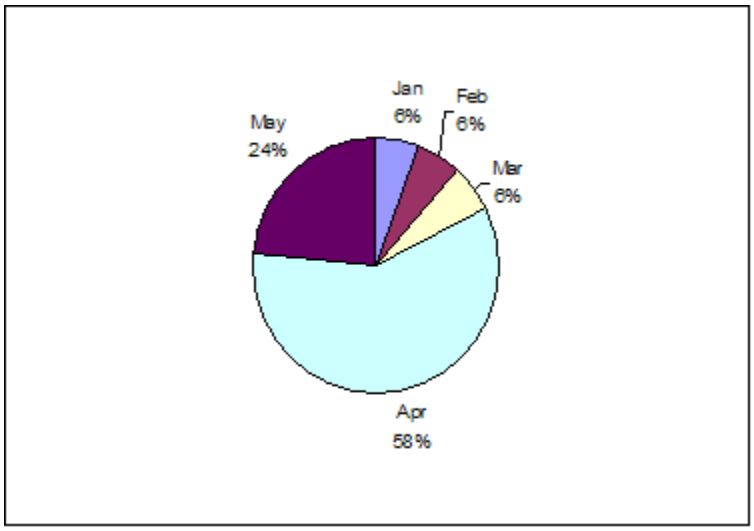

Figure 15: Distribution of positive traps in R Stream, by month, 2006-07.

The two larger rivers have the opposite problem with stream flows. Flows are often too high in winter for trap retrieval. On the other hand, flows stay high enough all summer to keep traps submerged, so trapping is done spring through fall rather than fall through spring.

Figure 16 shows the total number of positive traps per site in R Stream for the 2 year period 2006-07. There does appear to be a concentration of positives closer to the source, within about 300m. As water enters the stream along its course, the additional water will dilute the concentration of spores in the stream, assuming a single source of spores, so we could expect fewer positives farther from the source. R Stream both gains and loses water along its course, so dilution would not form a straightforward relationship with distance and may not cause a reduction in positives with increasing distance from source. Figure 12, for instance, shows no consistent relationship between discharge at the upstream and downstream ends of R Stream, and some segments of the stream were dry at the time of the last measurements, while others had higher than usual water levels.

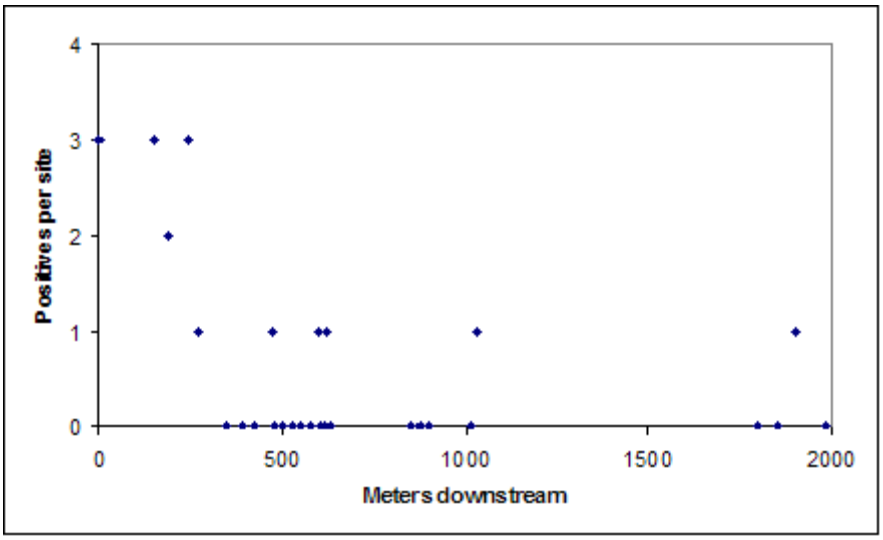

Figure 16: Proximity of positive traps to nursery.

Discussion

WSDA has conducted stream baiting for *P. ramorum* on R Stream since January 2006, and stream baiting continues into 2008. This paper looks at stream baiting data over a 2 year time span, from January of 2006 through December of 2007. Over that time span, 270 traps were retrieved, from a stream measuring less than 2 km in length; 17 were positive for *P. ramorum*, or 6%. These numbers do not allow for statistical analysis, or any firm conclusions. Other nurseries were also stream baited during this time period. Two other streams each produced one stream positive during this time period, with another positive right afterwards. One is associated with a positive nursery, while the source of the third stream positive is unknown. A comparison of soil types would be of interest, but again, the dataset is too small for firm conclusions. This thesis is therefore limited to observations on field data, and identification of areas for further study. In this section, possible explanations for the phenomena observed in the field are explored.

Precipitation

While the climate of western Washington is generally rainy, there are occasional extreme precipitation events, as well as extended dry periods in summer. Most precipitation falls from November to March, with daily totals generally no more than 25mm. Extreme precipitation events, which can be on the order of 125 mm or more in 24 hours, can produce significant runoff, soil erosion and stream flows, especially when rain falls on already saturated soil. Yearly average precipitation for Olympia is 1300mm, with monthly amounts averaging 200 mm falling from November through January (WRCC 2008).[15]

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[15]Data for Olympia are used due to Olympia's central location in western Washington.

Researchers in California have found a correlation between precipitation events and sporulation of *P. ramorum*, leading to positive finds in soil and streams (Davidson et al. 2005). Spring rain in particular, with its warmer temperatures, was associated with soil and stream positives, while temperatures alone are not as well correlated. Western Washington winters are both wet and cold, with average temperatures in the 2-4°C range (WRCC 2008). Peak season for stream positives in western Washington appears to be April and May, when precipitation is dropping off to between 50-75 mm per month, and temperatures average around 10°C. Figure 6 on page 66 shows that positive stream baits in R Stream follow simultaneous decreases in precipitation and increases in temperatures.

For water to enter the soil during precipitation events requires that the soil be able to accept infiltration. A soil's ability to accept infiltration depends on two factors: pore size at the soil surface and water content of the soil (Brady and Weil 2002). Water content, in turn, depends not only on pore volume but on antecedent precipitation. Soils with large pore volumes can hold more water than those with small pore volumes, and dry soils, i.e. at the beginning of the rainy season, can accept more infiltration than saturated soils, i.e. after prolonged precipitation. Infiltration is measured in depth/hr, and for most soils is faster at the beginning of a precipitation event and slows as the soil pores fill with water. Infiltration for a given soil, therefore, depends in part on precipitation history and on drainage class (Brady and Weil 2002).

As pore spaces drain, microbes adhere to the soil particles lining the pores (Hirst 1965). Continuous, rapid percolation of soil water from intense, prolonged rain is most likely to transport soil microbes such as spores of *P. ramorum*. These conditions are more likely to be met in coarse rather than fine soils, as a soil's hydraulic conductivity decreases four-fold with decrease in pore diameter (Brady and Weil 2002). Soils with rapid percolation should therefore sustain higher infiltration rates, and have more spores wash into the soil, than finer soils with lower infiltration and percolation rates.

Of the soils considered in this study, Harstine is the only coarse soil, with a gravelly sandy texture and 15-25% coarse fractions, where the other soils all have fine textures and no coarse fractions. Harstine soil ranks in the middle, however, in permeability, drainage and runoff production potential (hydrologic group), all of which will have some influence on infiltration rates.

Although precipitation may drop off in April, soil water content is generally still high. Spores that fall to the soil in early spring will still find a damp environment conducive to survival and to transport through soil water, given one good rainstorm.

It is true that few traps were in place in R Stream in summer and fall, and it might be possible that positives would be found if more traps were in the water. But due to the seasonality of precipitation in this area, there is little if any flow in a stream this small from early summer to midwinter. R Stream starts to run noticeably low some time in April, as precipitation and soil water content drop. Over the course of the summer, flow varies considerably, and a trap placed in sufficient water one week may be dry the next. The rainy season begins in earnest some time in November, but the soil's water holding capacity must first be filled before the stream starts to run again. Some positive traps have been found as early as January, but so far none in November or December; and there is insufficient water in a small stream from June to December to allow extensive trapping. The Sammamish and Green Rivers do have sufficient water in summer, and so were trapped over the summer months, but with no positives.

January 2006 was the start of stream baiting by WSDA, with the first traps placed in R Stream, and the first stream positives. January 2006 was also relatively warm and wet. Figure 6 on page 66 shows spikes in both temperature and precipitation, along with stream positives. January 2007 was preceded by elevated precipitation but not elevated temperatures, and no stream positives were found that winter in the baited streams: R Stream, M Creek, and the Green River. The first stream positives for 2007 follow a spike

in rainfall in April, along with rising temperatures. Extremes in rainfall were recorded for January 2006, November 2006, and December 2007, but only January 2006, with its warm temperatures, produced a positive stream bait, in R Stream at the base of the slope below the nursery.[16] It appears that successful trapping of *P. ramorum* in streams requires both some minimum temperature to allow sporulation, and sufficiently intense rainfall to transport spores.

For *Phytophthora* in general, the most critical environmental factor in sporulation is presence of free water (Erwin and Ribeiro 1996). Davidson et al. (2005) found that spore production by *P. ramorum* in northern California does not increase immediately with the beginning of the rainy season in fall, but lags behind precipitation, and is greatest in spring, when warm temperatures coincide with significant rainfall. The hypothesis is that inoculum reservoirs decrease over the dry summers, and must be rebuilt once dormancy of sporangia is broken by increased humidity. And while breaking of dormancy appears to be triggered by the presence of free water, the rate of production of sporangia appears to be temperature dependent, thus the correlation between sporulation and both temperature and precipitation (Davidson et al. 2005).

P. ramorum may be exhibiting a similar pattern in western Washington. Presence of free water should not be as limiting a factor in western Washington as it is in northern California. Precipitation in Fairfield, CA (site of Davidson's study) is 125 mm in January,[17] compared to 200 mm in Olympia, with an annual average of 580 mm in Fairfield versus 1300 mm in Olympia (WRCC 2008). Winter temperatures may be more limiting, averaging 3°C in Olympia compared to 9°C for Fairfield (WRCC 2008). The

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[16]The second positive in the Sammamish River, in January 2008, is not associated with warming temperatures, but only with extreme precipitation in December 2007 (Figure 7). The Sammamish River was not being trapped during the other extreme precipitation events, and its first positive followed the precipitation spike in April 2007.

[17]January is the rainiest month in both locations (WRCC 2008).

January 2006 positives in R Stream accompanied elevated temperatures averaging 6.6°C, along with 443 mm of rain, more than double the average for January (Figure 6 on page 66). November 2006 had similar conditions: average temperature of 6.2°C and 560 mm of rain, but no stream positives. December 2007 had average temperature of 3°C and 344 mm of rain, and no stream positives in R Stream. November is the beginning of the rainy season, and spores of *P. ramorum* may still be dormant from the dry summer. November 2006 in particular followed a very dry fall, with only 50 mm of rain in September and October in the area of R Stream. December 2007 was preceded by 320 mm of rain from September-November; it could be reasonably assumed that free water would not have been limiting.

Temperature, on the other hand, could well have been limiting to zoospore production. Davidson et al. (2005) found that zoospore production by *P. ramorum* was very low at 5°C. Winter temperatures in western Washington average around 3°C, and the average temperature in December 2007 was 3°C in the vicinity of R Stream. Zoospore production was maximized at temperatures around 15-20°C. April and May of 2006 and 2007 averaged 10°C and 13°C in the vicinity of R Stream, close to optimal temperatures for sporulation. June through September temperatures average between 15-20°C, but precipitation is minimal (as low as 61 mm total for the 4-month period in 2006) and the soil is dry. Without irrigation, it seems unlikely that any transport of spores through or over soil would occur during periods of low rainfall. If sporulation depends on both temperature and free water, then this could explain why stream positives in western Washington tend to occur in April and May, when the soil is still saturated and temperature is rising.

Overland stormflow

Stormflow, or runoff, is the precipitation that does not infiltrate the soil, but runs off along the surface (Knighton 1998). Subsurface stormflow, as the name suggests, flows just below the surface. Stormflow occurs when precipitation either falls faster than the soil can absorb it, or when the soil is already saturated and cannot absorb anymore. Stormflow generation is therefore related to infiltration, which is related to permeability and drainage class.

Stormflow generation potential is categorized under Hydrologic Group in Table 3 (see page 67), where A is lowest runoff potential and D is the highest (NRCS 2008). This is a categorization of runoff potential on level, bare, wet soil. Harstine soil is classed as C, or moderately high runoff potential, due to the presence of a shallow hardpan that impedes drainage. Once the soil is wet, the hardpan prevents downward drainage, and additional precipitation is likely to run off the surface without infiltrating the soil. Stormflow running off the surface will carry debris and pollutants with it, including microbes such as *P. ramorum* (Scheyer and Hipple 2005, Erwin and Ribeiro 2001). Group B soils have moderately low runoff potential, based on high permeability and no shallow hardpan or water table. Group D soils have high runoff potential, generally due to low permeability (usually clay soils), high water tables, or very shallow hardpans. All but one of the soils considered in this study are classed as D or C, with relatively high runoff potential. Most of these soils are flat, level soils that tend to pond in winter, with the water sitting on the surface for long periods, while Harstine is sloped, directing runoff into R Stream. Ponded water on flat, poorly draining soils does not necessarily flow into stream channels to drain, but drains vertically through the soil into the groundwater.[18] This water would be filtered of microbes as it passes through the soil (Brady and Weil 2002, Scheyer and Hipple 2005).

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[18]Ponded water is defined as non-flowing water sitting on the soil surface, while floodwater is water that flows over the soil surface without sitting (Charles Newling, Wetlands Training Institute, personal communication).

Stormwater contaminants (pesticides, fertilizers, metals) are often at highest concentrations in fall or winter, when the rainy season first begins (Brady and Weil 2002). But *P. ramorum* is not found in streams at this time; not until spring arrives, and with it the sporulation season. In R Stream, most stream positives have been found in March through May, when temperatures are rising but precipitation is falling off (Figure 6). Infested leaf matter and shed sporangia may be washing into the stream in fall, along with other stormwater contaminants. But if so, these sources of inoculum are being stored over winter, as stream baits are not picking up spores until spring, when conditions are more favorable for sporulation.

The Sammamish River positive finds do somewhat fit the pattern of stormwater contaminants, however. Figure 7 shows both positive finds occurring after major storms created large increases in monthly precipitation totals, and appear to have overwhelmed local drainage systems. The area adjacent to the Sammamish River trap is urban, with significant cover by impervious surfaces, and a storm drain system that appears to drain into the river about 15 meters upstream of the trap site. The December 2007 storms in particular appear to have caused a nearby stormwater pond to overflow, which could have allowed infested plant material in stormwater to wash into the river.

Soil Physical Characteristics and Water Features

Life forms in soil move by two different methods. Larger forms such as plant roots, earthworms, insects, vertebrates and fungal hyphae are able to push their way between soil particles and aggregates. Smaller forms such as nematodes, algae, bacteria, Protista and fungal spores move through the soil in soil water (Brady and Weil 2002). Soil water movement is limited by soil texture and structure. Microbial species movement through soil is, therefore, determined by the size and distribution of soil pores, which is in turn determined by soil texture and structure.

Soil Pores

Once water infiltrates the soil surface, it becomes soil water, occupying the spaces between soil particles, and is subject to forces exerted by soil particles, in particular capillary action (Brady and Weil 2002). Soil pores are the spaces between particles and between aggregates (Childs 1969). Two types of soil pores can be distinguished. Micropores are the pores between soil particles, and macropores are the pores between soil aggregates. Soil porosity (ϕ), the ratio of pore volume to soil volume, is not a fixed characteristic of a soil but changes with circumstances - compaction and tillage, in particular. Movement of soil water is determined by the soil's permeability, which is largely determined by the size, volume, and connectivity of soil pores (Deming 2006) and the resistance to flow offered by the pores (Childs 1969). Like porosity, permeability is not a fixed characteristic.

Loamy soils tend to have lower permeability than either clays or sands due to the mix of particle sizes - smaller particles fill in the pores between larger particles, reducing pore connectivity, while porosity (ϕ) may be the same. Permeability of soil pores increases and decreases by a power of 4, not linearly, with soil pore radius (Deming 2006), so soils with smaller particles filling in the pores between larger particles will have dramatically lower permeability. Conversely, soils with preferential flow channels, such as inter-aggregate cracks or large biopores, will have greater permeability than soils of similar textural class without such preferential flow channels (Brady and Weil 2002).

Soil Water

Soil water behaves very differently under unsaturated conditions as opposed to saturated conditions. Permeability of unsaturated soil is many times lower than the same soil in saturated conditions - air filled pores block water movement (Deming 2006), for example, and the thin films left as water drains are tightly held by surface tension (Childs

1969). As water drains from the soil, the larger pores drain first, having less surface area in contact with the water and therefore less surface tension (Brady and Weil 2002).

As the larger pores drain first, the water left in the soil is in smaller pores, which hold water more tightly, against the force of gravity (Brady and Weil 2002). As stated earlier, permeability changes by a factor of 4, not linearly, with soil pore radius (Deming 2006), so the rate at which water moves through drying soil drops rapidly as the larger pores drain first. Broadly speaking there are three critical points in soil moisture content, and three corresponding types of soil water. Saturation occurs when all available pores are filled with water (Brady and Weil 2002). Saturation includes gravitational and capillary water. As water drains through the force of gravity, the soil reaches its maximum water holding capacity. This point is referred to as field capacity (FC) (USDA 1990). This water is the available water (AW); the water available to plants. The third critical point is the wilting point. At the permanent wilting point (PWP), plants can no longer pull water out of the soil against the force holding water in the soil pores (Dahlgren 2006). This is the hygroscopic water. Table 3 lists the available water capacity (AWC) for each soil in this study. AWC can be thought of as an indirect measure of the relationship between gravitational water and available water in a particular soil - the higher the AWC, the less water held by gravitational forces, and it is the gravitational water that is responsible for transport of microbes through soil (Bruehl 1987).

In contrast to the variability of water content, soil matric potential (Ψ) is the measurement of the suction exerted by the soil on soil water, and is well correlated with fungal activity (Duniway 1983), while water content is not. The three critical water content points described above (Saturation, FC and PWP) occur at similar if not identical Ψ, regardless of soil texture. When all soil pores are filled with water at saturation, the matric potential $\Psi = 0$, and Ψ increases as soil drains. At FC, $\Psi = -0.03$MPa and most pores greater than 10 microns have drained (Duniway 1983). Zoospores of *P. ramorum* are about 10 microns in diameter, while the chlamydospores are about 50 microns

(Jennifer Falacy, WSDA, personal communication). In theory, when saturated soil dries to FC with drainage of gravitational water, transport of *P. ramorum* spores should cease. Fungal spore movement in soil water occurs mainly during saturation (Weste 1983), and with gravitational drainage (Bruehl 1987), or Ψ between 0 and -0.03MPa. At PWP, both gravitational and capillary water have drained, and Ψ = -1.5MPa. At higher Ψ, the pores large enough for fungal spores are dry (Duniway 1983). Duniway (1983) states that Ψ, both in the soil and in plant tissue, is a critical component of the environment to *Phytophthora spp*.

Fungal spore movement through soil water occurs in larger soil pores at higher permeability and lower Ψ. Larger soil pores drain fairly quickly, so fungal spore movement through soil probably only occurs for short periods of time following a precipitation event that saturates the soil (Hirst 1965). During western Washington winters, that period of saturation can be of long duration, as precipitation can be quite prolonged, with daily rain for two or three weeks at a time.

Erwin and Ribeiro (2001) state that zoospores of *Phytophthora* can move 35cm in saturated sand and 48cm in saturated loam, but do not move at all in saturated silt. One could therefore expect more transport of spores through sands and loams, such as Harstine gravelly sandy loam, than in soils such as Woodinville silt. In contrast to transport, survival of spores in soil is dependent on higher available water content (measured by mass) rather than gravitational water content (Fichtner et al. 2007). Soils in Fichtner's study site were gravelly clay loams and clay loams, with low AW and good drainage. Clays typically have little gravitational water, moderate available water, and a high percentage of hygroscopic water, which is unavailable for both plant use and microbial transport (Brady and Weil 2002). Survival in clay soils could therefore be more strongly tied to higher AW than in loam soils, as more of the water is tightly held as hygroscopic water. Similarly, survival in sandy soils could be as strongly tied to AW as in clay soils, as sandy soils have generally lower AWC than clays. In western Washington, however, it

appears that the dry season is short enough that soil moisture content should not be as limiting to survival as it is in California. Researchers in California have found summer soil survival to extend long enough to cover the typical summer dry season in western Washington (Fichtner et al. 2007, Davidson et al. 2005). Positive soil samples have been found in western Washington in August, but these were from irrigated nurseries, not forests as in California.

Biopores

Microbial movement in soil is generally considered to be negligible, due mainly to soil pores being very short and discontinuous. In loamy soils of mixed particle-sizes, pores can be very short, as their length is cut off by smaller particles settling in between larger particles, halting movement of microbial species (Paul and Clark 1989). Biopores, those created by life forms such as roots, earthworms, and moles, tend to be both larger in diameter and much longer than typical soil pores (Brady and Weil 2002). Biopores form preferential flow channels in soil, as water moves easier in larger pathways, as permeability increases 4 times with pore radius (Deming 2006). An old root channel, for instance, might measure as much as a few centimeters in diameter, rather than a few microns. Biopores can be responsible for much of the water movement in soil, as well as much of the movement of larger contaminants such as pesticides and microbes (Brady and Weil 2002).

Harstine soil showed signs of active mole and earthworm populations in the soil positive area and along R Stream, plus many mature trees grow in the area, sending large woody roots through the soil, and even into the stream channel. Biopores are likely to be numerous and continually created. None of the other sites in this study contained mature trees in the areas of interest, or evidence of any moles or significant earthworm numbers. Being air breathers, both moles and earthworms avoid continually saturated soils, such as Tukwila and Woodinville.

Hardpans

Of the limited number of streams in Washington state that have been found infested with *P. ramorum*, the only commonality in soil types is some kind of water-restrictive layer (Table 3). One soil is a glacial till/gravelly sandy loam, while the other is a raised bed of composted shavings sitting on a muck soil. The first has a glacial hardpan within about 1m of the surface, while the second has a permeable medium sitting on relatively impermeable muck, creating an artificial hardpan.

Hardpans are subsurface layers that restrict the downward movement of water through soil (Brady and Weil 2002). The key to this restriction is the relative permeabilities of the upper and lower layers. Any significant difference, in either direction, will impede drainage (Brady and Weil 2002). One of the characteristics of Harstine soil, setting it apart from even other glacial gravelly loams, is its shallow hardpan (USDA 1979). On level ground, this hardpan could cause ponding in heavy rains. On a slope, such as at Nursery Harstine, the hardpan intercepts downward-percolating water and directs it laterally, downslope. This hardpan was observed in the gully, forming ledges in the sides, often strong enough to stand on, especially at the bottom of the gully.

Hardpans are known to concentrate both percolating water, and microbes (Bruehl 1987, Erwin and Ribeiro 2001). Presence of hardpans has been found to be important in transport of other *Phytophthora*. In western Australia, the chlamydospores of *P. cinnamomi* travel downwards through the soil to the hardpan, where soil water collects and flows laterally (Shea et al. 1983). Zoospores of *P. cinnamomi* collect on top of the hardpan. Zoospores can also be found as deep as 2 meters below the hardpan, on top of an impervious clay layer (Kinal, Shearer, and Fairman 1993). Spores flow along the hardpan layer, traveling downslope as far as 2 meters in one study (Shea et al. 1983), and potentially as far as 120 meters (Kinal et al. 1993).

Some of the soil series are listed as having an artificial hardpan, or restrictive layer (Table 3). Harstine soil has a natural hardpan, created by glacial weight compacting soil particles. Nurseries Terric Medisaprist and Tukwila both use raised beds of composted shavings to display plant material, and it is the composted shavings that were cultured for, and found infested with, *P. ramorum.* The interface between the shavings and the muck soil underneath is of sufficiently different permeability to block drainage and create a restrictive layer, which acts as an artificial hardpan (Erwin and Ribeiro 2001, Brady and Weil 2002). Similarly, for Landscape Yelm, the infested soil is an imported landscaping mix laid on top of the native Yelm soil, creating an interface that blocks drainage and acts as an artificial hardpan. Water could be observed flowing from under the composted shavings in the nurseries, and standing in planting holes in Landscape Yelm.

Nursery Tukwila is on a muck soil that does not possess any of the characteristics of Harstine soil that appear to allow transport of spores. It has no coarse fragments, no slope, no hardpan, high runoff potential and high available water capacity. What Nursery Tukwila does possess, however, is numerous raised beds to raise nursery stock up off the wet mucky soil. The infested plants were sitting on one of these raised beds, and the composted shavings filling the bed were also infested. Composted shavings are of sufficiently different permeability from muck soil to create an artificial hardpan, blocking downward percolation and directing water laterally. Water could be seen running out the bottom of the raised bed and over the soil surface. This particular raised bed is alongside a drainage ditch, and is graded to direct runoff into the ditch. The associated stream positive is downstream of this ditch.

Groundwater

In deep soils, where there is no confining layer within the top 1.5m, gravitational water percolates downward until it reaches the water table and becomes part of the groundwater. Groundwater flows through soil and rock at a rate determined by the

saturated hydraulic conductivity.[19] A confining layer under the water table directs groundwater flow just as it does with subsurface stormflow. Groundwater can discharge into surface waters as baseflow, and carry any contaminants with it.

Groundwater is less likely than surface waters to receive some kinds of contaminants from the soil surface (Brady and Weil 2002). Before reaching the groundwater, soil water passes through a porous matrix (the soil), which acts as a filter. Soil pore spaces act as mechanical filters to restrict the size of particles that can pass through the soil and reach the groundwater. Soil chemical properties and biological communities likewise act as filters, to adsorb or metabolize contaminants before they reach the groundwater. The deeper, finer-textured, and more biologically active a soil is, the more thorough a filter it is. Shallow, coarse, and/or biologically inactive soils are poor filters and are more likely to allow contaminants of all kinds, including pathogens and other microbes, to reach the groundwater (Brady and Weil 2002). Harstine soil is a shallow, coarse soil and so should be expected to act as a poor filter of microbial contaminants, allowing them to flow through in gravitational water, and into R Stream. The shavings beds at nurseries Tukwila and Terric Medisaprist may also act as shallow, coarse soils, as they are only about 30cm deep, and made from large, macroscopic particles (composted wood shavings).

Slope

Precipitation falling on sloped soils generally does not infiltrate the soil as well as the same precipitation falling on a flat surface (Knighton 1998, Brady and Weil 2002). Sloped soils tend to produce more surface runoff than their level counterparts (USDA 1973). Surface runoff carries soil particles and debris with it in a similar manner to stream

[19]In soil, the saturated hydraulic conductivity is essentially the same as the permeability (Deming 2006).

flows, where sediment carrying capacity is dependent on velocity, which depends in part on slope (Knighton 1998). Surface runoff will pick up and carry debris such as *P. ramorum*-infested leaves and soil, where level soils may not produce sufficient runoff to transport surface debris.

Nursery Harstine is the only site in this study with an appreciable slope, about 6% or so from the top of the nursery to the bottom of the meadow. Nursery Woodinville has a very slight slope, so slight as to be unmeasurable with a clinometer. Most of the nursery is essentially flat, and water ponds in the winter, although Woodinville soil is not listed as ponding in soil survey data (USDA-SCS 1979). The other sites are all essentially flat.

In spring of 2008, at the time of this writing and outside of this study period, another infested nursery developed an infested stream, draining an infested recirculation pond. This nursery sits on Sara silt loam soil on a steep slope - approximately 18% at the infested area, as estimated by clinometer. This soil would normally have little runoff, except for the slope (Soil Survey Staff 2008). With the slope, it has very rapid runoff. It is a deep soil, with no hardpan, and a shallow perched water table in winter and early spring. In April 2008, water was observed draining from the infested area downhill towards a small intermittent stream, but this stream has not yet produced any positive stream bait traps. Instead, the nursery retention pond, which is in a different drainage area than the positive area, has produced a positive trap, along with the stream draining the pond. One main purpose of these retention ponds is to clean the water of pathogens (von Broembsen 2007), but apparently is not completely effective.

Soil Disturbance

Disturbed soils could affect transport of *P. ramorum* through increases in soil erosion and in surface runoff. Soil disturbance is not a prerequisite for stream contamination, as the initial stream positives in R Stream in 2006 appeared before any significant disturbance of the soil in the positive area of the nursery, through vegetation removal and soil fumigation. But in 2007, no stream positives were found in R Stream until May, and then only in traps that were downstream of bulldozing and earthmoving work done just prior to trap collection. The correlation suggests that soil disturbance can increase release of *P. ramorum* into streams, but is not conclusive.

Disturbed soils, in general, are more prone to erosional forces than undisturbed soils (Brady and Weil 2002). Sandy and gravelly soils, such as the glacial till soils, are labeled uncohesive - the individual mineral particles do not stick together very well (Knighton 1998). Uncohesive soils are more prone to detachment and erosion. Clay and organic materials are the usual binders in soils, and glacial till soils are low in both (USDA 1973). Individual soil particles, and the organic matter mixed in with loose soil particles, are more easily entrained by stormflow (runoff) than aggregated particles (Knighton 1998).

Vegetation cover greatly influences both erosion and amount of overland stormflow, and disturbed soils generally have had the vegetation removed (Brady and Weil 2002, WSFPB 1994). Forested areas create little overland stormflow, while bare soil can transform as much as half the received precipitation into overland stormflow (Sheyer and Hipple 2005). Vegetation reduces erosion and stormflow through interception of precipitation and through roots maintaining soil porosity (Brady and Weil 2002). Interception reduces the physical impact of raindrops on the soil surface, which can break up aggregates and reduce porosity through filling pores with small particles broken off the aggregates (Knighton 1998). Interception also allows the plant foliage to absorb and evaporate precipitation without it ever touching the ground. Western Washington's

coniferous forests can intercept and evaporate up to 90% of the precipitation from a typical winter rainstorm (King Co. DNRP 2007). Forest debris in the form of shed leaves and twigs also protects the soil surface from the physical impact of raindrops, as well as itself absorbing water; and is, according to Knighton (1998), the primary regulator of kinetic energy imparted to soil by raindrops.

Soil particle detachment (erosion) by raindrops depends on rainfall intensity, soil surface slope, and soil particle size, with particles in the large-silt and fine-sand size ranges (0.04-0.25mm) being most susceptible to detachment (Knighton 1998). Inundated soils are protected from detachment by raindrops. Harstine soil, with its sandy texture and significant slope, would have increased susceptibility to particle detachment in precipitation events, while Woodinville and Tukwila soils, with their much finer textures and no slopes, would be protected, especially while ponded.

Compacting or disturbing soils can also increase stormflow (Scheyer and Hipple 2005). Compaction collapses soil pores, reducing the space available for water and restricting its movement into and through soil (Brady and Weil 2002). Disturbance (grading, clearing, digging, tilling and plowing) removes the insulating layer of plant litter, kills roots and soil fauna, and disrupts soil pore size and continuity and can result in reduced infiltration, increasing overland stormflow. Removal of vegetation is generally accompanied by both compaction and disturbance of the soil.

The infested area of Nursery Harstine is in a wooded area. The shrub and groundcover layers have been cut back to make way for nursery stock, but the mature trees and some shrubs had been left in place to shelter shade-loving nursery stock such as rhododendrons. When the soil in this area was fumigated to eradicate *P. ramorum*, it was stripped bare of all vegetation, including a mature *Pseudotsuga menziesii*. This tree had been the main shelter for this area of the nursery, and its removal exposed the fumigated soil to the direct force of raindrops, rather than the gentle dripping of water working its

way through the tree's canopy. When autumn rains arrived after the fumigation, raindrops fell directly on soil that was very susceptible to particle detachment, erosion and increased stormflow generation due to sandy texture, slope, removal of protective vegetation, and the destruction of soil aggregates and the microbial and macroscopic soil community. And autumn rains arrived with a vengeance - precipitation in the R Stream area was only about 31 mm in October 2006, but 559 mm in November, much of it falling in just two days (WRCC 2008, OWSC 2006). Those two days of intense rain would cause quite a spike in Knighton's soil particle detachment equation, $D = aI^2s^c$, where I = rainfall intensity and s = slope. Soil particle detachment breaks up aggregates and could release *P. ramorum* propagules adhered to or sealed in aggregates.

Those two days of intense rain would also cause a spike in stormflow generation. The rational runoff equation estimates peak stormflow, with S = 0.278CIAw (Mitch and Gosselink 2000), where C = a runoff coefficient determined by land use,[20] soil type and vegetation, I = rainfall intensity (mm/hr), and Aw = drainage area. The NRCS has developed curve numbers (CNs) that describe the relative runoff production of various combinations of soil hydrologic group, land use and vegetation. CNs range from 98 for impervious areas to 30 for woods in good condition on hydrologic group A soils (USDA-NRCS 2007). Tables are given to estimate runoff from individual storms, based on precipitation, antecedent moisture conditions, and CN. There is no parameter for intensity of precipitation. Using these CN charts, the positive area of Nursery Harstine after fumigation would correspond to Woods, Poor Condition (understory and litter removed), on hydrologic group C soil (from Table 3), giving a CN of 77 in average soil moisture conditions, or 59 in dry conditions. Four inches of rain[21] under CN = 77 would give approximately 1.81" of runoff, or the equivalent of 1.81" of precipitation flowing directly

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[20]C varies from 0.1 to 0.2 in rural areas on sandy or gravelly soils (Mitch and Gosselink 2000).

[21]Approximately what fell in 24hrs in the storm in early November 2006, from the author's personal rain gauge in Olympia.

into the stream channel, picking up soil particles, organic matter, and plant debris in the process. Nursery Woodinville, in contrast, would be categorized as Urban Open Space, poor condition (grass less than 50%), on hydrologic group D, for a CN of 89. The same storm that delivered 4" of rain to Nursery Harstine probably delivered about two-thirds as much to Nursery Woodinville, for a runoff of 1.9". Figures 6 and 7 show comparative rainfall amounts. But, at Nursery Harstine, the runoff ran down the slope and into the channel, carrying contaminants with it, while at Nursery Woodinville, the runoff had nowhere to go on the flat terrain, so it ponded to as much as 30cm, which allowed contaminants to settle out.

Transport through soil water to new soil is important with root-rotting pathogens such as *P. cinnamomi* and *P. lateralis*, but *P. ramorum* is not a root rotting pathogen, although it is possible for *P. ramorum* to invade foliar hosts through their roots (Shishkoff 2007). Transport through soil might, therefore, seem unimportant in dispersal of *P. ramorum*. Transport through soil to stream water, however, and from there to new areas (as is done by *P. lateralis* [Hansen et al. 2000]), could spread *P. ramorum* over very long distances, and could be very important in dispersal of this invasive pathogen.

Stream Channel Morphology

To the extent that fungal propagules act as sediment load, understanding the behavior of sediment load in streams can help to understand how streams transport pathogens such as *P. ramorum*. The nature and behavior of a stream's sediment load can often be inferred by simple observation of the morphological type of the channel bed (pool, riffle, braided, etc.).

Stream segments can be classified into three basic sediment response types: sediment sources, sediment transport zones, and sediment deposition zones (WSFPB

1994). Although erosion, transport and deposition can occur in any stream segment, these general sediment response segments can be roughly differentiated by gradient. Source segments have gradients greater than 20%; transport, from 20-3%; and deposition zones, less than 3%. Sediment response type determines the particle sizes that form the channel bed, and thus the channel bed type. Pool-riffle segments can be either deposition or transport zones, with most deposition occurring in the pools, and some in the riffles (WSFPB 1994, Wohl 2000, Hoover et al. 2006). Riffles in R Stream were frequently observed to be collecting small bits of decomposing particulate organic matter (POM). Colluvial channels are generally source zones, so would be the areas where *P. ramorum* enters stream channels if they occur in proximity to infested areas. Step-pool and plane-bed segments are generally transport zones, so would transport sediments including *P. ramorum.* Some portions of these channels are capable of short-term storage of POM sediments.

Hydraulic roughness of a stream channel (bed-material height relative to water depth) also affects transport and storage of sediments (Nowell and Jumars 1984, Richardson et al. 2005, Hoover et al. 2006), and thus could affect transport and storage of inoculum of *P. ramorum*. Smaller streams and gravel-bed streams, such as R Stream, as well as riffle segments, such as are common in R Stream, tend to be rougher than larger streams (Knighton 1998), and so would deposit and store more of the POM sediments that are likely to be sources of *P. ramorum* inoculum. Gravel and cobble bed materials, such as found in most segments of R Stream, have numerous pockets and flow obstructions that trap and store fine sediments such as FPOM (Vogel 1994, Wohl 2000). Riffles in particular are composed of alternating ridges and troughs, and these troughs entrap fine sediments in vortices (Vogel 1994).

R Stream is the only stream in this study that presents distinct riffle-pool and step-pool reaches. The other streams are primarily sand bed streams, and often quite large, to the point that even very large bed material would not significantly affect the water column.

The Green and Sammamish Rivers in particular appear to be channelized sand-bed streams with flume-like configurations. In R Stream, riffle segments are shallow enough that the bed can be clearly seen, and bed material protrudes above the water surface at low flows. The water surface is visibly uneven and dimpled, an indication of hydraulic roughness and turbulent flow. Fine sediments in the silt, sand, and fine FPOM size classes can be seen collecting in the riffles.

Hyporheic flow

R Stream is an intermittent stream, meaning flow is much reduced in the dry season. Some segments go dry, while others continue to flow, or at least have some water in them. This suggests the existence of hyporheic flow, which is the portion of the water column that flows beneath the surface of the channel bed through spaces between sediment particles (Stanford and Ward 1993). Some segments of R Stream disappear beneath cedar root wads in summer, but are flowing through large open spaces, not between sediment particles - these are not hyporheic.

Gravel-bed rivers in recently glaciated areas in particular, such as R Stream, can have a significant portion of the streamflow flowing interstitially through coarse, highly permeable streambed sediments and abandoned stream channels (Stanford and Ward 1993). Streamflow enters the hyporheic zone at the upstream end of a floodplain and emerges at the downstream end, rejoining the above-ground streamflow, in response to vertical hydraulic gradients (Stanford and Ward 1993, Vogel 1994). On a much smaller scale, streamflow enters the streambed at the upstream side of the gravel ripples that form the base of riffles, and re-emerges at the downstream side (Creuze et al. 1994). Either pathway to the hyporheic zone could embed propagules of *P. ramorum* in streambed sediments and alluvial deposits, allowing for survival over the dry season and subsequent dispersal downstream in winter. Streamflow also flows laterally to enter gravel alluvial aquifers alongside stream channels, as shown by the presence of green and blue-green

algae and riparian organic detritus in these aquifers (Stanford and Ward 1993). Spores of *P. ramorum* could enter these alluvial aquifers as well, surviving long dry periods to re-enter the stream channel farther downstream and later in time, or be pumped to the surface in irrigation water.

None of the other streams included in this study are gravel-bed streams, but are composed of much finer sediments, and therefore not as likely to have significant hyporheic exchange flow in the segments potentially exposed to *P. ramorum* (Stanford and Ward 1993). M Creek also goes dry in the dry season, with isolated pools showing a trace of current. It is possible that this is an indication of hyporheic flow, but the shallow water table in this area suggests that the pools may be simply depressions in the stream bed that intersect the water table. The bed and banks of M Creek are composed of very fine, silty and clayey sediments, rather than the coarser, more permeable gravel, sand and cobble of R Stream. Plates 3 and 5 show soil profiles for Harstine and Woodinville soils, respectively, photographed at the stream banks.

Culverts

In R Stream, the first stream positive was found in a plunge pool at the downstream end of a culvert (Nathan Lubliner, WSDA, personal communication). Culverts affect the morphology of the stream at both the upstream and downstream ends. By their nature, culverts are inflexible, being constructed of concrete, steel or rigid plastic. Culverts on R stream tend to back up water on the upstream end, forming what Church (1996) describes as a dammed pool. Most culverts on R Stream form a dammed pool upstream. Culverts funnel the stream flow through a narrow area, creating a very fast current in the culvert itself, and carve out a plunge pool where this fast current empties at the downstream end. The waterfall exiting the culvert carves into the channel bed, forming a sediment source zone at the upstream end of the pool. Sediment collects only at the downstream, or tail, end of the pool, in contrast to dammed pools.

The stream positive associated with Nursery Tukwila is also in the plunge pool downstream of a culvert. Both the Green and the Sammamish Rivers are too large to have culverts. Bridges cross these rivers, and while they do affect flow, they do not do so to the same degree as a culvert.

Sediment & Sedimentation

Particulate Organic Matter

Leaves and particulate organic matter (POM) move in the water column in suspension, saltating along the channel bottom (Webster et al. 1999, Vogel 1994). Channel bottoms of small streams are relatively rough, being composed primarily of coarse particles that occupy a significant portion of the water depth (Nowell and Jumars 1984, Knighton 1998, Richardson et al. 2005, Hoover et al. 2006). This hydraulic roughness creates turbulent flow, including backwashes and eddies. Leaves and larger coarse particulate organic matter (CPOM) can be trapped by the current on larger, protruding stones (Hoover et al. 2006). Fine particulate organic matter (FPOM) is small enough to be entrapped by the backwashes, eddies, vortices, and current dead zones that form on the upstream and downstream sides of the same larger, protruding stones (Nowell and Jumars 1984, Vogel 1994, Wohl 2000).

Of the streams in this study, R Stream is the sole gravel bed stream, with riffle and braided reaches composed of cobbles and gravel that trap and store POM. The other streams (Green and Sammamish Rivers, Tukwila ditch, and M Creek) have sand beds. While bedforms in sand beds can store small amounts of POM, protruding upright clasts are much more efficient at trapping and storing POM (Hoover et al. 2006). Riffles, steps, and the tails of pools in R Stream were frequently observed collecting organic debris, leaf packs and small debris jams. LWD is also a major POM storage device (Richardson et al.

2005, Hoover et al. 2006), and R Stream is the only stream in this study with significant LWD.

Baited traps themselves can be flow obstructions in a stream as small as R Stream, often taking up half or more of the channel width, and most of the depth. POM and mineral particles collect in the traps - twigs, decomposing leaves, silt, sand, and small pebbles. Traps function as debris jams, potentially collecting *P. ramorum* inoculum in the form of infested POM, rather than (or in addition to) the bait attracting spores floating free in the water column. Traps also affect current and turbulence, which in turn will affect collection of FPOM (Nowell and Jumars 1984, Vogel 1994). Plate 8 shows traps in R Stream acting as debris jams, creating a collection of small leaf packs.

R Stream crosses numerous private property lines, some of which are marked by wire-mesh or barbed-wire fences. These fences also create debris jams. At one trap site, a wire-mesh fence has collected enough woody debris and attendant leaf packs to act as a weir, funneling the flow through a narrow gap in the organic mat. This forms a small narrow waterfall, which in turn creates a small plunge pool, which has produced a positive trap. The inoculum that infested the bait could have come from decomposition of infested leaves in the leaf mat. Grated culvert openings also develop debris jams and leaf mats, which could release inoculum downstream as the leaves decompose.

Larger rivers tend to have shorter retention times for POM (Giller and Malmqvist 1998). Significant flow obstructions are rare in larger rivers, due to the larger dimensions of the channel and higher discharges, faster currents, finer sediment at the channel boundary, and a general lack of trees large enough to block the channel in most riparian areas of most larger rivers. Riparian vegetation along larger rivers often tends more towards grasses and other herbaceous plants (Wohl 2000). Even where trees do grow along larger river channels, the channel is too wide to be blocked by a single tree. The Green and Sammamish Rivers in this study area, for instance, are vegetated almost entirely

by reed canarygrass and Himalayan blackberry, with only a few, scattered individual willows, cottonwoods and red alders, none of which will create anything like the LWD jams formed by conifers growing along headwater streams (Giller and Malmqvist 1998, Larry Dominguez, DNR, personal communication).

The Sammamish River has a fallen mature cottonwood tree (*Populus balsamifera ssp. trichocarpa*) about 100m upstream of the positive trap site, but even this large tree does not reach more than about a third of the way across the channel, and the current through the skeleton of branches is barely slowed and does not allow for creation of large leaf mats or debris jams. In fact, the current stripped the tree of bark and smaller branches within a few months of its falling into the river. Some POM has collected in the branches and along the trunk, but the residence time cannot be long in this current. Both rivers are used by boaters, so channel-spanning obstructions are not allowed to remain in place, even if they could form.

Weed beds can collect POM in larger rivers (Giller and Malmqvist 1998). Emergent aquatic vegetation does slow the current and reduce turbulence somewhat, and the tangle of vegetation physically traps POM. Water levels on both the Green and Sammamish Rivers inundate the vegetation growing along the channel banks. High water marks in the form of leaf and grassy stem mats can be seen tangled high in willow and alder branches. In contrast to the leaf mats that form in small headwater streams such as R Stream, these leaf mats seem unlikely to act as a long-term source of inoculum of *P. ramorum*. *P. ramorum* does not appear to survive more than a couple of months, if that, in leaf litter or dried rhododendron leaves (Fichtner et al. 2007). Infested leafy debris deposited among the blackberry and reed canarygrass, if also buried under deposited mineral sediments, could act as a long-term source of inoculum, and be remobilized in the next winter's high discharges. Winter discharges tend to strip the leaves off the vegetation growing along the channel, so it is unclear how much POM could be deposited in these

areas, although falling river stages definitely deposit a thin layer of very slick clay and silt, and FPOM could easily be incorporated in mineral sediment deposits.

The single positive trap site on the Sammamish River sits on a shallow cobbly bench alongside the bank. A thin, discontinuous layer of sediment collects in the lee of the cobbles. A mature red alder tree grows on the bank next to the trap site, and the fallen cottonwood is about 100m directly upstream. The remainder of the riparian vegetation is primarily reed canarygrass and Himalayan blackberry. It seems likely that the sediment collecting among the cobbles includes FPOM, and that the two trees act as flow obstructions to collect small quantities of POM. The red alder was observed to have numerous small leaf jams in its lower branches in February 2008, after the river had dropped from the December 2007 storm, and after the trap was retrieved and found positive in January 2008.

Sediment Storage

Streams not only move sediment, they also store it (Knighton 1998). Sediment is picked up in source zones, moved through transport zones, and stored in deposition zones. As is common with small streams, some segments of R Stream switch between transport and deposition as water levels fluctuate with the seasons. Sediment that is deposited in spring and stored over the summer can be remobilized in winter, transported to new depositional zones, and stored there for the next summer. Floodplains receive fine sediments carried by overbank flows in winter storms, and store these sediments until the next overbank flow remobilizes them. In this manner sediment is transported downstream in pulses (Knighton 1998).

Propagules of *P. ramorum* readily survive the summer dry season in northern California forests when buried 6cm deep in soil (Fichtner et al. 2007). Propagules in leaf litter do not survive long once the dry season arrives. Burial in soil enhances survival, and

may be aided by percolation from precipitation and by natural pedoturbation processes (i.e. incorporation by earthworms and moles). In the Netherlands, *P. ramorum* has been detected 20cm deep in sandy soil up to one year after removal of the above-ground portions of the infested plants (Aveskamp et al. 2005). Both Fichtner's and Aveskamp's studies found reduced recovery at the soil surface and in the litter layer compared to at depths. These findings illustrate the importance of soil in supporting propagules of *P. ramorum* in the absence of susceptible plant hosts.

Stream sediments are basically the same materials as soil. Soil is defined as mineral particles no more than 2 mm in diameter, loose enough to dig with hand tools, and capable of supporting plant life (Brady and Weil 2002). Sediments in streams and ponds are made of the same mineral particles as soil, are generally loose enough to be dug by hand, and can support plant life given appropriate water depth, chemistry and currents (Mitsch and Gosselink 2000). In fact, stream sediments generally originate as soil particles that fall or wash into a stream, or are carved through by a stream (Knighton 1998). For *Phytophthora* propagules, stream sediments (especially the coarser sediments, gravel and cobble, greater than 2 mm in diameter) should confer a survival advantage over soils, as they are more consistently wet than unsaturated zone soils, while better aerated than saturated zone soils.

R Stream possesses three separate sections with relatively significant floodplains. Summer flows retreat to narrow braided channels hidden under skunk cabbage, or dry up completely, but winter finds swaths as wide as 15 m inundated with flowing water. Fine sediments, thick with anaerobically decomposing POM judging from the smell, blanket the floodplains up to 30cm deep when flows retreat. Inoculum of *P. ramorum* could be incorporated into these sediments either in the POM, or carried into pore spaces by infiltrating water. The other streams in this study are too entrenched to have active floodplains. The two larger rivers in particular have been channelized and diked for flood control, separating them from historical floodplains. Woodinville, Nooksack and

Newberg soils along the Green River are described as subject to occasional flooding, suggesting the Green can overflow these sites (Table 3). But, there was no indication during the two year study period of any overbank flows by the Green or Sammamish Rivers.

In summer 2006 the infested area of Nursery Harstine was stripped of vegetation, and the soil fumigated. Then November 2006 brought record rainfall throughout western Washington, about 660 mm in the South Puget Sound area, with100 mm falling in one 24hr period.[22] Figures 6 and 7 show spikes in precipitation for this time period - 356 mm for the Green River, and 559 mm for R Stream. When stream baiting was resumed in late November after the worst of the storms had passed, the upper portion of R Stream, where it runs down a 6% slope from the positive area of the nursery, had been transformed from a wide, shallow, pool-riffle woodland stream into a narrow, deep, entrenched gully by extreme erosional forces on stripped, disturbed, sandy soil. Then the channel was about 30 cm wide and 1-2m deep. The original stream bed could still be seen in places alongside the new gully, in cobbled terraces encased in the gully walls. Of course, when the gully was carved out by the stream flows, what had been soil was now sediment being transported by stream flow. Water velocities increased greatly, to the point that WSDA field crews had trouble keeping the traps in the water - tie lines would snap, or the lead fishing weights would pull loose, or the traps would be tossed out of the water and hang up on the side of the gully. Figure 3 shows R Stream in the vicinity of the nursery, with relative positions of the positive area, gully, and meadow. Plate 9 shows the gully.

From the positive area in the nursery, R Stream flows down the 6% slope, and then levels out in the meadow. This is where much of the sediment removed from the gully was deposited. In the spring of 2007, major earthmoving work was done at the base of the gully and in the meadow, disturbing and releasing more sediment. A new channel for R

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[22]Unofficial data, from author's personal rain gauge in Olympia.

Stream was dug across the meadow, a culvert was installed for a new road, and two large pools connected by a waterfall were constructed from boulders brought on site. As a result of the creation of the new stream channel, the confluence of the two seasonal streams was moved downstream about 7m. The high, unstable banks of the gully in its lower portion, created by accumulations of sediment washed down from the nursery by the carving of the gully, were bulldozed into the channel to recreate the old configuration.

Bulldozing of the gully banks put huge amounts of sediment, originally carved from the gully just downstream of the positive area, back into the stream. This sediment was then transported further downstream. New sediment deposits accumulated in the meadow segment, and down to the new confluence. Even before the bulldozing, this new confluence received enough sediment during 2007 that the trap placed there in February was buried in March and was unrecoverable. Then, between the end of baiting in summer 2007 and restarting in winter 2008, the new confluence filled with sediment and moved downstream by another 7 m. The site has been so transformed by sediment deposition as to be unrecognizable. The traps in early 2007 had been tied off to a branch of vine maple growing horizontally across the channel, about 30 cm above the surface, at the point where the thalwegs of the two forks joined. When the site was rebaited in 2008, that branch was completely buried.

Some of this sediment may have originated in the meadow, but the meadow itself received large amounts of sediment washed down from the gully, and repeated storms may have pushed sediment and plant debris all the way from the nursery positive area, across the meadow, and into the main stem of the stream. Every time sediment and POM were relocated by the stream, spores of *P. ramorum* could have been released into the water column.

Some time during December 2006, one of the traps placed in the gully broke loose, and washed down the gully and over the bank at the bottom of the grade, where it was

buried in the accumulation of sediment forming at the top of the bank. This is the same area that was later bulldozed in April of 2007. It was not recovered until the end of March, when its rope was found exposed at the top of the bank. It had been buried in damp alluvium for nearly 4 months. The leaves were still in place and intact, and were brought back to the laboratory for culturing, but were negative. During the same time period that this trap was torn loose from its emplacement, a debris jam formed at about the same place in the gully, probably in the same rainstorm, and sent the stream over the bank, where it carved a temporary channel northward toward the small wetland at the head of the north fork of R Stream. A second trap was also lost from the gully some time in December of 2006. Its rope was later found emerging from the mud of the new ornamental pond in April of 2007, but the trap itself was buried too deeply to be recovered. The movements of these traps, and the channel shift at the debris jam, illustrate how *P. ramorum* - infected water and plant material could be carried to new locations by streams. The burial of the lost traps also illustrates how *P. ramorum*-infested plant material could be carried out of the nursery and stored in stream sediment, to be rereleased as the stream reworks the contaminated sediment.

Stream Discharge

The highest number of positive stream baits for the two year time period 2006-07 was in the smallest stream - 17 in R Stream, 2 in the Sammamish River, 1 in a ditch draining into the Sammamish, and none in either the Green River or its tributary M Creek. There are many reasons why this might be so, aside from discharge. For instance, M Creek was only baited for 5 months from October 2006 to February 2007, not during prime stream-baiting season, which appears to be April and May. Another possibility is that trapping was discontinuous on the Green River, and very few traps were put out, compared to the size of the river. In part, this was due to the sheer enormity of the task, i.e. lack of available labor and materials, and the high degree of vandalism of the traps that

were placed. R Stream had many more traps placed per kilometer of stream channel, in both time and space, than any other stream baited by WSDA in this time period.

Discharge is known to be associated with stream baiting success for other *Phytophthoras*, such as *P. cinnamomi* in South Africa (von Broembsen 1984). And, invasive species in the early stages of a biological invasion can be expected to occur in small numbers (Smith and Smith 1998), which can be very hard to detect (Perkins 1989, Keller 2001). Sampling design must account for the size of the population and the area being sampled (Keller 2001). In the case of stream baiting for *P. ramorum*, the area being sampled is the volume of water in the river - 0.01cms for R Stream, 13 cms for the Sammamish River, and 30 cms for the Green River.[23] The size of the population being sampled is of course unknown, but likely to be quite small, both in numbers and in microscopic size of the units being counted. The USDA-APHIS Survey Manual (2006) uses a disease incidence in host plants (one way of 'counting' a pathogen) of 0.5% to calculate sample set sizes for nursery inspectors, suggesting the expectation of very small populations of *P. ramorum*.

One possibly critical difference between R Stream and every other stream baited is the percent of channel width taken up by the traps. R Stream is small enough that the traps used by WSDA take up a significant portion of the channel width and depth, particularly in spring, when flows are reduced. It is not uncommon for the traps to lay flat on the bottom of the channel, and take up essentially all of the width and the depth - nearly all the water flow contacts the baits. This is not the case, however, for the Sammamish River - the channel at the single positive site is about 15m wide and has an unknown depth that is more than 2 meters, by visual estimation, although the trap itself sits on a shelf in water comparable in depth to that of deeper segments of R Stream. This site produced one

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[23]Average discharges for March, for the Sammamish and Green Rivers, from USGS (http://waterdata.usgs.gov/wa/nwis/). R Stream value from author's measurements taken in March and early April of 2008.

positive trap during the study period, a second directly after the study period (January 2008), and a third in April 2008.

In addition to the simple physical dimensions of the traps compared to the stream channels, the effects of currents on the traps should be considered as well. In the larger rivers, the current pulls the traps to the furthest extent of their tie lines and then holds them against the bank, where they are quickly covered with fine sediment in the sand and silt size ranges. The banks of these rivers are often vegetated with reed canarygrass, in which the traps get entangled. In either case, the baits in the traps are sheltered from the water column, reducing exposure to any spores of *P. ramorum* that might be floating by.

Turbulence could be important as well. In the smaller channels, traps will have a considerable effect on turbulence, which in turn will have a considerable effect on patterns of sedimentation of mineral particles and FPOM (Nowell and Jumars 1984, Vogel 1994). Larger channels will be relatively unaffected by the hydraulic roughness created by the traps.

There were no sites available on the larger rivers that were similar to those used on R Stream - the morphology of a high order, alluvial, urban river is almost entirely dissimilar to that of a low order, intermittent, semi-rural headwater stream (Knighton 1998). Traps on R Stream could easily be anchored in such a way as to keep them in the thalweg - by tying them off to a branch growing over the channel for instance, or a shrub growing out of a gravel bar, or laying a rock on the tie line midstream. There was no way to anchor traps midstream on the larger rivers - no trees reach across the channels, the bottom is too deep to reach, bridges are too high above the water, and boats use both rivers, ruling out any trapping that obstructs the channel.

Water Sampling

At some sites, water samples were taken rather than stream baits. Some nurseries have no neighboring stream; in these cases, puddles and rivulets were sampled with 250 ml bottles. No water samples were positive for *P. ramorum* in this study. Figure 12 shows estimates of how many liters of water in R Stream flow over various previously positive trap sites in March and April. The lowest estimate is about 20,000 liters per hour, over a trap measuring 40 cm wide, and assuming a 1 cm deep layer of water over the trap that a zoospore of *P. ramorum* could swim through to reach the baits, based on figures given for other species of *Phytophthora* by Weste (1983). If it takes about one minute to fill a 250 ml bottle, then a stream bait trap is sampling 333 L in the same time period. A 250 ml water sample simply does not compare to the 333 L/minute being sampled by a stream bait trap, so it is not surprising that no water samples came up positive in this study. Populations in the water column would have to be much higher than can be assumed for the beginnings of a biological invasion for water sampling to be effective. Even with well established species of *Phytophthora*, water and soil sampling often return false negative results (Erwin and Ribeiro 1996), so should not be assumed to be effective for a recently introduced species such as *P. ramorum*.

Some nurseries have retention ponds to capture runoff from the nursery and hold it for settling of contaminants and potential reuse for irrigation. These ponds had traps placed in them, similar to streams, but of course there is no current in retention ponds to pull spores past the traps. As much as possible, traps were placed close to inlets, to maximize the potential for detection of *P. ramorum*. While concentrations of *Phytophthora spp.* can be high in nursery irrigation runoff, and at retention pond inlets, concentrations are significantly reduced in water that is pumped from these ponds after a period of settling (MacDonald et al. 1994, von Broembsen 2007). No retention ponds were positive for *P. ramorum* in this study, but in April 2008 a nursery in southwestern

Washington produced positive stream bait traps at the outlet of its retention pond, and in the ditch draining the pond (COMTF 2008).

Conclusions

- Temperature and precipitation: seasonality of stream positives seems very strong, with April and May the best months for finding *P. ramorum* in streams in western Washington. January through March, stream positives tend to follow Pineapple Express storms, with warm temperatures and intense precipitation. In urban areas, stream positives follow storms that overwhelm stormwater drainage systems.

- Soil type: shallow hardpan, coarse soil, and slope appear to be conducive to escape of *P. ramorum* from nurseries into streams. More data are necessary to examine the relative effects of these three factors.

- Stream channel morphology: within this limited dataset, there appears to be a correlation between plunge pools and riffles, and successful trapping of *P. ramorum*. Successful trapping in these segments may be associated with POM storage.

- *P. ramorum* seems to require that almost the entire water column be sampled to be detected. It also appears to be stored in sediments in the channel, along the banks, and in floodplains, and that it is still infective when remobilized. Stream sediments could be acting as reservoirs of infective material in the absence of infected plant hosts.

- Soil disturbance is not essential to transport of *P. ramorum* to streams, but is probably a contributing factor.

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Color Plates

Plate 1: Baited stream bait traps.

Plate 2: Traps in R Stream.

Plate 3: Harstine soil profile.

Plate 4: R Stream in meadow.

Plate 5: Woodinville soil profile.

Plate 6: Traps in M Creek.

Plate 7: Flooding at Nursery Woodinville, February 2007.

Plate 8: Traps collecting leaf jams.

Plate 9: The gully on R Stream.