An examination of the soils supporting *Hackelia venusta,* Washington State's most endangered species

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Abstract

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Hackelia venusta (Showy stickseed) is an endemic, endangered species restricted to a small forested area in the eastern footslopes of the Cascade Mountains in Washington State. The purpose of this study was to characterize the soils supporting *H. venusta* according to their chemical and physical properties. The soils are shallow, well-drained and coarse-textured with low organic matter content. Percent base saturation is high although cation exchange capacity is low. Total and extractable N are low but similar to forest soils of other locations with similar site characteristics. Bray extractable P is high and may be somewhat attributed in part to small amounts of volcanic ash in the soil. However, given the history of fire in the study area it is most likely attributed to increased mineralization of P as a result of burning. A single, excavated *H. venusta* specimen revealed a shallow root system that demonstrated obvious strain against continuous erosion of surface materials. DNA extraction of several root and rhizosphere soil samples indicated that arbuscular mycorrhizal fungi may be associated with the species.

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INTRODUCTION

The Endangered Species Act, enacted by Congress in 1973 was designed to protect and recover endangered and threatened species and preserve critical habitat necessary for the survival of vulnerable species (USFWS 1973). The protection of rare and endangered species is critical and is directly related to their economic, cultural and ecological values. Particular attention must be paid to species whose exact contribution to each of these values is not wholly understood as the implications of their loss may extend far beyond their current perceived place in the ecosystem.

Hackelia venusta (*H. venusta*) is one of the most rare and endangered species endemic to Washington State. The species is particularly sensitive as a result of its extremely limited distribution and was included on the federal endangered species list in 2002 (USFW 2002). The current population is restricted to an area of roughly 10,100 m^2 in the eastern footslopes of the Cascade Mountains outside of Leavenworth, WA at an elevation of approximately 500 m. This area has been decreasing since the late 1960s at which time the population was found over a few square kilometers (Gentry and Carr 1976). By the mid-1980s the population was restricted to approximately 50,000 m^2.

While some research has been conducted exploring its distribution, habitat preferences (Carr, 1974; Gentry and Carr 1976), reproductive biology (Taylor 2008) and taxonomic differences from similar species (Harrod et al. 1999, Harrod et al. 2013 In Review), little has been done to characterize the below-ground environment including an examination of the root system as well as an analysis of the chemical and physical properties of the soils on which *H. venusta* resides. Effective management and preservation of *H. venusta* is contingent upon a better understanding of its below-ground site characteristics and overall ecological requirements.

The main purpose of this study was to characterize the below-ground habitat of *H. venusta* and to determine if there were any unique properties that could be contributing to the limited range of this population. This was achieved through an analysis of the physical and chemical properties of the soil, the implementation of a small erosion study to investigate erosion taking place within the core population and the excavation of one *H. venusta* individual so that the root system, including both its extent and morphology could be described. Roots and rhizosphere soil were also examined for the presence of mycorrhizal symbionts. A small sample of the leaves was collected for foliar analysis of nutrients. A better understanding of these properties will allow for the development of appropriate management decisions critical to the preservation and survival of this rare and endangered species.

CHAPTER 1. LITERATURE REVIEW

H. venusta is a member of Boraginaceae which is comprised of 145 genera and over 2,200 accepted scientific species names. Within the genus *Hackelia,* there are 39 accepted species names (The Plant List 2013). Species within *Hackelia* are widely distributed occurring in Europe, Asia, Central and South America, Mexico, and North America. Western North America is home to the most species and the greatest diversity of species within the genus (Gentry & Carr 1976). Broadly speaking, *Hackelia* tends to be a genus with limited geographic range and according to Gentry and Carr (1976), many are "narrow endemics". In fact, *H. venusta* is just one of several species within the genus that have been identified as having populations limited to just a few square miles (Gentry & Carr 1976).

Within the state of Washington there are at least 14 species of *Hackelia,* four of which (*H.cinerea, H.diffusa, H.hispida,* and *H. venusta*) are included in the Washington Natural Heritage Program's Field Guide to Selected Rare Plants (WNHP 1999). Of these four species, only *H. venusta* has been federally listed as threatened and endangered (USFWS 2013). *Hackelia venusta,* commonly referred to as "Showy stickseed", is a biennial herbaceous forb approximately 20 to 40 cm tall, with hirsute leaves ranging in length from 7 to 15 cm and multiple stems extending from a taproot (WNHP 2013). It has large, "showy" white flowers and nutlets that are 3 to 4.5 mm long covered in small prickles (Harrod et al. 1999).

In 1948, a voucher specimen of what was believed to be *H. venusta* was collected in Merritt, WA, a nearby town in Chelan County (USFWS 2002). However there is currently debate as to whether or not this specimen is actually *H. venusta* or *Hackelia diffusa var. arida* (Sagebrush stickseed) and was mis-identified at the time of collection (USFWS 2007). *H. diffusa var. arida* is found in the same area as *H. venusta*. Though easily confused with *H. venusta*, it is

distinguished by its taller stature, differences in leaf morphology, and smaller flower size (USFWS 2007). If the specimen is actually *H. venusta* it would imply that the population extended further than previously described. However, attempts to relocate the population where the Merritt specimen was collected have not been successful and to date no other specimens of *H. venusta* have been identified outside of the core population. In an effort to resolve the debate, the 2007 *Hackelia venusta* Recovery Plan proposes that the Merritt specimen, which is currently kept at the University of Washington herbarium, undergo genetic testing to determine its similarity or dissimilarity to the genetics of *H. venusta* (USFWS 2007).

The core population of *H. venusta* occurs within the Tumwater Botanical area originally established in 1938 by the Wenatchee National Forest. This area was first established for the protection of *Lewisia tweedyi*, a species no longer considered to be threatened. The Botanical area is now maintained and managed as a Late Successional Reserve allowing certain silvicultural and fire hazard reduction treatments to occur in an effort to preserve and protect *H. venusta* as well as *Silene seelyi* (Seely's catchfly), another rare species in the area (USDA/USDOI 1994).

H. venusta, first identified in the early 1920s, was listed as endangered by the Department of Natural Resources in Washington State in 1981, at which point the population was estimated at approximately 1,000 individuals. Between 1995 and 2000 there was an observable increase in the population size of *H. venusta* (USFWS 2007). In spite of the moderate increases seen between 1995 and 2000, the population as a whole declined from 1000 individuals to approximately 300 individuals and in 2002 the species was placed on the Federal Endangered species list (USFWS 2002). In 2004, the population was estimated to be between 572 and 772 individuals. The current population of *H. venusta* is estimated to be 477 (J.Arnett, Pers.comm.,

WA State Dept. of Natural Resources, 2013). In order for the plant to be declassified as federally endangered the population must be at or above 1,000 adult individuals for five years, show no signs of decline and young plants should be well-established and reproducing on their own (USFWS 2007).

Several other higher elevation populations (between 1,920 m and 2,255 m) of *Hackelia* have been described in the surrounding area (USFWS 2002). However these populations are considered to be morphologically and taxonomically distinct from *H. venusta* and are designated as *Hackelia taylori* (Harrod et al. 2013, In Review). These populations are smaller in stature than *H. venusta*, have blue flowers, and due to the presence of a snowpack in the spring, tend to flower in July while *H. venusta* typically flowers during the spring (USFWS 2002).

The ecosystem supporting *H. venusta* is best described by the description of the Douglas-fir zone on the Eastern side of the Cascade Mountains (Franklin & Dyrness 1973). This forest type has an overstory of Ponderosa pine (*Pinus ponderosa*) and Douglas-fir (*Pseudotsuga menziesii*) and an understory comprised of a variety of grasses, herbs, and shrubs including species such as *Penstemon subserratus* (Finetooth beardtongue), and *Phacelia hastata* (Silverleaf phacelia) (USFWS 2007). *H. venusta* is predominantly shade-intolerant and prefers to grow in areas of open canopy with low competition (USFWS 2002), though there are some plants higher in elevation that grow under the shade of large conifers (USFWS 2011). This sparsely vegetated environment preferred by *H. venusta* is maintained by wildfires that have historically preserved habitat with reduced competition from woody vegetation as well as substrates with little to no organic horizons (Agee 1993). Wildfires have been actively suppressed in this area in recent history encouraging the encroachment of shrubs and woody vegetation (USFWS 2007). The

history of fire suppression in the area is believed to be one of the major factors contributing to the loss of habitat and the resulting decline of this species (USFWS 2007).

Plants are found on steep slopes of loose gravel and talus with some clusters on rock outcrop ledges, and within the cracks of granitic cliffs (USFWS 2007) that range in aspect from south-southwest to west-northwest. These slopes are steep and are approximately 80% on average though in some places may exceed 100% (USFWS 2007). Casual observations of the soil from within the core population of *H. venusta* describe a weathered granitic "loamy sand or sandy loam" with up to 40% gravel (Gamon et al.1997; Taylor 2008). A 1995 survey of the Cashmere Mountain Area in Washington classifies the soils in the core population as primarily inceptisols that belong to part of the Icicle-Chumstick-Rock outcrop complex. This complex is developed on granitic colluvium mixed with varying amounts of volcanic ash on slopes ranging in steepness from 45%-90% slopes (Aho & Bieler 1989). Soils range in depth from shallow to deep, are well-drained, and have relatively shallow O horizons.

The volcanic ash deposited in the area is derived primarily from the eruptions of Glacier Peak in Washington beginning about 11,670 years ago, Mount Mazama (now Crater Lake) roughly 7,000 years ago, and some from the 1980 eruption of Mount Saint Helens as well (Bieler 1975; Busacca et al. 2001; Matz 1987; Soil Survey Staff 2013). The granitic material is derived from the Mount Stuart batholith, a pluton composed primarily of quartz diorite, though composition spans the full range of composition from two-pyroxene gabbro to granite (Erickson 1977).

The combination of steep slopes and loose, coarse soils make the site of the core population especially vulnerable to erosion and mass wasting. There was a landslide in the area in 1992 that closed the highway below the core population. Subsequent repairs to the road made

by the Washington State Department of transportation (WSDOT) killed 50 individual *H. venusta* plants (USFWS 2007).

Other factors have contributed to the increased susceptibility of the site to erosion. In 1994, moderate to high intensity wildfires passed through Tumwater canyon (Gaines et al. 1997; Kifner 1994). There was considerable mortality among both the understory and surrounding trees (Harrod 1994). Beyond their effects on vegetation mortality, wildfires also increase erosion. The reduced vegetation cover following a fire leads to a decrease in the amount of water taken up by vegetation and therefore an increase in the amount of water in the soil which can ultimately increase the overall instability of a soil. Additionally, wildfires consume organic material which typically protects soils from the impact of rain drops. The burning of organic material may also lead to hydrophobicity of soils which leads to increased erosion as well. Coarse-textured soils, such as those supporting *H. venusta* are more prone to becoming hydrophobic than soils with a finer texture (DeGomez 2002).

In an effort to protect this vulnerable location from further erosion, several preventative measures have been taken. In 2011, in conjunction with the Federal Highway Administration, the WSDOT led a slope stabilization project. For this project they not only removed trees and shrubs to generate more open habitat for *H. venusta* but they also removed "excess materials" from the slope to protect the plant from destruction by downward moving rocks, boulders. Cable netting was also installed over the lower slopes where the population is found. This netting is designed to protect the lower plants that are more accessible to people from further destruction (USFWS 2011). Additionally, a total of 34 snags, left behind from the fire that passed through the area in 1994, were felled in an effort to minimize disturbance to the area. Clearing the snags reduced potential damage to the plants and soil from wind throw and opened up the canopy as well as

creating a more suitable habitat for *H. venusta*. These combined efforts had the added benefit of protecting the roadway below the steep slopes from potential damage due to landslides. Human disturbance is another factor that plays a significant role in the population dynamics of *H. venusta* which should not be ignored. *H. venusta* has been subject to collection by scientists and rare-plant enthusiasts looking to enhance personal collections (USFWS 2002). Not only has collection of the plant directly reduced numbers of the population, but any visits to the highly erodible site dislodges materials that could potentially bury or crush plants further reducing the population size.

Steep slopes, shrub encroachment stemming from fire suppression, and human disturbance are not the only threats that exist to the survival and preservation of *H. venusta.* This species may also be threatened by the encroachment of *Mugolones cruciger*. *M. cruciger* is a weevil that was released as a bio-control agent for *Cynoglossum officinale* (Houndstoungue) in Canada beginning in 1997 (De Clerke-Floate et al. 2005). *C. officinale* is also a member of Boraginaceae and is found in Chelan county within close proximity to the *H. venusta* population. While the weevil has a strong preference for *C. officinale*, it has shown the ability to lay eggs and develop in several non-target species including two *Hackelia* species, *Hackelia floribunda* and *Hackelia deflexa* in the field (Andreas 2008b; H. Catton, Pers. comm., University of British Columbia, 2013). Moreover a 2004 study examining the potential threat of *M. cruciger* on *H. venusta* found that the weevil was able to partially develop on *H. venusta* (Andreas 2004). The weevil has not been released into the United States but appears to have made its way into Washington State (Rare Plant Press 2010). The *H. venusta* population is well within the known range of *M. cruciger* (Andreas 2008a).

Currently there are protective measures in place for *H. venusta* being implemented by the WSDOT. In 2003 the department developed a plan to address threats to *H. vensuta* that could be mitigated by changing WSDOT practices. As a result of the management plan, several steps have been taken including an examination of the effects of CalBan, a de-ice commonly applied to the roads during winter months composed primarily of diluted non-sodium salts (Chalker-Scott & Brickey 2004).This de-icer was believed to be associated with an increase in the presence of noxious weeds and the decline of *H. venusta* near the roadway (USFWS 2007). The study, conducted in 2004 at the Center for Urban Horticulture greenhouse, determined that there was a negative effect of the de-icer compound at a dilution ratio of 1:100 (Chalker-Scott & Brickey 2004). As a result of this study the WSDOT has been cooperating with the U.S. Fish and Wildlife Service, the United States Forest Service and the Washington Department of Natural Resources in an effort to reduce the impact of road management activities (USFWS 2007).

Furthermore, the Tumwater management plan addressed the need for best management practices regarding the use and application of herbicide and the removal of noxious weeds by hand. *Linaria dalmatica* (Dalmatian toadflax) and *Centaurea diffusa* (diffuse knapweed), are two weeds considered to be noxious in Washington State. Both weeds are listed as Class B weeds and prevention of new infestations of these species is considered a high priority with methods of doing so left up to the discretion of local authorities (WSNWC 2013). In 1999 and 2000, the U.S. Forest Service staff began pulling noxious weeds by hand and careful application of herbicide by hand to weeds in areas near the *H. venusta* habitat and adjacent to the highway below the core *H. venusta* population (USFWS 2007).

Beyond the protection of the current population, several efforts have been made to evaluate techniques to establish new individuals and increase the size of the overall population of

H. venusta. Edson et al (1996) determined that reintroduction of *H. venusta* into its natural habitat could be done successfully through the use of axillary shoot cultures that use minimal growth regulators and in a separate study (Edson et al. 1997) tested micropropagation techniques as a means of establishing new populations within the natural habitat of *H. venusta*. The individuals grew well in the greenhouse and survived in the field for up to 9 months after the initial planting (Edson et al.1997). In 1995, three separate populations were planted, each with a total of 136 individual *H. venusta* specimens grown using the micropropagation techniques adopted from Edson et al. (1997); two populations were planted in Tumwater Canyon and one population was planted in Icicle Creek Canyon. By 2003, the outplanting populations had decreased significantly: 18 individuals remained at the Icicle Canyon site and just one individual remained at one of the Tumwater Canyon sites (USFWS 2007). As of 2013, only three surviving individual at the Icicle Canyon outplanting location were observed.

CHAPTER 2. AN EXAMINATION OF THE SOILS SUPPORTING *HACKELIA VENUSTA, WASHINGTON STATE'S MOST ENDANGERED SPECIES*

2.1 Introduction

Hackelia venusta (*H. venusta*), commonly known as "showy stickseed" is an endemic species in the state of Washington and is currently one of the most endangered species within the state. Fewer than 500 hundred individuals make up the current population of *H. venusta* which is restricted to an area less than one hectare on the eastern slopes of the Cascades Mountains. Due to its narrow range and diminishing population, *H. venusta* has been included on the federal endangered species list since 2002 (USFWS 2002).

H. venusta is a biennial herbaceous forb that is between 20 to 40 cm tall, has hirsute leaves that are 7 to 15 cm in length and multiple stems that extend from a single taproot (WNHP 1999). It is known for its large, "showy" white flowers and small nutlets that are covered in small prickles that 3 to 4.5 mm long (Harrod et al. 1999).

There has been some research conducted regarding various aspects of the ecology of *H. venusta* including investigations into its range and distribution (Carr 1974; Gentry and Carr 1976), reproductive biology (Taylor 2008), and taxonomic differences (Harrod et al. 1999; Harrod et al. 2013, In Review), however there has not been any specific work done to describe the soils supporting the population and how they may be playing a role in the distribution and success of the population.

The objective of this study was to determine if there were any unique features of the below-ground environment that could help to explain the limited distribution and population of *H. venusta.* A better understanding of these properties is critical to developing appropriate

management techniques needed to ensure the preservation and survival of this rare and endangered species.

2.2 Materials and Methods

2.2.1 Study Area

The study was conducted primarily within the boundaries of the core population of *H. venusta*. The core population is located within an open ponderosa pine (*Pinus ponderosa*) and Douglas-fir (*Pseudotsuga menziesii*) forest of the eastern slopes of the Cascade Mountain range in Washington State (Figure 1). The core population is found between an approximate elevation range of 470 m and 820 m on loose, steep slopes averaging 80% (USFWS 2007). The plants are found in a range of aspects from SSW to WNW though most plants are located on slopes with a predominantly western aspect (USFWS 2007). The climate is characterized by warm, dry summers and cool, wet winters. July and August are the hottest months with average temperatures hovering near 30 °C while January and February are the coolest months with temperatures averaging between -8 and -6 °C. The area receives an average of 65 cm of rain per year with most falling between the months of October and March. Snowfall contributes an additional 240 cm of precipitation per year between November and March (Western Regional Climate Center 2013). Soils surrounding the core population are primarily inceptisols developed on a colluvial granitic parent material mixed with volcanic ash deposits (Aho & Bieler 1989).

2.2.2 Soil Sampling

A total of four sites were selected for characterization; Sites 1, 2, and 3 were located within the core population of *H. venusta,* Site 4 was located at the site of a 1995 outplanting of

the species situated in a nearby drainage for comparison to the core population (See Appendix). Sites were chosen based upon their accessibility and proximity to existing clusters of *H. venusta* within the core population and were selected to capture the variation within the landscape of the core population. All sites are located on predominantly south-facing aspects with slopes ranging from 38° to 40° (78% - 83%) and were located within an elevation range of 530 m to 580 m.

One soil pit approximately 20 cm wide was dug at each of the selected sites. Soil samples were collected every 10 cm until continuous or nearly continuous rock was reached. The soil pits at Sites 1 and 3 reached nearly continuous rock at a total depth of 30 cm while the pits at Sites 2 and 4 reached a total depth of 45 cm. If there was an O-horizon, that was sampled as well; Site 3 was the only site to have an O-horizon (Oi and Oe). One face of each soil pit (the uphill face) was cleaned so that horizons could be identified and described. Horizons were described based upon their depth, continuity, aggregate structure and consistency, roots, and any other pertinent information available including surrounding vegetation and rock cover. Samples were placed in bags and taken back to the University of Washington for analysis.

Bulk density samples were collected by depth where possible, however some depths were too coarse or friable and were not collected. A subsample from each collection depth was used for soil moisture analysis. Soil moisture samples were placed in tins and weighed immediately upon returning to the lab before oven drying for at least 24 hours at 105°C or until weight was constant.

Bulk soil samples were stored in the cold room (3 °C) until drying. All samples for chemical analysis were air-dried and analytical weights were corrected for moisture content. The gravimetric water content was calculated by subtracting the oven-dry weight from the wet

weight, divided by the dry weight and multiplied by 100. Dry weights were recorded and the wet and dry weights were then used to calculate the gravimetric water content of each sample.

Thermochron iButton temperature sensors were installed at each site to measure soil temperature at 10 cm depth; sensors were programmed to collect data every four hours. Sensor locations were marked with a wooden stake and data were downloaded upon each re-visit to the sites. In addition to temperature data, soil moisture samples were collected at 10cm depth from each site. These samples were collected upon each return visit then brought back to the lab for subsequent analysis.

2.2.3 Soil Analysis

Once air-dried, bulk samples were dry-sieved to determine particle size distribution. Samples were weighed and initially passed through a 2 mm sieve; the remaining coarse fraction was weighed and recorded as gravel and set aside. Samples were next passed through a 0.5 mm sieve and set aside. Finally, the remaining <0.5 mm fraction was passed through a 0.045 mm sieve. The coarse fraction was then combined with the 0.5 mm-2 mm fraction to total the sand fraction for the sample (0.045 mm-2 mm). The <0.045 mm fraction was weighed and recorded as the silt+clay fraction of the sample. The percent sand was calculated by dividing the weight of the sand by the total weight of the < 2 mm fraction; percent silt+clay was calculated by dividing the weight of the silt+clay fraction by the total weight of the <2 mm fraction. Particle size distribution was calculated by dividing the weight of each particle size class by the total weight of the bulk sample. The < 2 mm soil fractions were then recombined for chemical analysis. Bulk density was calculated by dividing the mass of the air-dried soil by the volume of the core taken.

Soil pH was measured using a 1:1 ratio using 10 g of soil to 10 g of deionized water (Page et al. 1982). A Radiometer PHM92 pH Meter was used to measure the pH. The pH of the organic horizon (Oi) from Site 3 was measured using the saturated paste method. One blank plus two samples were duplicated at random for quality control. Extractable N was measured using a 2*M* KCl solution (Page et al. 1982). The filtered solution was then analyzed for NH_4^+ and NO_3^- using an autoanalyzer. Extractable phosphorus was determined according to the Bray 1 method using a 1:7 ratio (soil to solution) and filtered using Whatman #42 filter paper (Cade-Menun 1997; Page et al. 1982). One blank plus two samples were duplicated at random for quality control for both extractable N and P analysis. Initial Bray extracts revealed elevated P levels in the soil and as a result, the Bray P extraction method was completed twice on all of the soil samples for quality control. Values reported are averaged between the two tests. A metals digest following the methods outlined in EPA method 3050b was conducted to determine the total phosphorus content in the soil (Edgell 1989; Page et al. 1982). Phosphorus and nitrogen samples were analyzed using an ICP-AES.

In an effort to better explain the high levels of extractable P in the soil, small subsamples of the 0-10 cm depth and 10-20 cm depths were treated with peroxide to remove organic matter, sieved to 0.5 mm and examined with a petrographic microscope to look for volcanic ash. Total carbon and nitrogen were analyzed using a CHN analyzer 2400 model, Perkins Elmer Co. Base saturation was measured using unbuffered 1*M* NH_4Cl with a 1:10 ratio of soils to solution. The leachate was then analyzed using an ICP-AES to measure amounts of base cations. Following an ethanol rinse 1*M* KCl was added and the resulting leachate was analyzed to determine cation exchange capacity (Page et al. 1982; Skinner, et al. 2001).

2.2.4 Erosion Measurements

One set of erosion pins was installed at each site approximately two meters from the nearest *H. venusta* plant in a location determined least likely to be disturbed by people. Pins were installed so that the head of the pin was flush with the surface of the ground (See Appendix A.2). While all site locations had a similar amount of slope, the two erosion pins installed at site three were placed on a near-level shelf near the base of a large Douglas-fir tree; this was the only site with an O-horizon.

Three size classes (<2 mm, 2-5 mm, and >5 mm) of fluorescent willemite (a zinc silicate) with calcite (calcium carbonate) were evenly distributed between the two erosion pins (Fowler 1969; Traynor 1983). Upon each site visit, notes were made regarding the movement of the particles though it wasn't until late November that the particles were effectively observed with an ultra violet light and the distance downslope was quantitatively measured.
Photos were taken to capture the proportion of particles remaining between erosion pins (See Appendix A.3-A.5). From the photos, the remaining amount of each size class was estimated. These estimates were used in conjunction with the measured downslope movement of visible particles to assign a relative class of erosion. A relative index of erosion was created based upon the amount of soil removed from beneath the erosion pins, the measured distances travelled by the fluorescent particles, and the relative amount of fluorescent particles remaining between erosion pins.

2.2.5 Plant Root Assessment

One *H. venusta* specimen grown in the greenhouse was used for an initial examination of the root structure of the specimen. The plant was approximately four years old and had been

grown in an incubation chamber with temperatures alternating between 15 °C and 6 °C every twelve hours. It was grown in the same container the entire time. The plant was removed from its pot and the roots were placed under gently running deionized water to remove soil and expose the root structure. The roots were very fine and tightly entangled making it difficult to fully expose the root system without damaging the roots. A portion of the root system was examined under a high-power dissecting microscope (using magnification from 10X to 400X) to look for any mycorrhizal structures (See Appendix). Additionally, a fungal stain was applied to a portion of the roots (lactophenol ink) to determine the presence of fungi associated with the roots. Prior to sampling, it was determined that only one individual *H. venusta* specimen would be excavated. The sample was collected in early June to coincide with the phenology of the plant; this is typically the time when the fruit of *H. venusta* is mature. The criteria for selection included the plant's location within the core population, its proximity to other *H. venusta* plants, the maturity and the number of stems on the plant (L. Malmquist., Pers. comm., Wenatchee River Ranger District, 2012; W. Gibble, Univeristy of Washington Botanic Gardens, Pers. comm., 2012).

The selected *H. venusta* specimen was located approximately 30-40 m upslope of Site 1, had two main stems and there were no other *H. venusta* plants within two meters in any direction. The plant was located on a slope similar to that of Site 1 with similar substrate cover. Excavation was done by hand using a small trowel to remove bulk soil and a small brush to expose the root system, taking extra care to avoid damaging the finer roots. A majority of the root system was recovered although at depth some of the finer roots undoubtedly broke off.

Once the specimen was fully excavated, the length of the main taproot and the longest branch off of the main taproot were measured. The overall height of the plant was measured as

well. After the measurements were taken, a composite sample of leaves including a few from the basal rosette and main stem were collected and placed in a Ziploc bag. Several small pieces of the root were clipped and placed in a separate Ziploc bag and both samples were returned to the lab.

Additional samples of both the roots and leaves from *H. venusta* were collected from the sole *H. venusta* individual located at site four. A portion of the roots was carefully exposed and a small sample was clipped from the exposed roots and then the roots were reburied. The root sample was placed in an additional Ziploc bag to be analyzed separately from the roots collected at the excavation site. A small sample of the leaves were collected as well though these were combined in the same bag as the leaves collected from the excavation site for a composite analysis of metals within the leaves. At both the excavation site and Site 4, a small sample of the rhizosphere was collected. The roots and rhizosphere from all sites were collected for DNA sequencing to determine whether or not arbuscular mycorrhizal fungi (AMF) is present. One additional root sample and rhizosphere sample was collected from an *H. venusta* located just above Site 3.

2.2.6 DNA Extraction

DNA from the roots and rhizosphere was extracted and sequenced using the MPBio Fast DNA Spin Kit to determine whether or not AMF were present (Verbruggen et al. 2012). Big leaf maple (*Acer macrophyllum*) roots known to contain AMF were used as the control.
A small sample of root, rhizosphere, or root and rhizosphere was placed in MP Lysing matrix E and DNA was extracted following the MPBio Fast DNA Spin Kit protocol provided by the

manufacturer. Tubes were then placed in the fast prep machine to break down the tissue resulting in the DNA extract.

A nested PCR was done following the DNA extraction. The first PCR was conducted using NDL22 and 0061 as the eukaryote-specific primers which target the variable D2 region of the large ribosomal subunit (M.Khorsani, Pers. comm University of Washington, 2013; Stuckenbrock & Rosendahl 2005). NDL22 (forward primer) has the following sequence: TGGTCCGTGTTTCAAGACG; 0061(reverse primer) has the following sequence: AGCATATCAATAAGCGGAGGA. For the second PCR the primers FLR3 and FLR4 were used. FLR3 (forward primer) has the following sequence: TTGAAAGGGAAACGATTGAAGT and FLR4 (reverse primer) has the following sequence: TACGTCAACATCCTTAACGAA. Each PCR assay was performed in a final volume of 50 µl: 19 µl water, 2.0 µl template DNA, 2.0 µl forward primer, 2.0 µl reverse primer, 25 µl master mix (GoTaq® Green Master Mix by Promega). Amplification conditions for the first PCR was completed using an initial denaturation at 95 °C for 1 minute, followed by 30 cycles each consisting of 1 minute at 95 °C, 1 minute at 53 °C (annealing temperature), 1 minute at 72 °C and a then final extension at 72 °C for 5 minutes. The first PCR products were then used as templates for the second PCR. Amplification for the second PCR was completed using an initial denaturation at 95 °C for 1 minute, followed by 25 cycles each consisting of 1 minute at 95 °C, 1 minute at 56 °C (annealing temperature), 1 min at 72 °C and a final extension at 72 °C for 5 minutes.

The resulting PCR products were separated by electrophoresis in 0.8% agarose gel in 1X TBE buffer (Sambrook et al., 1989). Gels were stained with Ethidium bromide and photographed under UV light. Inclusion of the large ribosomal subunit enhances both the resolution at the species level and increases the strength of the overall phylogenetic analysis (Kruger et al. 2009).

The second PCR was conducted using the primer pair FLR3 and FLR4 which is specific to fungal phylum Glomeromycota. Final PCR products that had 500 base-pairs indicated the existence of AMF. Products from the second PCR were purified using the PCR purification kit by Qigen and sent to DNA sequencing facilities at the University of Washington to be sequenced. The sequenced DNA extract was analyzed using FinchTV software (version1.4) to determine their similarity to other published sequences within the kingdom Glomeromycota.

2.2.7 Foliar analysis

Leaves were dried to a constant weight, divided into larger and smaller and analyzed separately for total carbon and nitrogen to see if there was any difference between the two sizes. The remaining samples were composited to determine other nutrients as there was insufficient remaining foliage to analyze the "big leaves" and "small leaves" separately. The combined sample was digested using HNO_3 and H_2O_2 according to EPA method 3050 and analyzed using ICP-AES (Edgell 1989; Page et al. 1982) though volumes were adjusted to account for the small amount of sample.

2.3 Results

2.3.1 Soil Profile Description

The soil profile supporting the *H. venusta* population consists of O, A, Bw, BC, and C horizons with some variation (See Appendix 7). Where there was an O-horizon it was an Oi horizon 1.5 cm thick made up primarily of Douglas-fir needles with an Oe horizon approximately 1.5 cm thick. The A horizon ranged in depth from 5 cm to 10 cm and is a dark yellowish brown with many fine roots and few medium roots. The A horizon at Site 3 was

slightly darker due to a higher organic matter content. Aggregates are small friable crumb structures. Overall the A horizon had a clear, smooth boundary, though in places the A horizon gradually graded into an AB with a less definitive boundary. The Bw horizon ranged between 5 cm to 20 cm, sometimes reaching a thickness of 30 cm. Aggregates are fine, very friable crumb to subangular blocky structure. Roots are predominantly fine with some medium-sized as well. In some pits a high gravel and cobble content made excavation of the horizon difficult. The Bw horizon typically transitioned into a BC horizon, with a gradual, wavy boundary in some profiles. The BC horizon, where present, ranged in depth from 20 cm to 45 cm before reaching continuous or nearly continuous bedrock. The C horizon, where present, is composed of granitic gravel, stone and cobbles with no evidence of weathering. None of the pits reached completely continuous bedrock.

Based upon the percentages of sand and silt+clay the soil texture is best described as a sand (Table 1; Shoeneberger 1998). Bulk density, where measured, was moderate and well-below the root-limiting bulk density of a sandy soil (Brady & Weil 2010). This was made especially clear when digging the soil pits for soil sampling; the soil was so loose that it was difficult to maintain a clean face as soil kept sloughing off into the pit as soon as any material was removed from the side.

Gravimetric soil moisture content was particularly low, ranging from 0.7 to 3.8% between June and October (Figure 2). The soil moisture increased dramatically by November, ranging from 7 to 25% with the highest moisture contents observed at Site 3. Maximum soil temperatures at 10 cm depth were recorded in mid-August with Site 4 reaching the highest temperature at 29.3°C (Figure 3). At Site 4, the soil temperature and the air temperature were nearly equal while at Site 3 the soil temperature was considerably cooler than the air temperature

(Figure 4). As air temperatures began to cool, soil temperatures began to cool as well and the differences between pits began to diminish by late September.

2.3.2 Chemical Analyses

The four sites showed little variation in chemical composition in spite of differences in location (Table 2). The low carbon and nitrogen concentration and content, and cation exchange capacity suggest low fertility though typical of soils with high sand contents (Brady & Weil 2010). While the CEC is low, the base saturation is relatively high (ranging between 52-77%). The pH is slightly acidic throughout the profile across the four sites. While slightly acidic, the pH is typical of coniferous forest soils (Moghaddas & Stephens 2007; Erickson et al. 2005). Extractable nitrogen content is low with extractable nitrate levels somewhat higher (averages ranging between 2.4 $\mu g\ g^{-1}$ and 5.0 $\mu g\ g^{-1}$) than extractable ammonium values (averages ranging between1.9 $\mu g\ g^{-1}$ and 2.7 $\mu g\ g^{-1}$) for depths between 0 and 40 cm. At 45 cm the extractable ammonium content exceed the extractable nitrate content. Total phosphorus content was highest in the O-horizon sample and tended to decrease with depth. Extractable phosphorus is high ranging between 87 $\mu g\ g^{-1}$ and 113 $\mu g\ g^{-1}$ across all four sites.

2.3.3 Erosion Study

The four site locations highlighted the range of erosion occurring within the core population of *H. venusta* with every site location exhibiting signs of erosion. Based upon the movement of willemite particles applied between the erosion pins, Site 4 showed the greatest amount of surface erosion; six months following the initial application, none of the willemite particles remained. By contrast, nearly 100 percent of the fluorescent particles remained at Site

3. Sites 1 and 2 were intermediate with Site 2 showing more movement of willemite particles than Site 1. Approximately 40 to 50% of the total particles remained between pins at Site 1 and between 30 to 40% remained between pins at Site 2. In both cases, the majority of the remaining particles were of the intermediate size class (2-5 mm). Observation of the erosion of soil from around the erosion pins correlates fairly well with these observations. An average of 0.5 cm was eroded away from the top two erosion pins at Site 1 compared to an average of 1.9 cm at Site 2 and 0.6 cm from the top two erosion pins at Site 4. See appendix for examples of erosion.

2.3.4. Root system structure and morphology

The excavated plant was approximately 27 cm tall. The basal leaves of the *H. venusta* selected for excavation were buried beneath several centimeters of sand and gravel. Excavation of the specimen did not reveal an extensive root system. One main taproot extended to a depth of 20-22 cm and the longest branch of the main taproot extended a length of 23 cm before the roots were so small they broke during excavation. Further, the root system did not extend straight down. There was a definite curve to the taproot and the taproots were actually bent back upward and into the slope (Figure 5).

2.3.5. DNA Extraction

Some of the roots from the *H. venusta* grown in the greenhouse stained blue indicating a fungal presence but there were no AMF structures evident when roots were examined beneath a high-powered microscope (See Appendix). It is unlikely that the soil used for greenhouse plantings of the *H. venusta* had any inoculum in the soil which would explain the lack of AMF.

DNA extracts taken from the roots and soil collected in the field had inconsistent results. The amplification results for the Bigleaf maple root control samples proved the existence of the AMF and were used to ensure the quality of the test. Neither the roots nor the rhizosphere soil from the excavated *H. venusta* specimen indicated the presence of AMF. Additionally, rhizosphere soil and root samples collected near Site 3 showed no amplification of AMF. The rhizosphere soil collected at Site 4 (the outplanting site) showed no amplification however the root sample from Site 4 did indicate the presence of AMF in the sample. The roots from Site 4 showed amplification and there was a 99% match for uncultured Glomeromycota, the phylum in which AMF are found (Blaszkowski & Czerniawska 2011; Schubler et al. 2001).

2.4 Discussion

2.4.1 Soil and plant properties

2.4.1.1 Soil texture and moisture content

Soil texture is often indicative of a soil's moisture content and water availability (Kimmins 1996; Pritchett & Fisher 1979). The soil supporting the *H. venusta* population is a coarse-textured soil with low organic matter content. Organic matter content can significantly contribute to the water holding capacity and moisture content of sandy soils (Perez et al.1998). Low organic matter content in this soil is likely contributing to the low soil moisture values as well. Soils comprised of mostly silt or clay have more micropores and higher water retention compared to coarse-textured soil which have more macropores and lower water retention. Coarse-textured soils with their larger pore spaces also create an aerated environment providing the oxygen necessary for root growth (Gill & Miller 1956; Troeh & Thompson 1993).

The gravimetric soil moisture contents at 10 cm depth determined for June, July, September and October are low, ranging in value from 0.7% to 3.8% across all four site locations. While coarse-textured soils typically have lower moisture contents, it is likely that the calculated gravimetric water content values for the soil supporting *H. venusta* underestimate the actual moisture content of the soil. When digging the soil felt moist to the touch and the moisture increased with depth. The soil was only sampled to a depth of 10 cm for moisture content so it is likely that the increased moisture at depth was not captured in the sampling. Furthermore, the rock content (rock is included in the coarse fraction that is greater than 2 mm) of the soil, which averaged 27% across all four sites at the 0-10 cm depth, is likely suppressing the gravimetric water content; the actual soil moisture available in soil pores may be greater than indicated (Reinhart 1961).

The soil moisture content in forest soils as determined by gravimetric water content is affected by the presence of coarse materials in the soil (Fleming et al. 1993). Coile (1952) showed that determination of soil moisture based on oven-dry weights for samples containing coarse and fine fractions alike, underestimates the amount of the soil moisture content. The presence of gravel and stone in a sample contribute to an increased sample weight and not necessarily an increased water weight. As a result the calculated gravimetric soil moisture values across the four sites do not necessarily reflect what was observed in the field or what is available to the plant.

However, the relatively high rock content of the soil may also be responsible for contributing to the moisture observed in the soils at the time of sampling. At 10-20 cm depth, the average rock and gravel content ranged from 22% to 64% with an average of approximately 51% across all four depths. The presence of rock fragments has been shown to significantly influence

the moisture holding capacity of a soil (Hanson and Blevins 1979; Cousin & Coutadeur 2003). Gravel reduces evaporative loss of water by inhibiting the capillary movement of water to the surface of the soil (Unger 1971). On high-altitude talus slopes, one study found that when compared to bare, sandy soil, a layer of gravel greatly reduced the amount of water lost via evaporation (Weaver 1919) while another study found that the overall soil moisture content is up to 20 times higher in soils beneath a layer of stone fragments versus soils beneath bare, sandy talus (Perez 1991).

While the gravimetric water content for the top 10cm of the soil is relatively low, it is higher than lower altitude sites situated in shrub-steppe environments east of the core population (Taylor 2008). This low yet adequate moisture content may also be attributed to the steep slopes of the sites which serve to shade the soils (Taylor 2008).

Soil moisture content is an important soil property regulating plant growth and observations indicate that *H. venusta* is difficult to grow in the greenhouse and is particularly sensitive to moisture variation (Pers.Comm. W. Gibble, University of Washington, 2013). Plants performed better when grown in clay pots instead of plastic pots but were still vulnerable to fluctuations in water content. According to Taylor (2008), a watering regime that keeps the leaves of the plant dry and maintains a moist but not saturated soil that keeps the roots from drying out, is essential to the survival of the seedlings. This further suggests that the coarse texture of the soil supporting *H. venusta* is crucial to regulating the moisture content of the soil.

These low moisture contents correspond to the maximum air temperatures, below average precipitation rates (NOAA 2013) and high soil temperatures recorded for the moisture sample collection times. Soil temperatures are influenced by both the aboveground air temperature and the rock content of a soil. The rock content of the soil also plays a significant role in the

movement of heat within a soil (Poesen & Lavee 1994). Subsurface soil temperatures tend to lag behind air temperatures particularly in coarse-textured soils with low moisture; wet, compacted soils transfer heat much more quickly than loose, dry soils (Brady & Weil 2010; Pritchett et al. 1979). The movement of heat within a soil also influences the movement of moisture within the soil. The differential heating between rock fragments and the surrounding fine fraction can lead to the accumulation of moisture beneath the cooler rock fragments. As a result, pockets of moisture are formed in an otherwise warm, dry soil creating potentially crucial microsites supporting plant growth (Poesen & Lavee 1994). This is a potential factor influencing *H. venusta*'s growth on rock outcrops and ledges.

2.4.1.2 Cation exchange capacity and exchangeable bases

The soils supporting *H. venusta* are relatively undeveloped, as evidenced by the coarse texture, weak aggregation and subtle differences between horizons. The low cation exchange capacity is not surprising in the young, weakly developed soil with low organic matter content where *H. venusta* is found. While there was litter on the ground at all site locations, Site 3 was the only one with an identifiable O horizon. The other sites had scattered, sparse accumulations of litter on the surface of the soil. Total percent carbon ranges from 0.5%-2% across all sites and depths. In sandy soils and soils with low secondary clay content, organic matter content is responsible for much of the cation exchange capacity (Daniels et al. 1987, Schlesinger 1997). While CEC for this soil is low, the relatively high percent base saturation suggests that many of the exchange sites are full and have reduced acidity in the soil as more CEC sites are filled with nonacid cations instead of H^+ ions (Brady & Weil 2010). Further, because the CEC sites are so low, even a high percent base saturation does not indicate a high availability of base cations.

2.4.1.3 Phosphorus

Phosphorus in soils exists in both organic and inorganic forms (Shen et al. 2011). In general, total phosphorus values in soil range between 500 and 800 μg g^{-1} (Stevenson & Cole 1999). While total phosphorus values may be quite high, only a small portion is available for plant uptake (Yang et al. 2012). Most phosphorus occurs in soil as orthophosphate (Mengel & Kirkby 1979). Total phosphorus content and extractable phosphorus content varies across a range of vegetation and soil types as. In the soils supporting *H. venusta*, extractable phosphorus content makes up an average of 15-17% of the total phosphorus content (Figure 6).

The values for the extractable phosphorus content in the mineral soil as determined by the Bray method are high across all four sites in the soils supporting *H. venusta*. It has been reported that on average, anywhere from 25-30 μg g^{-1} of extractable P is ideal to support plant growth (Kovar & Pierzynski 2009) and the average extractable P content for *H. venusta* soils ranged between 86.8 μg g^{-1} to 112.9 μg g^{-1}across all four sites and depths, far exceeding the optimum values reported in Kovar & Pierzynski.

Compton et al. (1998) examined phosphorus content in soils beneath adjacent Douglas-fir and Red alder (*Alnus rubra*) stands in the Cedar River watershed located on the Western side of the Cascades, approximately 60 km southeast of Seattle, Washington. Soil from 0-7 cm depth within the Douglas-fir stand had a total P value of 1,612 μg g^{-1} and 1,134 μg g^{-1} within the Red Alder stand. Similar values can be observed further south in the Oregon Cascades. Spears et al. (2001) found total P down to 10 cm depth ranged between approximately 1,200 to 1,600 μg g^{-1} in soils developed from volcanic ash and pumice (Andic dytrudepts) beneath Ceanothus (*Ceanothus velutinus*) and Douglas-fir respectively in the H.J. Andrews Experimental forest.

Extractable P is a measure of the labile phosphorus within the soil. These values vary greatly between the different extraction techniques but also within extraction techniques as well. The Bray method for determining extractable phosphorus content is suitable for soils with slightly acidic pH values (Kalra & Maynard 1991). P is limited in availability at lower pH (as it tends to bind with iron and aluminum and become insoluble.

Extractable P content is highly variable depending on site locations. Extractable P from 0-7 cm depth in the Douglas-fir and Red alder stands studied by Compton et al. was154 μg g^{-1} and 4.6 μg g^{-1} respectively. Vitousek et al. (1982) found Bray extractable P to range from 2.5 μg g^{-1} in Pacific silver fir stands to 85 μg g^{-1} in Coastal hemlock stands within the Pacific Northwest at 0-10 cm depth. The average Bray extractable P content for *H. venusta* at 0-10 cm depth was greater than all of the 17 forest types distributed across the United States that were sampled by Vitousek et al. (1982) (Figure 7).

Because very little phosphorus is deposited by atmospheric deposition, phosphorus in soils is made available mainly through weathering of primary minerals in the soil. Given that the parent materials of this soil are largely granitic there is likely some other mechanism that would explain the elevated phosphorus levels. Two important factors may be playing a role in the elevated levels of extractable P in the *H. venusta* soils: fire history and inputs from volcanic ash. Historically, fire has played an integral role in maintaining the ecosystem supporting *H. venusta* (Agee 1993). As recently as 1994 moderate to high intensity wildfires passed through the core population after years of fire suppression in the area (Gaines et al. 1997; Kifner 1994). Fire increases the availability of phosphorus through the mineralization of organic P present in soils in the form of organic matter to plant-available orthophosphate ($H_2PO_4^{-2}$) (Cade-Menun et al. 2000; Neary et al. 2005) and by increasing the pH of soil (Certini 2005). Given the history of fire

suppression in the area there was likely a significant build-up of organic material on the surface of the soil resulting in the mineralization of high amounts of P.

The literature suggests that extractable P following fire is variable and site specific. Results from a study of burned slash piles in Eucalyptus forests in Australia found that the mineralization of P increased with increasing fire severity but that the effects were diminished with depth; the greatest effects were observed in the moderately to severely burned surface soils at the 0-2.5 cm depth with Bray extractable P values of 11.79 $\mu g\ g^{-1}$ and 13.13 $\mu g\ g^{-1}$ respectively (Romanya et al. 1994). The fire that burned through the core population was of moderate to high intensity. In a mixed-conifer stand within the Teakettle Experimental Forest, soils developed on decomposed granite (Dystric xeropsamment) showed extractable P values as high as 1,166 to 1,275 $\mu g\ g^{-1}$ (original values reported in ppm) in the 0-10 cm following prescribed burns in plots that had received variable thinning treatments (Wayman & North 2007).

There is some discrepancy concerning the amount of time the increased P levels will persist in a soil. Some research suggests that the increase is usually short-term and does not endure in the system (Certini 2005); others find that increased levels may persist years after a fire has passed through. One study examining the effects of an understory burn in a jack pine forest (*Pinus banksiana*) did find elevated extractable P levels in the mineral soil 10 years after a fire (Lynham et al. 1998). Hatten et al. (2012) found elevated levels of available P (Bray extractable P) up to 15 years after a prescribed burn in the Malheur National forest located in the Blue Mountains of eastern Oregon on soils that developed from andesite and basalt with inputs of volcanic ash. This study found that available P increased in the A horizons from nearly 50 $\mu g\ g^{-1}$ in the control plots to approximately 70-80 $\mu g\ g^{-1}$ in prescribed burn plots. While these levels

remained elevated above the control 15 years after the burns, levels showed signs of decrease 5 years after the prescribed burns.

The presence of volcanic ash may also be contributing to the high extractable P in the soil. Volcanic activity in the Cascade Mountain range of the Pacific Northwest from Glacier Peak, Mount Mazama, and Mount Saint Helens has deposited variable amounts of ash over the area (Aho & Bieler 1989; Bieler 1975; Busacca 2001; Matz 1987). Eruptions from Mount Mazama and Mount St. Helens are reported to have limited amounts of apatite, one of the most common sources of inorganic phosphorus (Mullineaux et al. 1975; Sarna-Wojcicki et al. 1981).

Allophane, imogolite, and ferrihydrite are short-range order minerals that are formed through the weathering of volcanic ash. These minerals help to increase the anion exchange capacity thus increasing the soil's capacity to adsorb plant-available phosphorus (McDaniel & Wilson 2007). In neutral to acidic soils, phosphorus tends to adsorb to these amorphous materials. As pH decreases, iron and aluminum phosphates precipitate out of solution becoming unavailable to plants for uptake (Troeh & Thompson 1993). The soils supporting *H. venusta* are slightly acidic averaging between 5.8-5.9 across all depths at all four sites which would favor the adsorption of phosphorus by amorphous minerals rather than precipitation of phosphorus into insoluble forms.

The question in this particular case is how much volcanic ash is in the soil? The ash that was deposited in the inland Pacific Northwest is found in variable amounts and is susceptible to erosion by water, wind and bioturbation from local fauna (Kimsey et al. 2007). In a study conducted by Busacca et al. (2001), the amount of volcanic glass in the Columbia Plateau (an area just to the east of the location of the core population of *H. venusta*) averaged 12.1% though the range extended between 5.9% for samples in the southwestern portion of the plateau to

27.5% in the northeastern portion of the plateau. According a soil survey conducted in 1995, the soils within the core population of *H. venusta* are mapped as part of an Icicle-Chumstick-Rock outcop complex (Aho & Bieler 1989). Both the Icicle series and the Chumstick series contain ash mixed in but neither soil meets the criteria for a volcanic ash soil (Soil Survey Staff 2010). This suggests that while volcanic ash is present in the soil, it is neither a defining nor the dominant feature of the soil.

While there were trace amounts of volcanic glass apparent upon examination with the petrographic microscope, the dominant mineral assemblage was granitic. This further supports that volcanic ash inputs are present though likely limited at this particular site.

Given the steep slopes of the site and the amount of active erosion taking place on the surface, it is likely that there was a lot of mixing of the soils resulting in the incorporation of ash (both fire and volcanic) into the soils. This is further supported by the lack of a consistent decrease in phosphorus content with depth; while overall phosphorus content decreases with depth, there are slight increases observed in the 10-20 and 20-30 cm depths. However, the amount of erosion would also suggest that much of the surface material (and associated nutrients) has moved. This would suggest that the phosphorus levels of the toe slopes could actually be higher than those observed in this particular study (all samples were taken from the backslope).

The foliar P content was 1.7 mg g^{-1} which falls well within a range of the foliar P values for 134 species examined from the Mediterranean Basin (Stock & Verboome 2012) which ranged between 0.4 and 3.4 mg g^{-1} and averaged 1.3 mg g^{-1}. In a similar study conducted by Foulds (1993) 368 different plant species were examined from 39 different habitats in Southwest

Australia. Of these 368 species, foliar P contents ranged between 0.9 and 1.6 mg g^{-1} for herbaceous species.

2.4.1.4 Nitrogen

Extractable ammonium and nitrate nitrogen for the *H .venusta* soils falls within the range of extractable N content for other forest soils. Similar coarse-textured soil formed in decomposing granite beneath mixed-conifer stands in the Sierra Nevada Mountains had a 2*M* KCl extractable ammonium nitrogen content that was similar, though slightly lower than the *H. venusta* soils (Figure 7; Erickson et al. 2005). In the 1982 study conducted by Vitousek et al. (1982) extractable N content was examined for 17 different forest types. Within these forests, extractable N content ranged from 0.2 μg g^{-1} to 30 μg g^{-1} for NH_4^+ and NO_3^- respectively for depths ranging from 10-15 cm. The *H. venusta* soils fell within the lower end of this range with values for NH_4^+ averaging 2.7 μg g^{-1} and 5 μg g^{-1} for NO_3^- (Figure 8).

Ammonium levels were lower than nitrate with the exception of the 40-45cm depth (Table 2). Available nitrogen is influenced by several environmental factors including temperature, precipitation, vegetation growth, and fire history. Samples were collected between May 21st and June 19th and soil temperatures averaged 16.3°C across all four sites (Figure 3). When temperatures are low, it is expected that amounts of NH_4^+ and NO_3^- will be lower as mineralization rates are slowed as well. Soil moisture during this time period was particularly low as well, ranging between 1.9% and 2.7%; such low moisture levels would also favor decreased mineralization rates and thus a decrease in the amount of ammonium in the soil (Troeh & Thompson 1993). In addition to low soil moisture, levels of NH_4^+ and NO_3^- may be decreased

overall as a result of plant uptake (Stevenson 1999) as soil samples were collected when *H. venusta* was flowering and presumably taking up more inorganic nitrogen.

Fire greatly influences the cycling of nitrogen within a soil and the intensity of the fire determines the fate of the nitrogen already in the soil. Nitrogen volatilizes at temperatures less than 200°C (Perry 1994). Therefore, during high intensity fires in which temperature may exceed 1100°C at the surface of the soil much of the organic nitrogen is lost to the atmosphere due to volatilization. During moderate intensity fires, most of the organic nitrogen in the soil is converted to inorganic forms (Certini 2005). While ammonium is the product of the combustion of organic forms of nitrogen, nitrate is released via the nitrification of ammonium. Elevated levels of NO_3-N compared to NH_4-N observed in the *H. venusta* soils may result from the nitrification of ammonium produced during wildfires that passed through the core population. However, it is surprising that the NO_3-N levels are higher than NH_4-N given both the mobility of NO_3^- and its tendency to leach down the soil profile while ammonium is more likely to be held in the soil by negatively charged surfaces and organic matter. Ammonium that isn't fixed in the interlayer spaces of clays tends to be nitrified into nitrate (Certini 2005). Further, the presence of small amounts of volcanic ash in the soil may increase the ability of this soil to retain nitrate in spite of the coarse texture and resulting increased drainage.

Despite relatively low nitrogen in the soil, C and N analysis on new leaves of *H. venusta* did not indicate any deficiency in N content as total nitrogen was 2.8%. The leaves appeared to be healthy upon observation and showed no visible signs of nitrogen deficiencies such as chlorosis or stunted growth (Kimmins 1997). Plants that are adapted to more infertile sites may not always display a nutrient deficiency but rather will grow at a slower rate as a result of a lower nutrient availability (Schlesinger 1997; Clarkson and Hanson 1980).

2.4.2 Erosion and Root Morphology

The soil supporting *H. venusta* has developed on very steep, granitic talus slopes where erosion is an active force shaping the surrounding landscape. Erosion of a landscape is directly related to a variety of factors including the amount of vegetative cover, the texture of a soil, and the amount of moisture in a soil. Erosion is also directly influenced by the slope (and length of slope) of a given landscape (Wischmeier et al. 1965). In addition to the steep incline of the slopes, there is correlation between parent material and erodibility of a soil. Granitic substrates weather to coarse fragments with little to no aggregation making them a highly erodible parent material (Rider et al. 2005). A study conducted examining the erodibility of eight different parent materials in California found that soils developed on granitic substrates tend to be more erosive than other parent materials including metamorphic, sedimentary, and alluvial substrates (André & Anderson1961).

The differences in observed erosion in this this study are attributed to differences in vegetative cover, presence or absence of an O-horizon, and slight variations in topography at the individual site locations. Site 3 is especially well-protected when compared to the other locations. The erosion pins were placed on a relatively flat shelf 1-2 m downslope from a granitic cliff face and beneath the cover of a large Douglas-fir tree. As a result, there was a significant amount of organic matter protecting the willemite particles from water erosion and the relatively flat surface limited erosion due to gravity. Conversely, there is little vegetation cover and no significant accumulation of organic matter to offer protection from erosion at the other three site locations. Additionally, Site 4 has an unofficial climber's trail that runs through the area; some of the erosion could therefore be attributed to human disturbance.

The limited rooting depth observed in the excavated *H . venusta* is likely attributed to the relatively shallow soil that the *H. venusta* is growing in. Additionally, the erosion of surface materials appears to be pushing the aboveground portion of the plant downslope as the base of the excavated plant was buried beneath several centimeters of sand and gravel and the taproot was arched upslope.

Root structure may also be influenced by the nutrients available in a soil (Clarkson and Hanson 1980). Increased growth and fine branching in the roots of *Hordeum vulgare* was demonstrated when growing in phosphorus deficient soils. In addition, increased lateral branching of the *H. vulgare* roots was exhibited when increased levels of N or P were applied to specific portions of the root system (Hackett 1968) However, in the case of *H. venusta*, it seems more likely that the shape and structure of the root system is influenced more by erosion than by nutrient availability as the foliar analysis did not indicate any nutrient deficiencies within the plant.

According to Gentry and Carr (1976) upon examination of 21 species of *Hackelia*, all can be classified as either tetraploid or diploid; *H. venusta* is a tetraploid species (Hipkins 2003). Within these 21 species, basic habitat preferences can be ascribed based upon the tetraploid and diploid distinction. Tetraploid species tend to be found on more unstable and rocky substrates including talus and rock slides and many tetraploid species have a tendency to divide their taproots in these dynamic environments which allows the plant to survive as two separate individuals (Gentry & Carr 1976). This particular feature was not observed upon excavation of the individual *H. venusta* specimen. However, observations made by Taylor (2008) did note divided taproots occurring within 2” pots.

In the field, *H. venusta* is observed growing on both unstable slopes and more secure rock outcrops and crevices. While *H. venusta* is clearly adapted for the dynamic slopes on which it occurs, results of the erosion may place additional stresses on the plant. Erosion at the soil surface decreases soil productivity by reducing organic matter, increasing run-off and decreasing water infiltration, and as a result, and limits the depth of a soil (Troeh & Thompson 1993). Furthermore, the continuous movement of materials downslope puts a considerable strain on the growth of the root system itself. The shallow nature of the soils prevents a deeply-rooted taproot and the constant movement of surface materials forces the plant to expend energy sending roots upslope as the base of the plant is slowly moved downslope by erosion.

2.4.3 DNA Extraction

Even considering the elevated extractable phosphorus content of the soil, the lack of AMF in the majority of the samples collected is somewhat striking. Research indicates that AMF play a critical role in the uptake of phosphorus and that AMF associations occur in 70% - 90% of all vascular terrestrial plants (Smith & Read 2008). In ecosystems with limited phosphorus availability, mycorrhizal fungi associations are commonly identifiable in the roots and or the soil (Clarkson & Hanson 1980; Kimmins 1997). However, even elevated levels of phosphorus in a soil do not inhibit the growth of AMF (Menge et al. 1978). There are several factors that could have contributed to the inconsistencies in the results including spore abundance in the soil and uneven infection of the roots within the samples that were collected.

Amplification for AMF in the roots at Site 4 does not conclusively indicate the presence of an AMF symbiosis with the *H. venusta* roots nor does the absence of amplification rule out the possibility of an association within the other samples. AMF spores may be present in the soil and

detected through DNA extract but may not indicate a currently active symbiosis (Kruger et al. 2009). Conversely, spores may be present in the soil but their abundance so low that they are not able to be detected. If the overall spore abundance was low in the soil at the time of sampling, it could explain why no AMF was detected in any of the soil samples. Some AMF tend to sporulate in the late spring while others tend to sporulate in late summer (Bever et al. 2001). Gemma et al. (1989) studied five species of AMF found in sand dunes located in Dartmouth, MA and observed seasonal variation in the abundance of spores. This seasonal abundance of spores has been observed in several other studies including another sand dune ecosystem (Giovanetti 1985) and several agricultural studies (Saif & Khan 1975; Sutton & Barron 1972) with maximum spore abundance found to be related to the end of the growing season of the host plant. These studies were conducted on annual species and spore abundance may differ phenologically between annual and perennial species (Gemma et al. 1989; Sylvia 1986).

The use of group-specific primers has been developed in an effort to filter out glomalean fungi (AMF) from other types of fungus, however there are still some issues. For example, DNA extraction of AMF specific colonizers within a soil is made difficult by the presence of organic materials and can often be contaminated by the presence of other pathogenic and saprophytic fungi (Redecker & Weimken 2003). Given the coarse, sandy texture of the soil supporting *H. venusta*, the likelihood of organic material preventing the extraction of the fungal mycelium is probably low (Koltai & Kapulnik 2010).

The root and soil samples for this study were collected towards the end of the growing season of *H. venusta* and Site 4 was the only site where the roots collected were found to have AMF; none of the soil samples were reported to have any AMF. However, this does not necessarily mean that the other plants from which root samples were taken do not have an AMF

association. It is possible that some parts of the rooting system could still remain uninfected with AMF as colonization can and often is restricted to only some parts of the root (Kapulnik & Douds 2000) thus the samples that were collected may simply be the portions of the root that were not infected.

Furthermore, colonization of the root can be influenced by the amount of phosphorus in the soil. While the levels of extractable P in the *H. venusta* are very high *H. venusta* may still be taking advantage of an AMF association. However, high levels of phosphorus in the soil can lower the percentage of the roots that are colonized in the plant. This is the result of increased root growth which decreases the proportion of colonized to non-colonized root length (Smith et al. 2011). Moreover, increased levels of phosphorus in the soil can lead to a decrease in the development of arbuscules and a decrease in the total AMF biomass in both the roots and the soil (Smith & Read 2008). It is interesting that the roots collected from Site 4 were the only ones to show any indication of the presence of AMF as the extractable phosphorus content at this site was lower than the other three sites.

2.5 Conclusions

Based upon the results of the physical and chemical analyses of the soil there does not appear to be any particular advantage afforded to *H. venusta* that would lead to such a localized and restricted population. Extractable P content is high and is likely attributed to the fire history in the area. The presence of limited amounts of volcanic ash in the soil which may be contributing to the elevated phosphorus levels is not restricted to the range of *H. venusta.* Rather the varying amounts of volcanic ash can be seen in soils across the state of Washington. Instead

it would seem that the fire history in the area is responsible for the elevated levels of extractable P in the soil.

The coarse-textured soil, while subject to erosion, provides adequate moisture and good drainage necessary for plant growth. Though soil cation exchange capacity and extractable nitrogen content are relatively low, the plant shows no signs of any nutrient deficiencies. Given the results of the physical and chemical analysis, it is likely that the lack of stable soils is precisely what allows *H. venusta* to survive. Reduced competition affords *H. venusta* more light and the plant has less competitors for already limited nutrients and moisture. The root system of the excavated *H. venusta* revealed a smaller, much shallower root system than originally hypothesized. Although not extensive, the root system is able to adapt to a variety of substrates including the loose slopes that dominate the landscape within the core population as well as the small ledges and crevices within rock outcrops that are scattered throughout. The results from the DNA extractions were conflicting, with none of the rhizosphere soil samples indicating the presence of AMF and only the roots from Site 4 indicating the presence of AMF.

CHAPTER 3. CONCLUSIONS AND MANAGEMENT IMPLICATIONS

The physical and chemical analyses of the soil did not reveal any one feature that could serve to explain the extremely localized and limited population of *H. venusta.* The elevated extractable P values in the soil are one of the more striking features of the soil. While volcanic ash in the soil may be contributing to elevated levels of extractable phosphorus in the soil, volcanic ash is found throughout the region and is not limited to the area of the *H. venusta* population. Furthermore, the amount of volcanic ash in the soil is low and not a dominant feature of the soil. Rather, it seems likely that the elevated values of extractable P in the soil are a result of the site's fire history. The coarse texture of the soil, while making it more prone to erosion, provides good drainage and adequate moisture to support plant growth. Soil mineral nitrogen content and cation exchange capacity are relatively low though foliar analyses revealed no deficiencies. Existing nutrient levels are clearly adequate to support the limited *H. venusta* population.

It seems likely that the limited stability of the soil provides a less competitive environment in which *H. venusta* can survive. Reduced competition affords *H. venusta* more light and the plant has less competitors for already limited nutrients and moisture. The root system of the excavated *H. venusta* revealed a smaller, much shallower root system than originally hypothesized. The root system is able to adapt to the loose slopes that dominate the landscape within the core population as well as take advantage of the small ledges and crevices within rock outcrops that are scattered throughout the core population. The results from the DNA extractions were conflicting with none of the rhizosphere soil samples indicating the presence of AMF and only the roots from Site 4 indicating the presence of AMF.

For future outplantings, choosing sites with similar soil properties should be considered. The biggest threat to the survival of this species appears not to lie in the ability of the soils to support the plant but rather in the protection of plants from further disturbance events. While the 1995 outplanting site (Site 4) is similar to the other three site locations in terms of physical and chemical composition, its location within a high traffic area made it more susceptible to the effects of site disturbance. By selecting more stable locations, such as rock outcrops and ledges, the newly planted seedlings may stand a better chance at survival. Further, by selecting sites that are less accessible to the general population may protect the plant from additional human disturbance.

Future outplanting efforts may wish to consider experimenting with instituting erosion control techniques such as wattles and/or stabilization fabrics. Wattles were historically composed of interwoven twigs and sticks and traditionally used for the construction of walls and fences (Grey & Leiser 1982). Wattles are staked into the ground which anchors them in place and further supports stabilization of the surface soils by decreasing the formation of surface erosion features such as rills and reduces the effects of gravitational erosion (Haigh et al. 2002). These bundles of woody materials help to dissipate the energy of water flowing downslope as well as trap sediments being carried by water downslope (Grey & Leiser 1982). Wattles installed above newly planted seedlings would protect them from debris moving down slope while those installed below the seedlings would help to secure the slope and prevent the downward movement of the slope.

The use of small wattles may be a cost-effective and field efficient method of stabilizing newly outplanted individuals of *H. venusta*. While there isn't a great accumulation of organic material throughout the core population as a whole, there may be adequate materials nearby to

construct wattles. There are large clusters of woody shrubs, *Ceanothus velutinus* in particular, that could provide material for the construction of wattles in the field. Naturally occurring downed logs and other coarse woody debris, though sporadic on site, could also be positioned in such a way as to create wattle-like structures.

Wattles on the site can then be stabilized using wooden stakes which are light-weight and allow for easy transport of these materials into the core population. Construction stakes are cut from lumber and come in a variety of lengths. These stakes are light-weight and offer a cost-effective solution for anchoring the wattles in place. If available, scrap wood could be cut down to the appropriate length which could lower the cost even further.

In conjunction with the construction of low-cost, site-specific wattles, the use of stabilizing fabrics may further protect the newly planted seedlings. Coir is a geotextile composed of fibers from the husks of coconuts and offers natural. This bio-degradable material can assist in the stabilization of the soil and encourage vegetative growth (Lekha 2004). *H. venusta* seedlings planted within a small matrix of coir may be more protected allowing them to anchor and firmly establish their root system. Small sections of coir laid down between wattles placed upslope and downslope of the outplanting site would offer a more protected environment for the *H. venusta* seedlings potentially increasing their chance of survival in the long term. Over time the wattles and the coir fabric will naturally degrade contributing to increased organic matter content in the soil which will further stabilize the surface (Lekha 2004).

The application of a layer of organic materials in lieu of the use of geotextiles, may be another affordable approach to the stabilization and protection of the microsites where the newly outplanted *H. venusta* individuals will be. Mulches are used for a variety of purposes including the maintenance of soil moisture by reducing evaporative water loss near the surface of the soil.

Additionally mulches increase the organic matter content of the soil and reduce soil erosion by absorbing the impact of rain drops and increasing the water-holding capacity of the soil. Finally, mulches have the added benefit of suppress competition from surrounding vegetation which may increase the chance of survival of the newly planted *H. venusta* seedlings (Brady & Weil 2010; Morgan 2005; Schroth & Sinclair 2003). Given the steep slopes of the site, mulches should be applied in conjunction with wattles to prevent the erosion of the mulch downslope.

A monitoring program developed for any newly established *H. venusta* individuals should take into account the risk of physical damage to the site and to individual species themselves. As Taylor (2008) suggested, prior to outplanting it would be prudent to test outplanting techniques in areas near the forest ranger station in Wenatchee. This would allow for experimentation to determine the most suitable habitat for establishing new *H. venusta* individuals while also preventing further site degradation as a result of multiple visits by field technicians.

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Catton, Haley. PhD Candidate. Department of Biology, University of British Columbia Okanogan, Kelowna, BC, Canada.

Gibble, Wendy. 2013. Rare Care Program Manager. University of Washington Botanic Gardens, University of Washington, Seattle, Washington.

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Malmquist, Lauri. 2013. Botany Program Manager. Wenatchee River Ranger District, Leavenworth, Washington.

TABLES

Table 1 Physical properties of soil by depth. Results are averaged across all four sites. Standard deviation is shown in parentheses.

Depth (cm)	Bulk Density (g cm^{-3})	%Silt+Clay (<0.05mm)	%Sand (0.05 mm-2 mm)	%Gravel (>2 mm)
0-10	1.31	3.6 (1.2)	96.4 (1.2)	27 (3.5)
10-20	1.28	3.4 (1.4)	96.6 (1.4)	51 (19.2)
20-30	1.28	2.9 (1.0)	97.1 (1.0)	24 (6.2)
30-40	--	2.6 (0.3)	97.4 (0.3)	15 (6.1)
40-45	--	2.6 (0.6)	97.4 (0.4)	31 (19.0)

Table 2 Soil chemical properties. Results were averaged across all four site locations. Standard deviation is shown in parentheses.

Depth (cm)	pH	Total C (%)	Total N (%)	C:N	%Base Saturation	Cation Exchange Capacity ($cmol_c\ kg^{-1}$)	Extractable N		Total P ($\mu g\ g^{-1}$)	Extractable P
							NO_3-N ($\mu g\ g^{-1}$)	NH_4-N ($\mu g\ g^{-1}$)		PO_4-P ($\mu g\ g^{-1}$)
O horizon†	5.2	27.6	0.90	33	--	--	--	--	848.0	--
0-10	5.8	2.0 (0.63)	0.08 (0.06)	25	75 (10.6)	7.7 (4.9)	5.0 (2.3)	2.7 (1.0)	666.0 (47.3)	98.1 (78.2)
10-20	5.9	0.9 (0.02)	0.04 (0.02)	22.5	72 (16.8)	4.6 (2.2)	4.0 (2.2)	2.5 (1.2)	680.0 (45.3)	100.9 (43.9)
20-30	5.9	0.9 (0.01)	0.04 (0.01)	22.5	71 (10.6)	4.4 (2.4)	4.0 (1.4)	2.7 (0.9)	684.9 (64.9)	112.9 (100.5)
30-40	5.9	0.6 (0.00)	0.02 (0.00)	30	77 (2.5)	2.8 (0.7)	4.2 (0.2)	1.9 (0.0)	604.7 (24.4)	106.1 (0.3)
40-45	5.8	0.5 (0.01)	0.02 (0.01)	25	52 (39.6)	3.0 (8.2)	2.4 (0.1)	5.0 (1.8)	631.6 (25.7)	86.8 (7.2)

† Only one O-horizon was identified and collected.

FIGURES

Figure 1 View looking southeast from within the core population inChelan County. There is a small *H. venusta* individual in the bottom left-hand corner of the photograph. *J. Vance.*

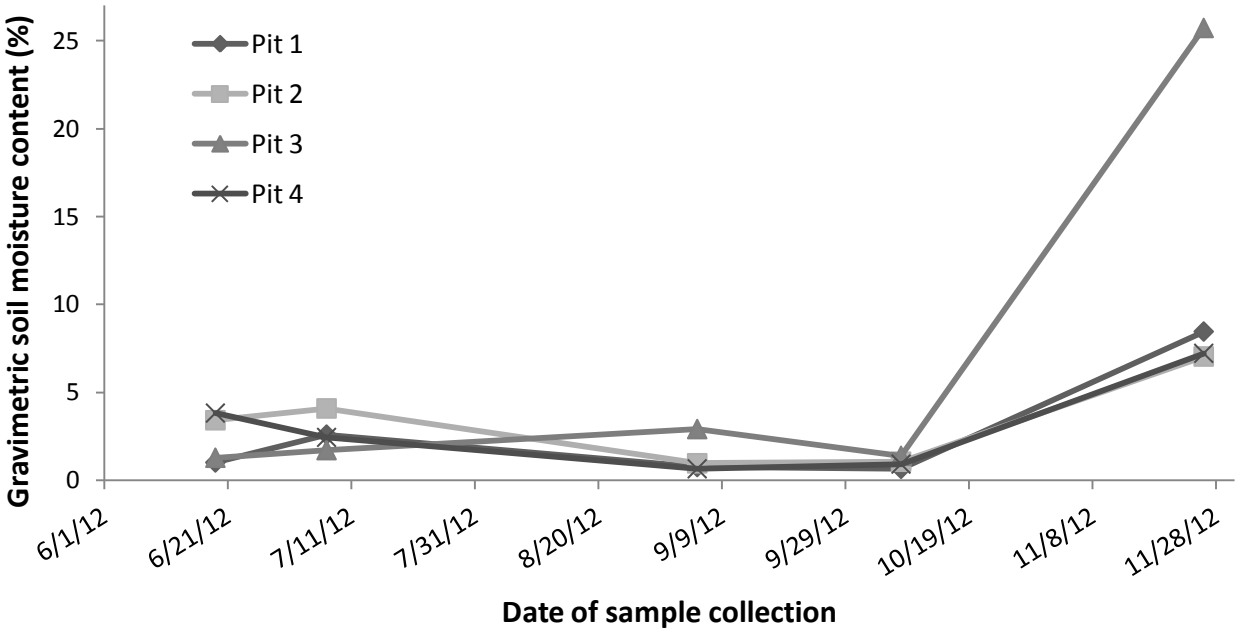

Figure 2 Soil moisture content at 10cm depth for all four site locations. Low soil moisture values are likely underestimated due to the high gravel content in the samples. Samples show dramatic increases in moisture in samples collected in November due to low temperatures and precipitation during the previous week. The increase in soil moisture at Site 3 relative to the other sites may be further explained by the protection of an O-horizon as well as over-story vegetation.

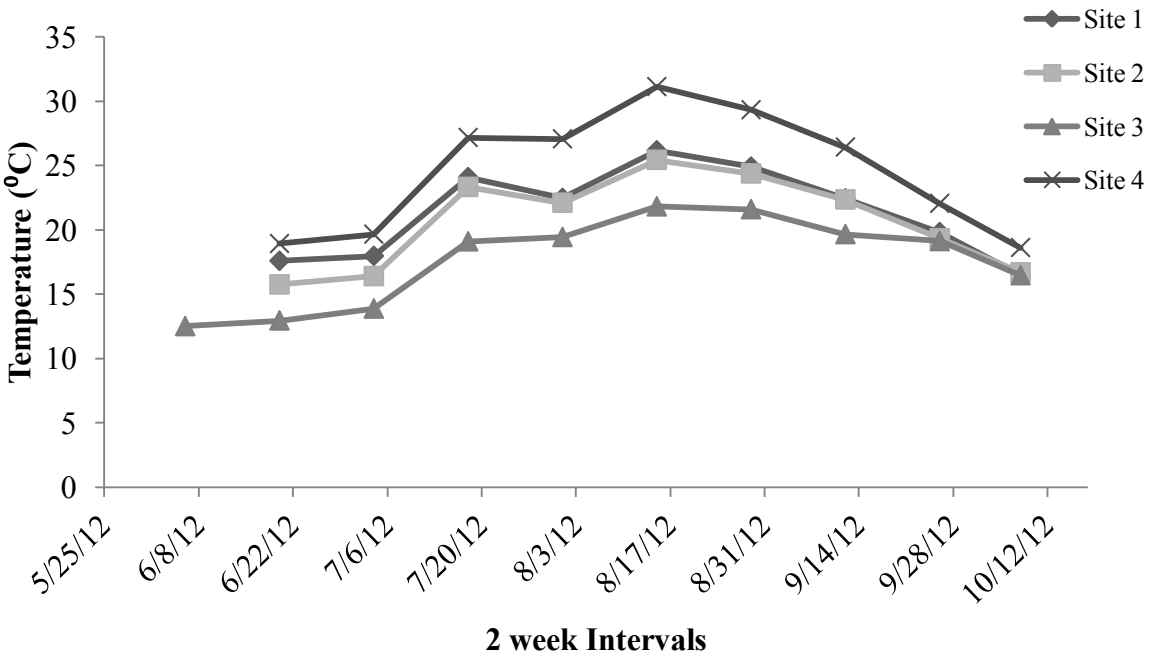

Figure 3 Soil temperatures measured at 10cm depth. Values shown are averaged over two week intervals between May and October, 2012.

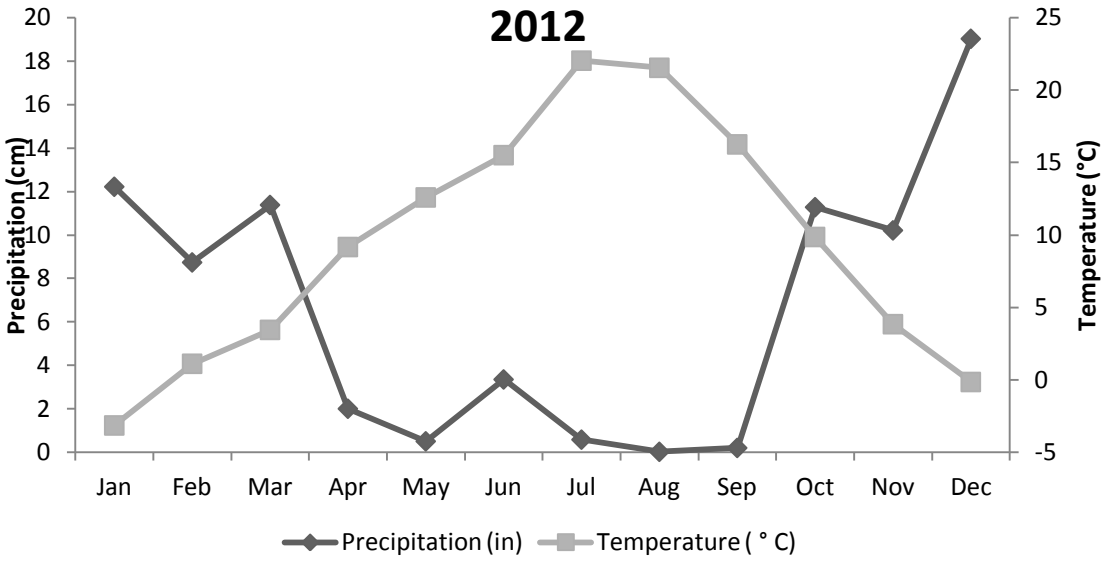

Figure 4 Average monthly precipitation and temperature data from the Leavenworth Weather station (#454572) for 2012. *Source: NOAA: National Climatic Data Center (2013). Retrieved April 1, 2013 from http://www.ncdc.noaa.gov/cdo-services/services*

Figure 5 The main taproot shows obvious strain from overall downward movement of soil particles**.** *D. Zabowski*

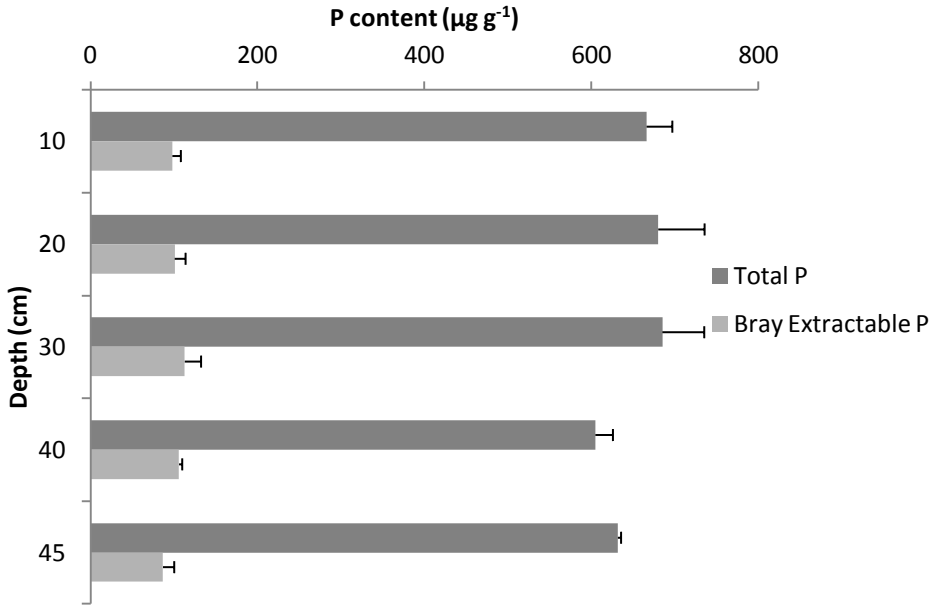

Figure 6 Total phosphorus and extractable phosphorus for soil supporting *H. venusta*. Extractable P comprises an average of 15-17% of the total P in this soil. Values are averaged across all four site locations. Error bars indicate standard deviation.

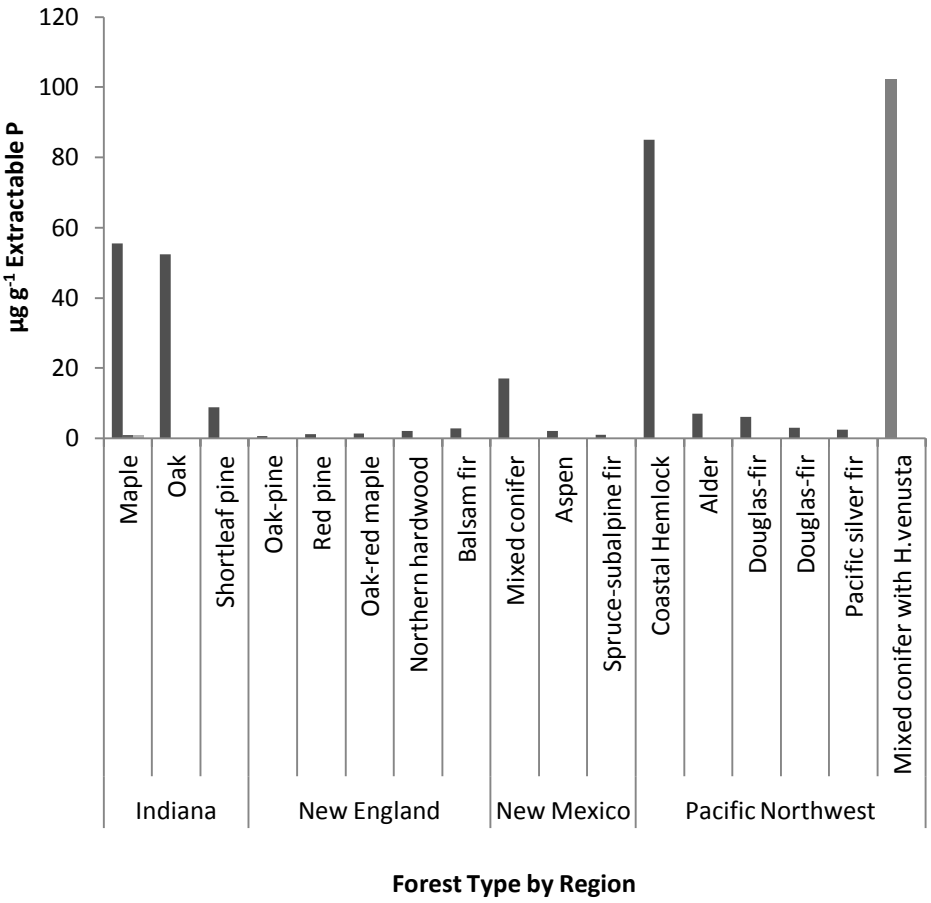

Figure 7 Extractable P contents of 17 forest types across the United States down for the 0-15 cm depth as determined by Vitousek et al. (1982) compared to the soils supporting *H. venusta* (shown on the far right in grey). While the extractable P values in the soils supporting *H. venusta* are significantly higher than reported optimum values (Kovar et al. 2009), and the values are considerably higher than many of the forests examined by Vitousek et al.

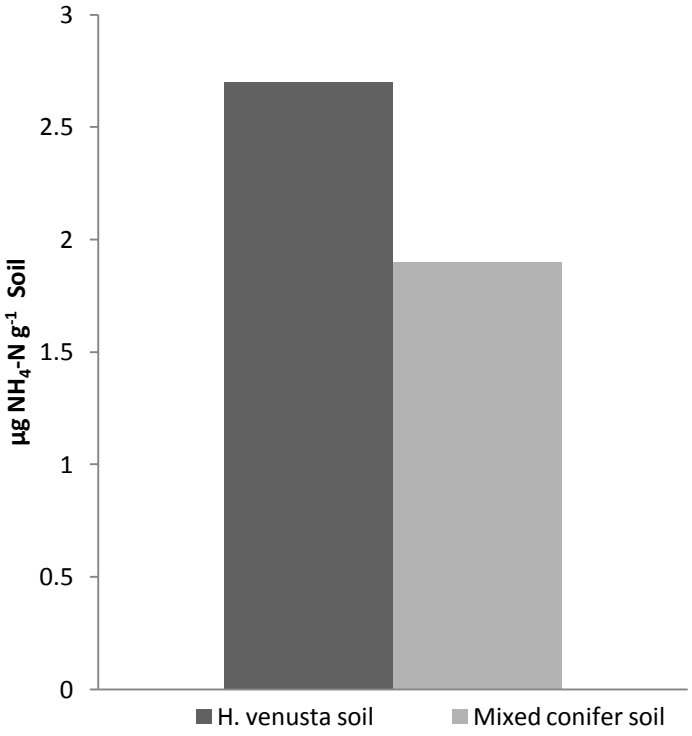

Figure 8 2*M* KCl extractable N for soil in H.venusta shown here relative to similar soil type at a similar depth (15cm) in the Sierra Nevada Mountains of California. The mixed conifer soil displayed here is from a similar coarse-textured, decomposing granitic soil in the Teakettle Experimental forest, located in the Sierra Nevada mountain range in California. *H.venusta* soils had approximately 2.7µg g^{-1} at 10 cm compared to approximately 1.9 µg g^{-1} in the soils taken from the Sierra Nevada Mountains (Erickson et al. 2005).

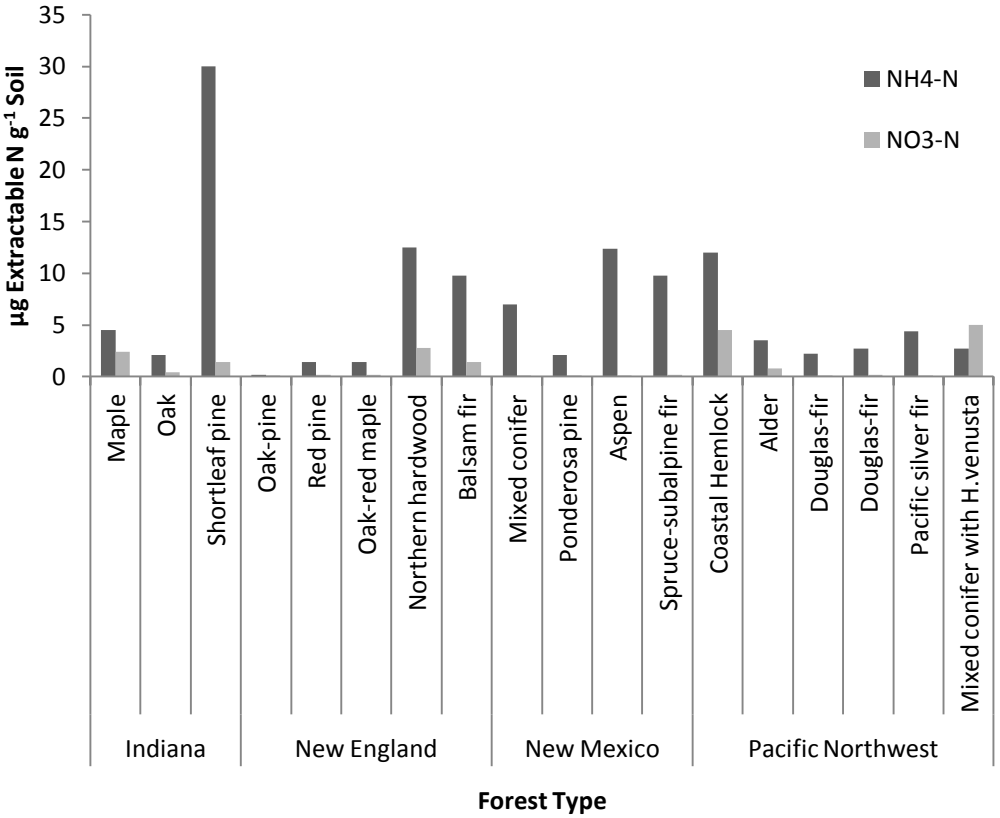

Figure 9 2*M* KCl extractable N contents of 17 forest types across the United States as determined by Vitousek et al. (1982) compared with the soils supporting *H. venusta* (shown on the far right, in grey). Extractable N (NO3-N and NH4-N) values in the soils supporting *H. venusta* fall within the range of the other values reported in Vitousek et al.

APPENDICES

Appendix A Figures

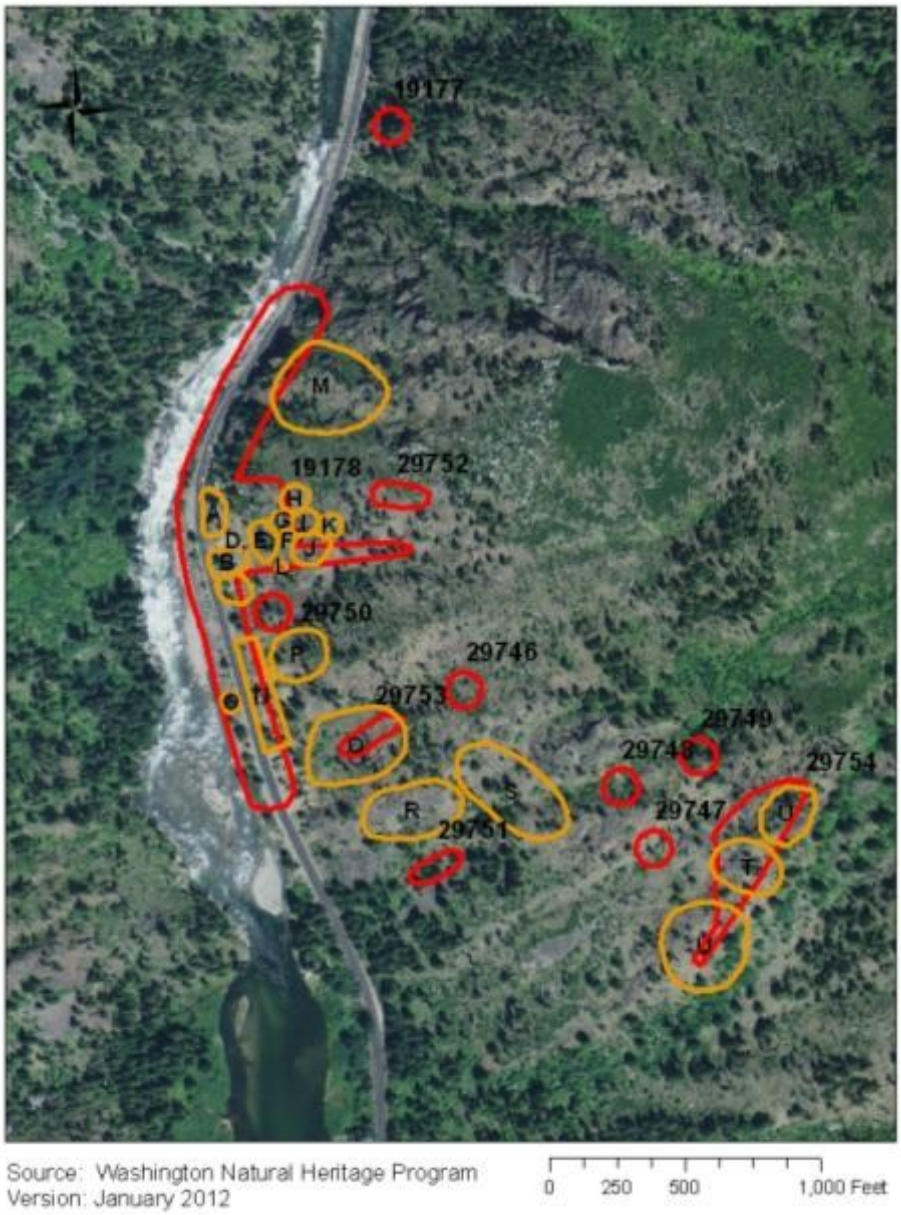

Figure A.1. Map displaying location of sampling sites and their corresponding mapped population units. Site 1 B, Site 2 falls in between polygons P and Qand Site 3 falls within polygon S. Site 4, the site of the 1995 outplanting, is not displayed on the map.

Figure A.2(Left) Erosion pins installed at Site 1 which has a slope of 38 degrees. Pins were marked with flagging to aid in recovery upon revisits. 6 months following the application of the fluorescent mineral particles, approximately 40-50% of the particles remained. Erosion pins are marked with yellow flagging.

Figure A.3(Right) 6months following the application of fluorescent mineral particles at Site 2, approximately 30-40% of the particles were observed. The majority of mineral particles remaining were between 2-5 mm (the intermediate size class of the applied minerals). Erosion pins are marked with yellow flagging.

Figure A.4(Left) 6 months following the application of fluorescent mineral particles, no particles of willemite remaining between erosion pins at Site 4.

Figure A.5 (top)Willemite particles distributed between erosion pins at Site 3. This location was had a significant accumulation of organic matter relative to the other sites and was situated on a relatively flat shelf of the slope.(bottom) Willemite particles distributed between erosion pins at site 3 fluorescing beneath the application of ultra violet. Willemite fluoresces to green and calcite can be seen as the red fluorescence

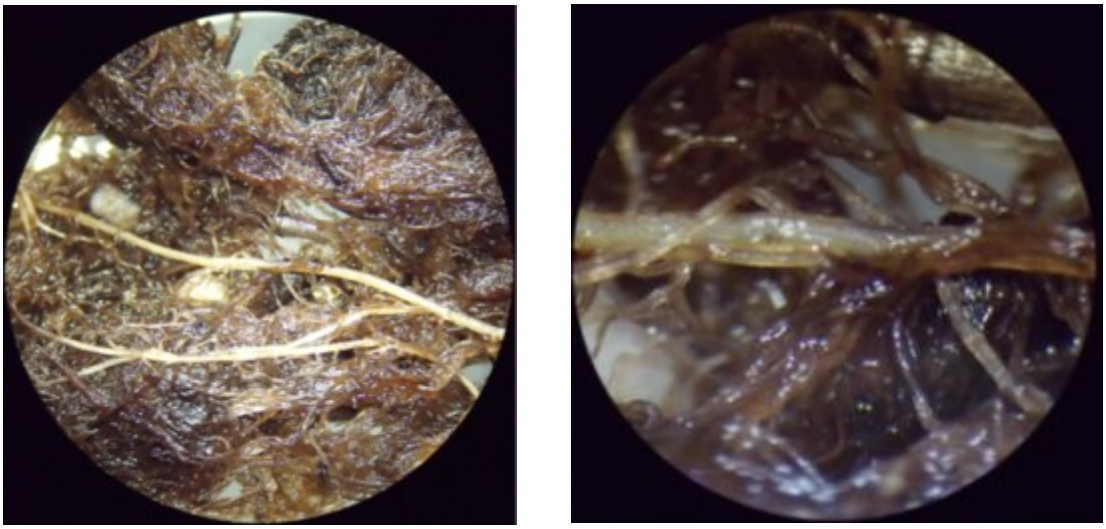

Figure A.6 *H. venusta* roots grown in the greenhouse at the University of Washington. Images taken at 15X (top left), 30X (top right) and 400X (bottom left and right). No mycorrhizal features were detected. *R. Edmonds*

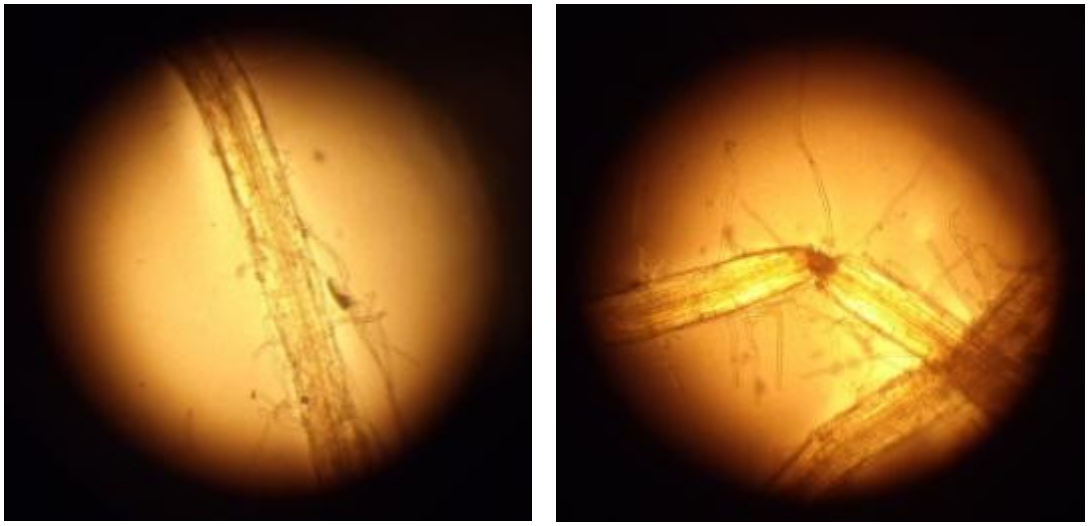

Figure A.7 Characteristic soil profile typical of the four site locations examined.

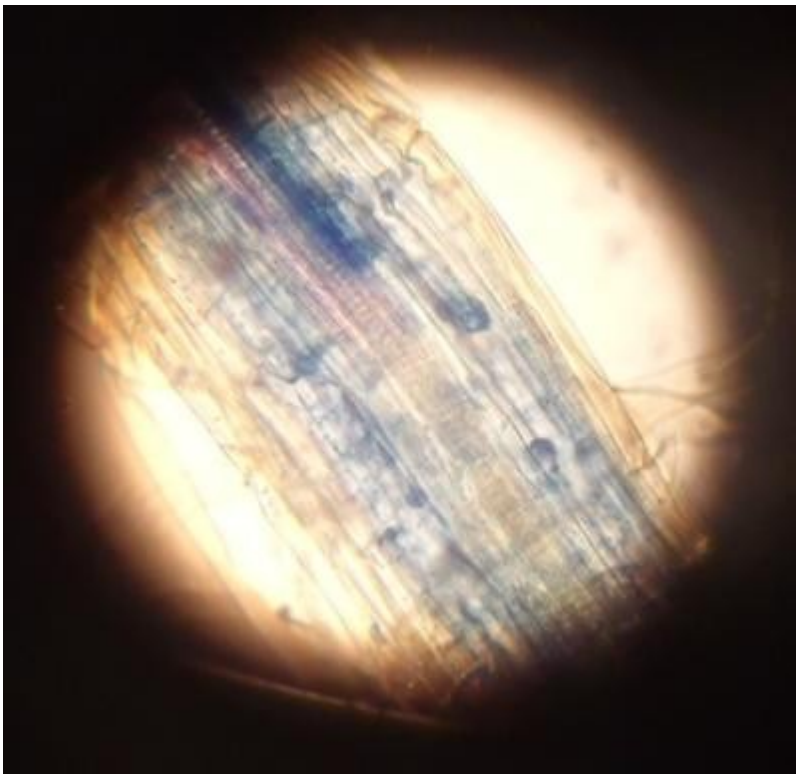

Figure A.8 Roots from *H. venusta* specimen grown in the greenhouse at the University of Washington. Lactophenol ink stain indicating the presence of fungal root association however no AMF structures were identified. Photo taken at 400X magnification with a dissecting microscope. *R. Edmonds*

Appendix B. Tables

Table B.1 Raw data for determination of particle size distribution in 10cm depth increments in *H. venusta* soils. Gravel is defined as the coarse fraction that is > 2 mm but less than 5mm. Any large rocks were removed prior to sieving. Only Site 1 (20 cm depth increment) and Site 3 (20 cm depth increment) had rocks in the sample weighing 408.8 g and 325.6 g respectively. Percent gravel was calculated by dividing the gravel weight by the total weight of the air-dried sample. Percent sand and percent silt+clay was calculated by dividing the weight of the sand fraction by the weight of the < 2mm fraction.

Site	Sample Depth (cm)	Total Weight (g)	Silt+Clay <0.045 mm (g)	Sand Weight 0.045 mm - 2 mm (g)	Gravel Weight > 2mm (g)	% Gravel	% Sand	% Silt+Clay
1	10	537.16	15	369.61	152.55	28.4	96.1	3.9
1	20	852.32	12.46	348.08	491.78	57.7	96.5	3.5
1	30	441.72	9.57	346.58	85.57	19.4	97.3	2.7
1	40	405.42	10.18	351.42	43.82	10.8	97.2	2.8
1	45	222.46	5.59	177.79	39.08	17.6	97.0	3.0
2	10	776.47	10.93	586.87	178.67	23.0	98.2	1.8
2	20	648.03	7.3	497.99	142.74	22.0	98.6	1.4
2	30	483.76	6.48	354.15	123.13	25.5	98.2	1.8
2	40	518.5	9.81	408.16	100.53	19.4	97.7	2.3
2	45	397.09	4.78	215.66	176.65	44.5	97.8	2.2
3	10	573.93	20.03	402.58	151.32	26.4	95.3	4.7
3	20	560.34	8.72	191.58	360.04	64.3	95.6	4.4
3	30	474.35	12.28	370.99	91.08	19.2	96.8	3.2
4	10	422.51	10.85	278.9	132.76	31.4	96.3	3.7
4	20	809.01	14.49	323.97	470.55	58.2	95.7	4.3
4	30	210.19	5.84	136.58	67.77	32.2	95.9	4.1

Table B.2 Results from chemical analyses for all four site locations.

Site	Depth (cm)	pH	%C	%N	CEC ($cmol_c$/ 100g)	%BS	Extractable N		Extract -able P	Total P ($\mu g\ g^{-1}$)
							NO_3-N ($\mu g\ g^{-1}$)	NH_4-N ($\mu g\ g^{-1}$)	PO_4-P ($\mu g\ g^{-1}$)	
1	10	5.9	2.07	0.08	7.91	69.7	5.44	4.12	149.1	678
1	20	6.0	1.31	0.04	4.53	79.2	3.39	3.85	149.7	650
1	30	6.0	1.24	0.04	3.97	75.1	4.22	3.60	151.2	604
1	40	6.0	0.58	0.02	3.33	79.2	4.29	1.92	130.9	637
1	45	5.7	0.61	0.02	2.99	79.7	2.45	6.27	117.9	678
2	10	5.5	1.73	0.06	3.60	63.2	2.47	2.06	94.2	539
2	20	5.5	0.67	0.02	2.21	65.9	3.12	2.93	115.5	661
2	30	5.7	0.64	0.02	2.73	70.4	2.35	1.48	91.3	579
2	40	5.8	0.65	0.02	2.31	75.7	4.07	1.95	78.6	605
2	45	5.9	0.45	0.01	14.62	23.7	2.27	3.72	66.3	626
3	O horizon	5.2	27.62	0.85	--	--	--	--	--	848
3	10	5.9	1.25	0.05	4.64	86.0	4.24	2.84	107.6	708
3	20	5.6	0.81	0.04	4.10	51.6	2.29	1.87	113.5	818
3	30	5.5	0.97	0.06	3.10	56.6	3.68	2.92	175.9	708
4	10	6.0	2.74	0.18	14.48	82.3	7.84	1.97	57.5	739
4	20	6.4	0.92	0.07	7.55	90.4	7.29	1.19	32.2	734
4	30	6.4	0.75	0.05	7.91	81.7	5.76	2.83	41.9	693

Table B.3 Gravimetric soil moisture at 10cm depth content for all four site locations. Gravimetric water content was determined by subtracting the oven-dry weight of the sample from the wet weight of the sample, divided by the oven-dry weight of the sample, then multiplied by 100.

Site	Date of Collection	Tin Tare Weight (g)	Tin+Soil Wet (g)	Wet Subsample Weight (g)	Tin+Soil Oven Dry (g)	Dry Subsample Weight (g)	% Moisture
1	6/19/2012	6.4	14.4	8.0	14.3	7.9	1.0
1	7/7/2012	6.4	24.2	17.8	23.8	17.3	2.6
1	9/5/2012	30.3	97.4	67.1	96.9	66.6	0.8
1	10/8/2012	17.0	63.6	46.6	63.3	46.3	0.6
1	11/26/2012	27.3	163.2	135.9	152.6	125.3	8.5
2	6/19/2012	6.3	16.3	10.0	16.0	9.7	3.4
2	7/7/2012	6.3	18.8	12.5	18.3	12.0	4.1
2	9/5/2012	28.1	132.9	104.8	131.9	103.8	1.0
2	10/8/2012	17.0	45.8	28.8	45.5	28.5	1.1
2	11/26/2012	30.2	118.4	88.2	112.6	82.4	7.0
3	6/19/2012	6.4	15.1	8.7	15.0	8.6	1.3
3	7/7/2012	6.3	23.0	16.6	22.7	16.3	1.7
3	9/5/2012	29.4	68.3	38.9	67.2	37.8	2.9
3	10/8/2012	15.8	37.7	21.9	37.4	21.6	1.4
3	11/26/2012	28.2	88.3	60.1	76.0	47.8	25.7
4	6/19/2012	10.2	24.6	14.4	24.1	13.9	3.8
4	7/7/2012	6.5	22.4	16.0	22.1	15.6	2.4
4	9/5/2012	27.8	136.2	108.4	135.5	107.7	0.6
4	10/8/2012	16.1	60.1	44.0	59.7	43.6	0.9
4	11/26/2012	29.5	88.9	59.4	84.9	55.4	7.2