OREGON DEPARTMENT OF AGRICULTURE

NATIVE PLANT CONSERVATION PROGRAM

Evaluating allelopathic affects of pennyroyal (*Mentha pulegium*) on two native plant species

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for

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Introduction

Even prior to 2005's explosive spread, populations of pennyroyal (*Mentha puligeum*) were present in wetlands throughout southwestern Oregon. Although pennyroyal was previously reported as occurring "occasionally in disturbed sites in the West Cascades" (Hitchcock and Cronquist 1973), the distribution of this weed has increased steadily for the last 20 years.

Specimens collected from Douglas County as early as 1928 are stored in the Oregon State University (OSU) herbarium, although most collections were made during the 1970's and later. Twelve counties from Multnomah to Curry currently support documented infestations of pennyroyal, although the vast majority of populations of this species occur in Lane and Douglas Counties. Anecdotal information from local botanists suggests that species diversity decreases in pennyroyal infested areas, and recent literature indicates that pennyroyal may displace native plants, especially in vernal pool habitats (Warner 2000).

Figure 1. Specimen of *Mentha puligeum* (from Woodville 1793).

As well as impacting native habitats, pennyroyal can displace forage crops. Because this species is unpalatable and poisonous to cattle, pastures with pennyroyal infestations provide little forage, and hay made from infested fields is of poor quality (Fuller and McClintock 1986, Warner 2000). Human deaths of both adults and infants have resulted from the ingestion of pennyroyal based teas (Bakerink et al.1998, Deans 2006). However, despite its potentially lethal hepatoxic effects, pennyroyal continues to be used medicinally as an abortifacient or emmenagogue (Anderson et al. 1996).

Due to its popularity as a medicinal herb, pennyroyal has been introduced into North America many times (Grieve 1959; Figure 1). Because this species exhibits substantial genetic diversity, even within a limited geographic area (Fadhel and Boussaid 2004), each introduction probably increased the diversity of North American populations, resulting in a broad genetic base from which our current populations evolved. This extensive genetic diversity has allowed pennyroyal to persist in a variety of habitats in North America, and eventually to become the adaptable and aggressive colonizer of most types of seasonally wet habitats that it is today.

Species of *Mentha* frequently produce interspecific hybrids, with some species able to interbreed with many of their congeners (Chambers 1982). Oregon's native field mint (*Mentha arvensis*) is also an adaptable hybridizer (Gill et al.1973), and interspecific hybrids between this native and introduced pennyroyal have been suspected to occur (Amsberry 2001). Pennyroyal is reported to have spontaneously hybridized with *M. spicata* (spearmint) in Brazil, creating the "well known *poejo de praia*", a new taxon with purported medicinal uses (Martins et al. 2004). As Oregon is the national leader in peppermint production and ranks fourth in spearmint production, with over 25,000 acres of these two crops harvested in 2005 (NASS 2006), pennyroyal's potential for hybridization with mint crops, as well as native mints, is cause for concern.

Pennyroyal plants are perennial and spread quickly by rhizomatous growth; they are also precocious and prolific seed producers (Pancetta 1985). Efficient water absorption, combined with fast growth and high reproductive rates, contribute to the ability of this species to successfully outcompete horticultural crops (Krohn and Feree 2005); these competitive traits probably also promote displacement of native plants by naturalized pennyroyal infestations. In addition to being exceptionally competitive, pennyroyal is potentially allelopathic. Acetone extracts of pennyroyal exhibited high bioactivity against agricultural weeds (Solymosi 1994), probably due to pennyroyal's production of pulegone, a volatile oil which also causes fatalities of livestock and humans (Fuller and McClintock 1986).

Although the production of deleterious excretions by roots was originally documented long ago (Schreiner and Reed 1907), recent studies indicate that allelopathy is more important to the success of invasive weeds than previously believed (Calloway and Ascheoug 2000, Bais et al. 2003, Hierro and Callaway 2003). The increase of pennyroyal due to its superior competitive abilities, and its allelopathic interactions with native plants, may be contributing to the decline of native wetland vegetation in southwestern Oregon, and may be the cause of reductions in population sizes of native wetland species (Amsberry and Meinke 2006).

Objective

The objective of this project is to evaluate the effects of the exotic invasive *Mentha puligeum* on the state and federally endangered species *Plagiobothrys hirtus* (rough popcorn flower), and *Perideridia erythrorhiza* (red-root yampah), a state listed and Bureau of Land Management Sensitive species.

Methods

Evaluating the effect of pennyroyal root extract on seed germination of Plagiobothrys hirtus (2007). In 2007, ten seeds of *P. hirtus* were placed in each of 60 Petri dishes lined with filter paper. Seeds were previously collected from the created population of *P. hirtus* at the Westgate site at Bureau of Land Management's North Bank Habitat Management Area (NBHMA; see appendix for seed collection site location). Twenty Petri dishes were watered with an extract of pennyroyal produced by soaking ground roots collected from cultivated plants in distilled water. An additional twenty dishes were watered with an extract produced by soaking whole, un-ground roots overnight in distilled water, and twenty were watered with un-amended distilled water as a control. Because seeds of this species do not require any pre-treatment to induce germination, Petri dishes were placed directly in the greenhouse.

As the surface of the filter paper dried, seeds were re-wetted with the appropriate extract, and the number of germinated seeds was recorded each day. Seeds were counted as ‘germinated’ when the radical could clearly be seen emerging from the seed coat). A one-way ANOVA in Statgraphics 5.1 was used to analyze differences in the proportion of germinants among treated and control dishes.

Evaluating the effect of pennyroyal root extract on seed germination of Perideridia erythrorhiza (2008). Seeds of another southern Oregon wetland native plant, *Perideridia erythrorhiza* (red-root yampah) were treated in a similar manner. Seeds for the initial trial (completed in 2007) were collected in 1999 at a naturally occurring population near Sutherlin. (See appendix for seed collection locations.) However, as seeds of this species require an extended chilling period to initiate germination, filter paper-lined Petri dishes containing these seeds were moistened with the appropriate extract and placed in a seed germination chamber at 5º C for seven weeks until germination was expected to begin (Roberts 2000). As the filter paper dried, it was rewetted with extract or distilled water. Once the appropriate time period for stratification had passed, Petri dishes were inspected daily, and the number of germinated seeds was recorded.

However, seeds used in this initial trial germinated very poorly, probably due to their long-term storage in suboptimal conditions. Reliable data could not be collected from this first trial, so additional seed was collected in from the Yampah Flat population of this species at the NBHMA in late 2007. Germination testing - using the same treatment and stratification methodology used in 2007 - was repeated in 2008 (Figure 2). A one-way ANOVA in Statgraphics 5.1 was used to analyze differences in the proportion of germinants among treated and control dishes.

As is typical of the seeds of plants in the Apiaceae during cold moist stratification, many seeds developed moldy exteriors during their time in the growth chamber (Roberts 2000). To provide data useful for evaluating any potential impact of these mold infestations on germination, the mold level for each dish was quantified on an arbitrary scale of 0 to 2 (0 = no mold visible, 1 = some seeds in dish moldy, 2 = all or most of seeds in dish moldy.)

Figure 2. Treated seeds of *Perideridia erythrorhiza* were chilled at 5º in germination chambers at Oregon State University until germination began to occur. Inset shows preparation of pennyroyal root extract. Photos by K. Amsberry.

Evaluating the effect of pennyroyal on the growth of Plagiobothrys hirtus (2007). In order to evaluate the relative effects of competition and allelopathy by pennyroyal on *P. hirtus*, 28 1 L (5" round plastic) pots were filled with pure silica sand amended with Gro-safe® powdered activated carbon, and planted with one *Plagiobothrys hirtu*s seedling and one pennyroyal seedling. Because activated carbon has a high affinity for adsorbing to organic compounds, addition of this amendment to growing media deactivates compounds with allelopathic activity. Plants of the target species growing in this amended medium were not subject to allelopathic affects of companion plants, and interacted with their co-potted associates only through competition (Ridenour and Calloway 2001).

Twenty eight additional pots were filled with un-amended sand and planted with one plant each of both study species, and a second set of 56 pots (28 of each of the two soil types) was planted with two *P. hirtus* seedlings. Seedlings of pennyroyal and *P. hirtus* were initially grown in separate flats on sand, and were transplanted into the 1 L study pots when they were 1-2 cm tall. Seeds for this study were collected previously from an introduced population of *P. hirtus* at the NBHMA in Douglas County (Amsberry and Meinke 2006), and from a naturally occurring population of pennyroyal in the same site. Subsequent to transplantation into the study media, seedlings were grown in a heat-secured greenhouse, where they were watered daily, and fertilized with Dyna-Gro® (a commercially available formulation of Hoagland's solution) at the manufacturer's recommended strength (for weekly feedings) once per week.

Plants were removed from their pots after 11 weeks, and sand was carefully washed from their roots. Plants were air-dried for 10 weeks, then weighed (to +/- 0.001 gram) using an Ohaus Precision Standard balance. Entire plants, including both above and below ground biomass, were weighed. A multi-factor ANOVA in Statgraphics 5.1 was used to analyze the effect of a same species or pennyroyal companion planting on growth (as measured by biomass) of *P. hirtus* plants in the presence or absence of carbon (Ramsey and Schafer 2002).

Evaluating the effect of pennyroyal on the growth of P. hirtus (2008). In 2008, the growth comparison was repeated using a similar experimental design (Figures 3-5). However, two changes in methodology were implemented. In 2007, study plants were grown under minimal nutrient resources; in 2008, we were interested in evaluating allelopathic effects under a nutrient-rich fertilization regime. To achieve this goal, plants in this second study were fertilized daily with Dyna-Gro® (at the recommended concentration for daily feeding), rather than weekly as was done in the 2007 trial. To further increase nutrient levels, growing medium was made up of a 70/30 sand/Vermiculite mix, rather than pure sand. Vermiculite is an inert soil amendment that does not contain significant nutrients, but helps holds water and fertilizer, and is easy to remove from roots. Twenty-three pots were planted with each of the four treatments (sand/Vermiculite with a mixed planting, sand/Vermiculite with two *P. hirtus*

Figure 3. Seeds of pennyroyal and *P. hirtus* were initially started in flats of sand, then transplanted to pots for the comparative growth portion of the study. Photo by T. Maddux.

plants, carbon-amended soil with a mixed planting, and carbon-amended soil with two *P. hirtus* plants).

Additionally, 46 pots were each planted with one *P. hirtus* seedling, 23 in sand/Vermiculite, and 23 with activated carbon amendment added. The biomass of these two groups was compared using a one-way ANOVA in Statgraphics 5.1 to identify any potential effect of carbon itself on plant growth in the absence of inter- or intra- specific competitors.

Finally, in order to evaluate the effect of inter- and intra-specific competition on plants of *P. hirtus* under "natural conditions," 30 pots were filled with a 50/50 mixture of commercial peat moss and native soil collected from a site supporting a *P. hirtus* population. These pots were then planted with one *P. hirtus* plant, two *P. hirtus* plants, or a *P. hirtus* plant with a pennyroyal companion. Plants were fertilized with Dyna-Gro® at the weekly feeding rate, and were harvested and weighed using the same methodology used in other portions of this study. Data were analyzed using a one-way ANOVA in Statgraphics 5.1 to evaluate the effect of companion plantings on the growth of *P. hirtus* plants on the native soil mix.

Figure 4. Pots of pennyroyal and *P. hirtus* (shown here soon after potting was completed) were grown in the greenhouse until mature and ready for harvest. Pots with un-amended soil appear white, while those with activated carbon amendment are gray. Photo by M. Carr.

Figure 5. Mature plants were washed to remove soil, placed in paper bags to air dry for several weeks, and weighed when completely desiccated. Photo by M. Carr.

Results

Evaluating the effect of pennyroyal root extract on seed germination of Plagiobothrys hirtus (2007). Both pennyroyal root extracts significantly reduced the germination of *P. hirtus* seeds ($p = 0.008$; Figure 6). The more concentrated solution (the ground root extract) produced a greater effect, but the difference between the effects of the two extracts was not significant. As well as reducing the number of seeds germinating, treatment with extract also reduced the size and vigor of seedlings (Figure 7).

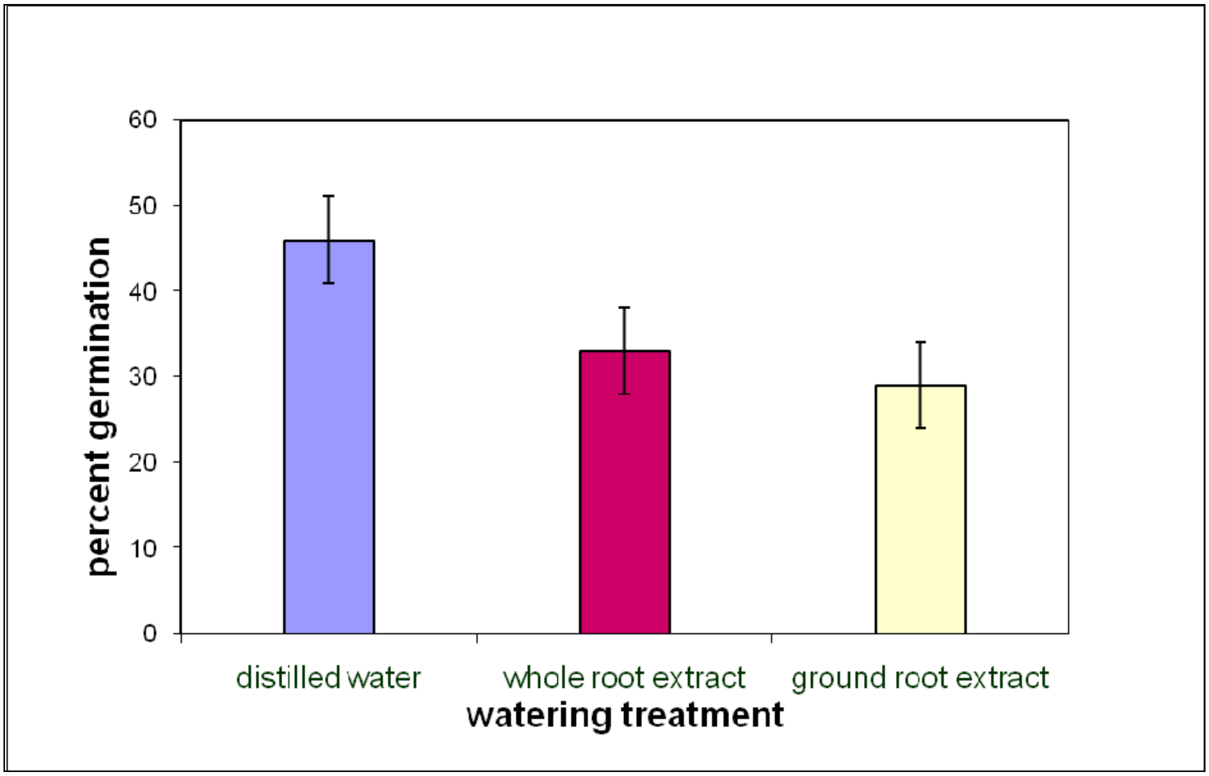

Figure 6. Pennyroyal root extracts reduced seed germination in *Plagiobothrys hirtus*. Error bars represent confidence intervals calculated using Fisher's least significant difference. n = 20 for each treatment.

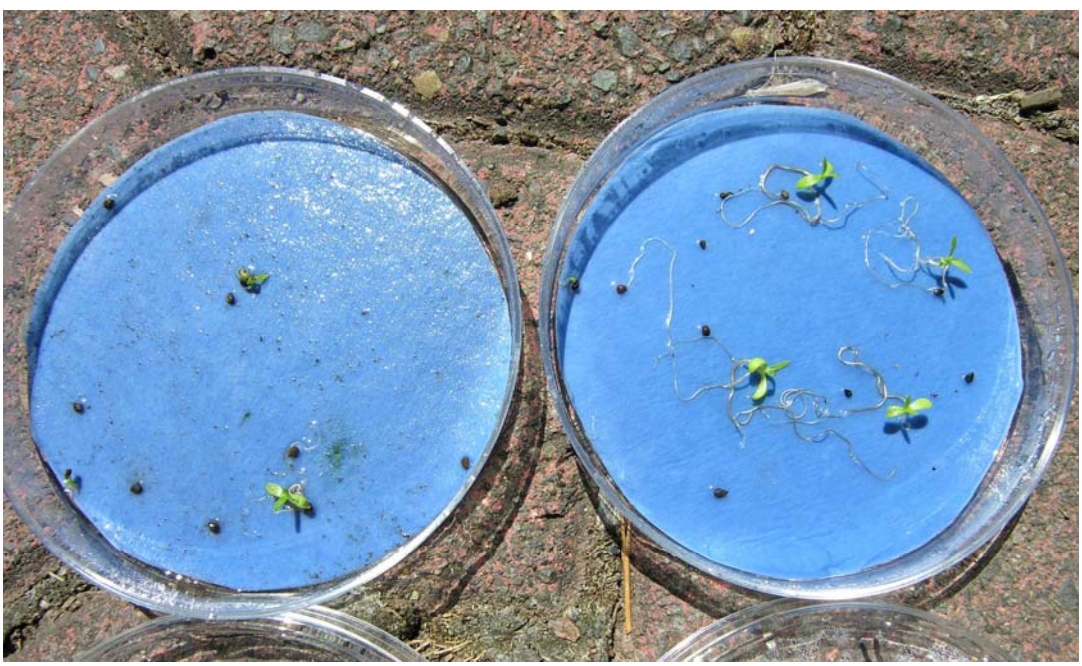

Figure 7. Seedlings watered with extract (left) were smaller and less vigorous than those watered with distilled water (right). Photo by K. Amsberry.

Evaluating the effect of pennyroyal root extract on seed germination of Perideridia erythrorhiza (2008). In the 2008 trial, newly collected seed of *P. erythrorhiza* germinated after stratification, as expected. Watering treatment significantly affected rates of seed germination ($p = 0.040$; Figure 8). As expected, the ground root extract reduced germination below the level seen in control dishes that were watered with distilled water. However, the seeds watered with the whole root extract (presumably with a lower concentration of active ingredients than the extract prepared from ground roots) actually germinated better than those treated with distilled water. Seed in dishes watered with distilled water were also much more likely to develop mold infestations (Figure 9).

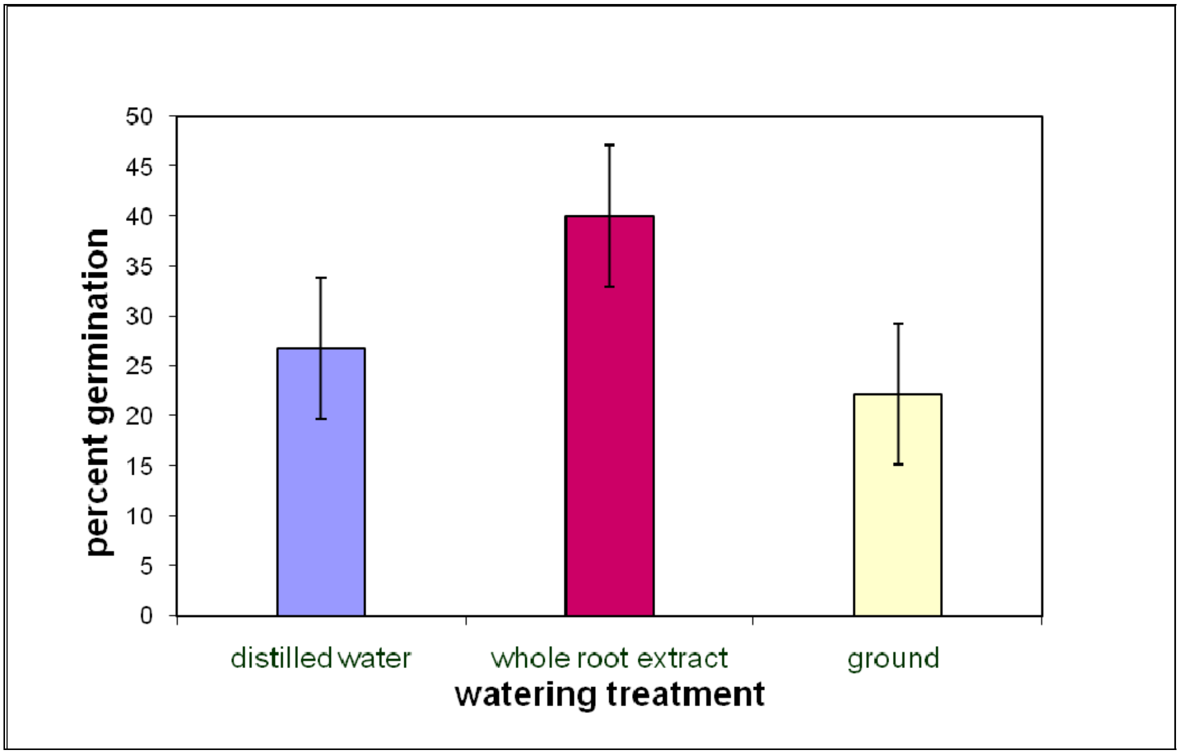

Figure 8. Pennyroyal extract prepared from ground roots reduced seed germination in *Perideridia erythrorhiza*, while extract prepared from whole roots increased germination. Error bars represent confidence intervals calculated using Fisher's least significant difference. n = 20 for each treatment.

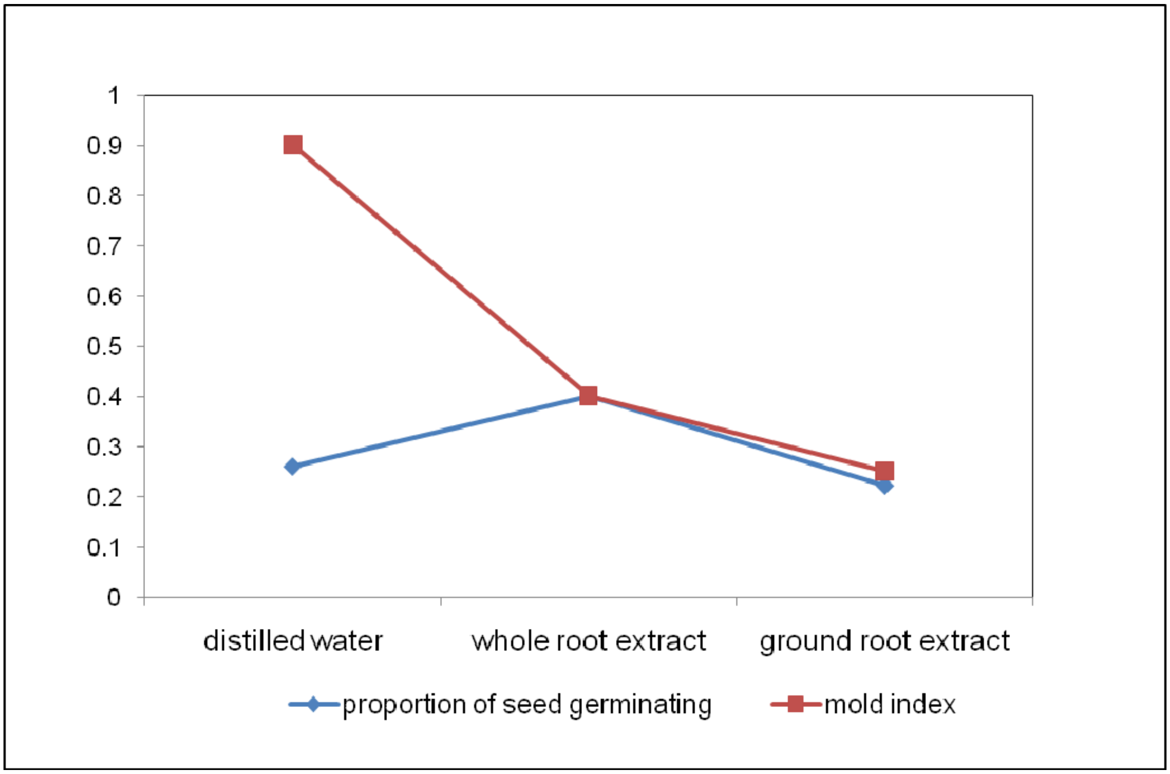

Figure 9. Mold grew prolifically in the untreated dishes of *Perideridia erythrorhiza* seeds, with progressive decreases in mold index as the concentration of the pennyroyal root extracts increased.

Evaluating the effect of pennyroyal on the growth of Plagiobothrys hirtus (2007). In the 2007 study, plants of *P. hirtus* grew larger (as measured by dry weight) when grown with pennyroyal as a companion planting than they did when grown with another plant of the same species, and this difference was significant ($p < 0.001$; Figure 10). The addition of carbon to the growing medium significantly increased plant biomass for both species ($p < 0.001$) in this initial study, and the interaction between the two treatments (pennyroyal companion planting and carbon amendment) was also significant ($p = 0.049$).

The greater increase in growth of *P. hirtus* resulting from the elimination of allelopathic chemicals (through the addition of carbon) in the mixed, as compared to conspecific, plantings allows for an estimated quantification of the effect of interspecific allelopathy. Part of the weight increase shown by *P. hirtus* plants in interspecific plantings in our study was due to the positive effect of carbon, and to the rare species' strong interspecific competitive abilities. The additional mean weight increase is due to the elimination of the allelopathic effects produced by pennyroyal on the growth of *P. hirtus* (Table 1).

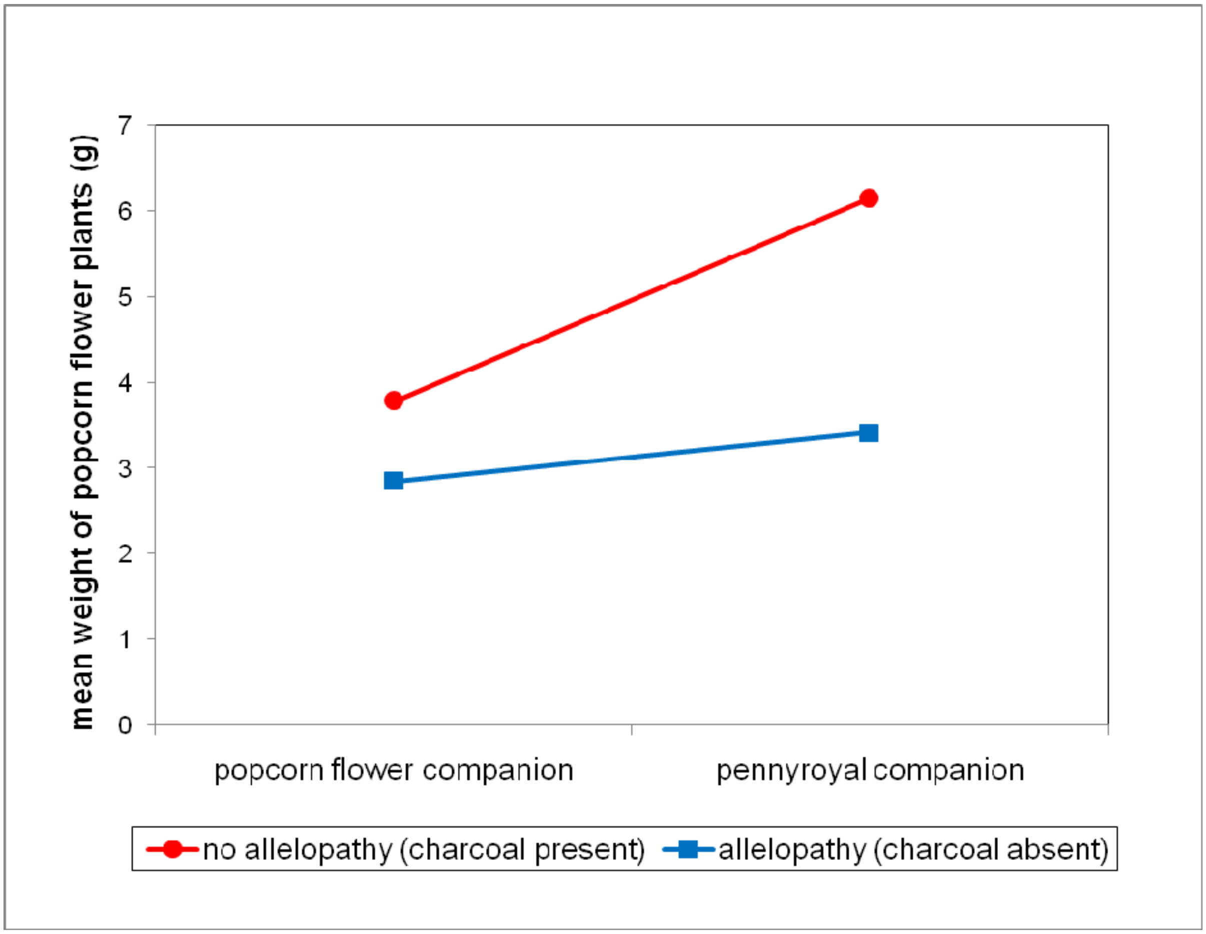

Figure 10. Grown under low nutrient conditions, plants of *P. hirtus* grew larger in the presence of pennyroyal than when grown with a conspecific companion, and the addition of carbon to the soil medium also increased growth. Most importantly, the effect of both treatments applied simultaneously was greater than the combined effect of the individual treatments.

Evaluating the effect of pennyroyal on the growth of P. hirtus (2008). The 2008 biomass comparison of *P. hirtus* plants grown with and without pennyroyal, and on amended and un-amended soils, used methodology similar to the 2007 study. However, plants were grown with more frequent fertilization. As expected, higher nutrient levels resulted in larger plants, with the overall mean weight of *P. hirtus* plants in all four treatments almost doubling (overall mean increase of 3.421 g) when compared to those grown in 2007. In this second study, addition of carbon did not significantly affect plant growth ($p = 0.130$; Figure 11), but choice of companion planting did ($p < 0.001$). The interaction of companion planting and soil amendment also continued to be suggestive ($p = 0.092$), although it was not longer significant at the 0.05 level. The comparison of solitary plants of *P. hirtus* grown on amended and un-amended soils that was included in this year's work also documented no effect of carbon on plant growth ($p = 0.442$). In the absence of any competitors, these solitary plants grew very large, with a overall mean weight of 10.180 g.

Plagiobothrys hirtus plants grown on the native soil/peat moss mix also grew well, with companion planting (pennyroyal, *P. hirtus,* or no companion) significantly affecting biomass ($p < 0.001$). *P. hirtus* plants grown without companions were the largest, with a mean weight of 5.372 g, while those grown with a conspecific companion were the smallest (2.419 g). The mean weight of *P. hirtus* plants grown with pennyroyal was not significantly different from those grown without companion plantings ($p < 0.05$), suggesting that competition and/or allelopathic interactions with pennyroyal may not be an important component affecting the success of this species under natural conditions (Figure 12).

Table 1. Difference in mean weights of *P. hirtus* plants grown with a carbon amended soil and/or a pennyroyal companion planting, as compared to plants grown in sand with conspecific companions.

Treatment	2007	2008
Carbon soil amendment only	+ 0.93	- 0.06
Pennyroyal companion planting only	+ 0.56	+ 2.12
Cumulative effect of both treatments, added separately	+ 1.49	+ 2.06
Both treatments simultaneously	+ 2.30	+ 3.31
Additional mean increase in weight for both treatments applied simultaneously	**+ 0.82**	**+ 1.25**

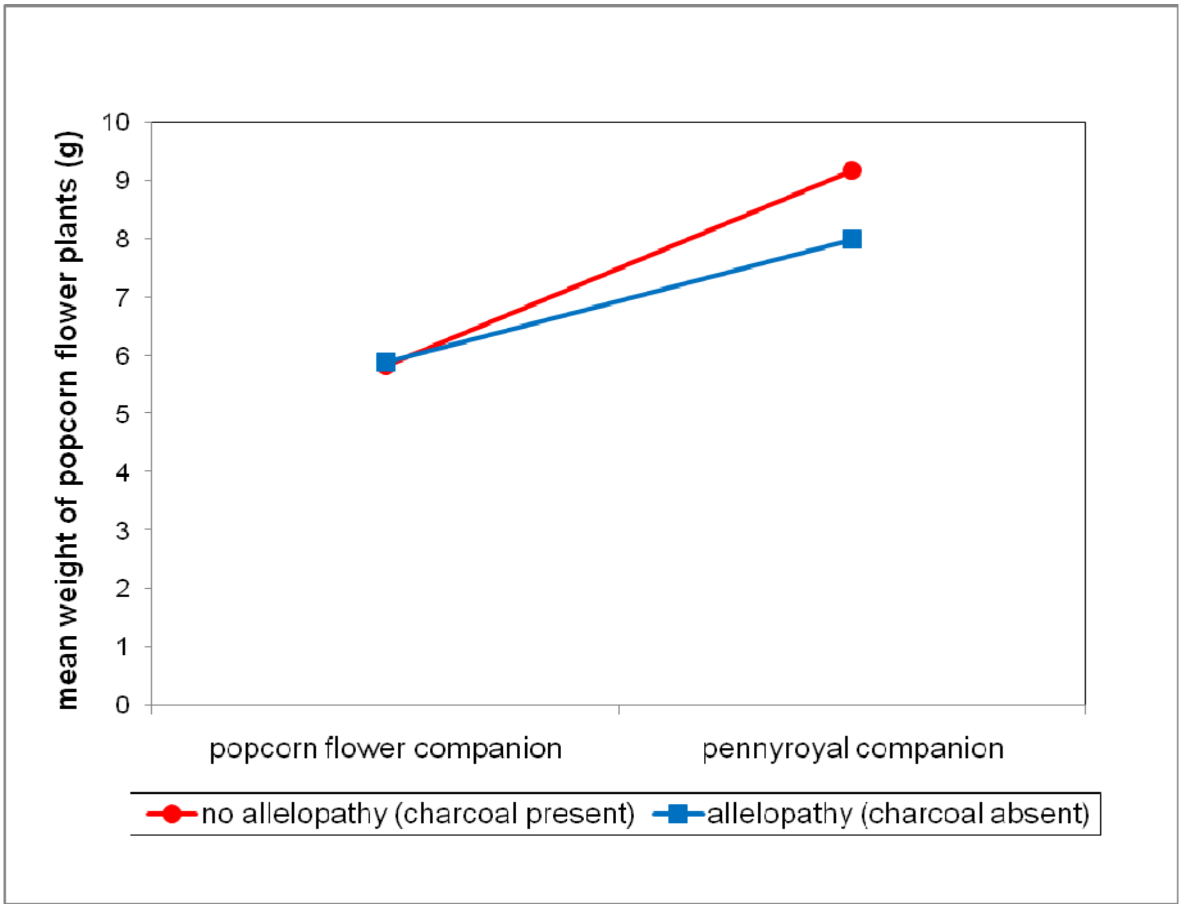

Figure 11. Grown under high nutrient conditions, plants of *P. hirtus* grew larger in the presence of pennyroyal than when grown with a conspecific companion, while the addition of carbon to the soil medium did not affect growth. As it had in the 2007 study, the effect of both treatments applied simultaneously was greater than the combined effect of the individual treatments.

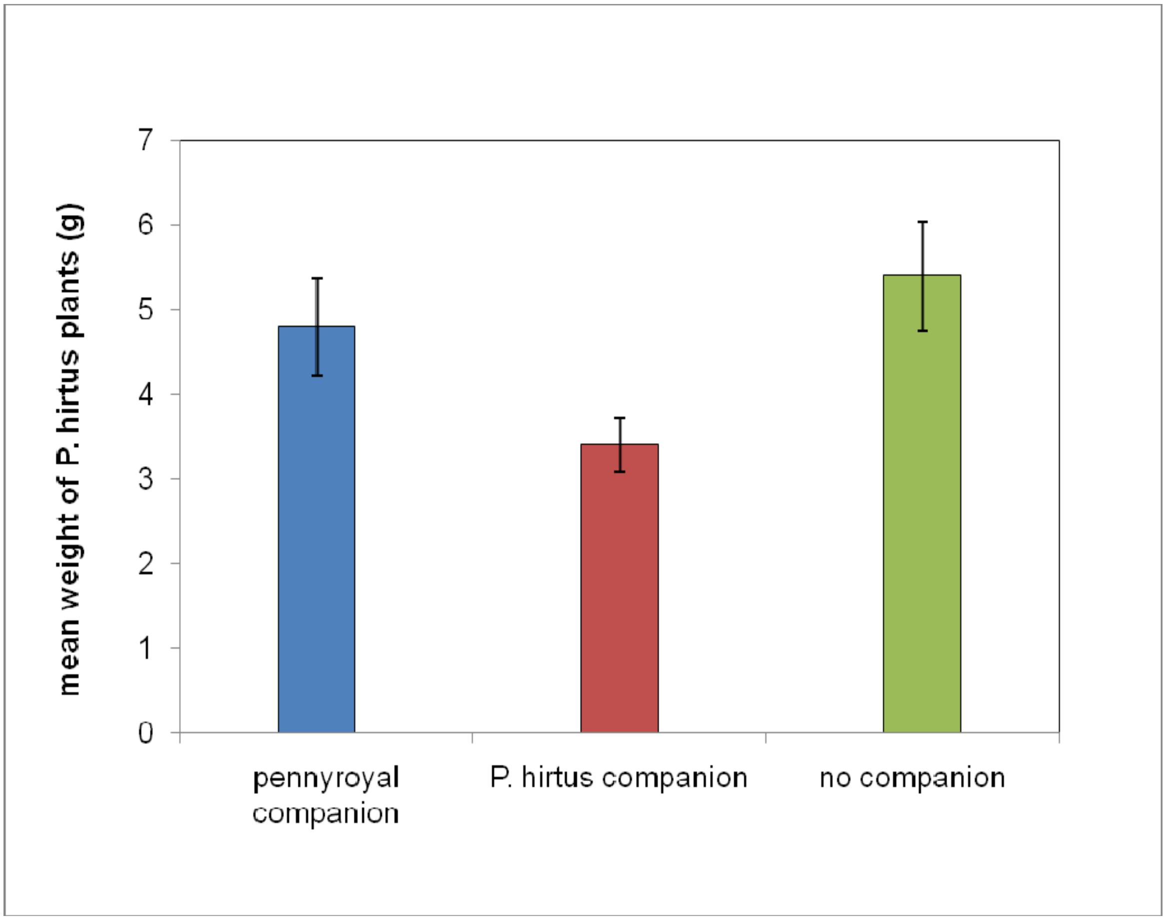

Figure 12. When grown on native soil, conspecific plantings of *P. hirtus* produced the smallest size plants.

Discussion

Our results indicate that pennyroyal has a detrimental effect on germination and growth of *Plagiobthrys hirtus*, one of our rarest native plants, and that this effect is due, at least in part, to allelopathy. Our documentation of pennyroyal's ability to secrete compounds which discourage the growth of neighboring plants is not surprising, as other studies demonstrate similar results for a wide range of invading weeds. Other studies evaluating the effect of weed extracts on seed germination and growth document negative effects of solutions produced by a variety of extraction techniques. Aqueous extracts of the ground roots of quackgrass rhizomes (*Elymus repens*) reduced germination of wheat seedlings, as well as shortened the length of roots and cotyledons of germinating seeds (Letourneau and Heggeness 1957). In a later study, solutions produced by extracting air-dried diffuse knapweed roots (*Centaurea diffusa*) with a series of organic solvents significantly reduced germination of rye grass seed, as well as inhibited seedling growth (Muir and Majak 1983). In an Indian study, watering bean seeds with a root leachate of the South American native plant *Parthenium hysterophorus* (false ragweed, an invasive weed in India) reduced bean seed germination and growth (Kanchan and Jayachandra 1979).

Pennyroyal's presumed toxic effects on other plants resulted in the inclusion of this species in a series of 430 plant species tested for use as botanical weed control agents (Solymosi 1994). As expected, pennyroyal was one of the 12 most active, and a crude extract of this plant was recommended as suitable for "direct practical application" for agricultural weed control. The negative effect of compounds released by pennyroyal on the germination of *P. hirtus* seeds, as observed in our study, may contribute to the this weed's success in invading wetlands previously inhabited by the rare species. Seeds of both taxa germinate without pre-treatment when wetted, placing them in direct competition with each other when autumn rains begin. Inhibited germination of seeds of *P. hirtus* places this species at a disadvantage early in the growing season, and allows available habitat to be colonized by the invading weed.

However, our results also indicate that, once germinated, plants of *P. hirtus* can survive in the presence of pennyroyal. In both years of our study, plants of *P. hirtus* grew larger in the presence of pennyroyal than they did when paired with a conspecific plant, suggesting that intra-specific competition may be as important to mature plants of this species as competition (and allelopathic interactions) from other species, even invasives such as pennyroyal. The importance of intra-specific competition to the population dynamics of another endangered wetland plant (*Scirpus ancistrochaetus* - northeastern bulrush) has also been documented. In this study, plants emerging in high densities grew poorly, especially under high nutrient regimes (Lentz 1999). The reduction in plant growth due to intra-specific competition among plants of *P. hirtus*, combined with low germination due to allelopathic effects of pennyroyal, may be the cause of the low numbers and decreased size of *P. hirtus* plants observed at the NBHMA in some years.

Additionally, in the first 'low nutrient' year of our study, the removal of allelopathic chemicals by the addition of carbon to the cultivation medium resulted in a greater increase in growth of plants of *P. hirtus* than can be explained by the positive effect of carbon and the relative competitive ability of the rare species against pennyroyal. Although mature plants of *P. hirtus* competed well against pennyroyal under the conditions present in our study, allelopathic chemicals produced and released into the growing medium by pennyroyal also played a part in the interaction between the weed and native plant, decreasing the potential size increase of the native. The ecological value of the increase in size of plants of *P. hirtus* that is eliminated by pennyroyal's allelopathic effect is unknown, and may be important under some environmental conditions.

In the second year of the study, in which plant cultivation methodology included a higher nutrient regime, the positive effect of carbon was no longer present. The positive effect that occurred under low nutrient conditions was probably due to the small amount of nutrients provided by the carbon itself – this small effect was no longer measureable when more nutrients were available. Because the effect of carbon itself was not present in the second year of the study, *P. hirtus* plants grown with conspecific companions on amended and un-amended soils were not significantly different in size. However, *P. hirtus* plants grown with pennyroyal on amended and un-amended soil did differ, with the increase in plant growth in the carbon-added medium due to the removal of allelopathic effects. These results corroborate those of our 2007 evaluation, and demonstrate that allelopathy contributes to the interaction between pennyroyal and *P. hirtus*.

This second study again documents the effect of intra-specific, relative to inter-specific, interactions on the growth of *P. hirtus*. Under both high and low nutrients regimes on artificial media, and when grown in native soil mix, plants of *P. hirtus* grew better when paired with pennyroyal than when paired with a plant of their own species. Intra-specific competition affects other annual, biennial and short-lived perennial plants, with reductions in biomass (Mack and Harper 1977), plant size (Waller 1981), and survival (Suzuki et al. 2003) attributed to the negative attributes of growing near conspecific neighbors.

Like the germination of *P. hirtus* seeds, germination of seeds of *Perideridia erythrorhiza* was inhibited by treatment with pennyroyal root extracts. However, the anti-fungal properties of these extracts (Deans 2006) confounded evaluation of these inhibitory effects. Seeds watered with distilled water germinated poorly due to excessive mold infestations, while those watered with concentrated extract (from ground roots) also germinated poorly due to inhibition by components in the extract. Seeds watered with the less concentrated extract from whole roots benefited from the reduction in mold, and did not experience the negative effect of the concentrated root extract, promoting germination at the highest rate.

Due to the ability of activated carbon to sequester allelopathic compounds produced by exotic plants and reduce their effects, this substance has the potential to reduce weed growth under field conditions (Kulmatiski and Beard 2006). When tested in plots dominated by *Bromus tectorum* (cheat grass) and *Centaurea diffusa* (diffuse knapweed), the addition of activated carbon at a rate of 1% by mass in the top 10 cm of soil reduced the cover of these two exotics. Activated carbon treatment also increased the cover of many native species, although this effect was not always consistent. Because allelopathic chemicals produced by pennyroyal reduced germination and growth of *P. hirtus*, addition of activated carbon to field sites that support these two species has the potential to reduce cover of the weed, with a subsequent increase in the native.

Complex biotic and abiotic interactions are the source of observed annual changes in *P. hirtus* densities at Douglas County field sites (Maddux et al. 2006), and these interactions also probably determine temporal changes in the extent of pennyroyal colonization. Native wetland plants are adapted to the wide temporal variation in abiotic conditions characteristic of vernal pools (Noe 2002), and generally recover from short-term population declines. However, several years of sub-optimal conditions, combined with the added stresses associated with pennyroyal colonization, could contribute to an increased probability for extirpation or extinction for rare plant populations. The size and vigor of pennyroyal populations peaked at the NBHMA sites in 2005, and *P. hirtus* and *Perideridia erythrorhiza* populations exhibited an all-time low during this and the following year. Fortunately weather conditions in 2006 - 2008 did not favor further pennyroyal colonization, and populations of both native species increased. At the Westgate site, the introduced population of *P. hirtus* has now returned to its pre-2005 size, (with a record number of plants recorded for 2008) and *P. erythrorhiza* plants are again growing and reproducing. However, despite the fortuitous recovery of the created populations in this site, pennyroyal infestations continue to have the potential to threaten the long-term viability of both created and naturally occurring populations of native wetland plants.

Summary

- Extracts of pennyroyal roots reduced germination and seedling vigor of *P. hirtus.*

- Allelopathic chemicals produced by pennyroyal affected growth of *P. hirtus*.

- Under the conditions used in our study, plants of *P. hirtus* grew larger when grown with a companion planting of pennyroyal than when grown with a conspecific associate.

- Extracts of pennyroyal roots reduced germination of seeds of *Perideridia erythrorhiza*, although these extracts also reduced mold growth, confounding evaluation of their effect on seed germination.

- Pennyroyal potentially reduces viability of created and naturally occurring populations of *P. hirtus*, although the interactions between the two species are complex.

- Addition of activated carbon to wetland field sites has potential to reduce the negative effects of pennyroyal on *P. hirtus* and improve viability of native wetland vegetation.

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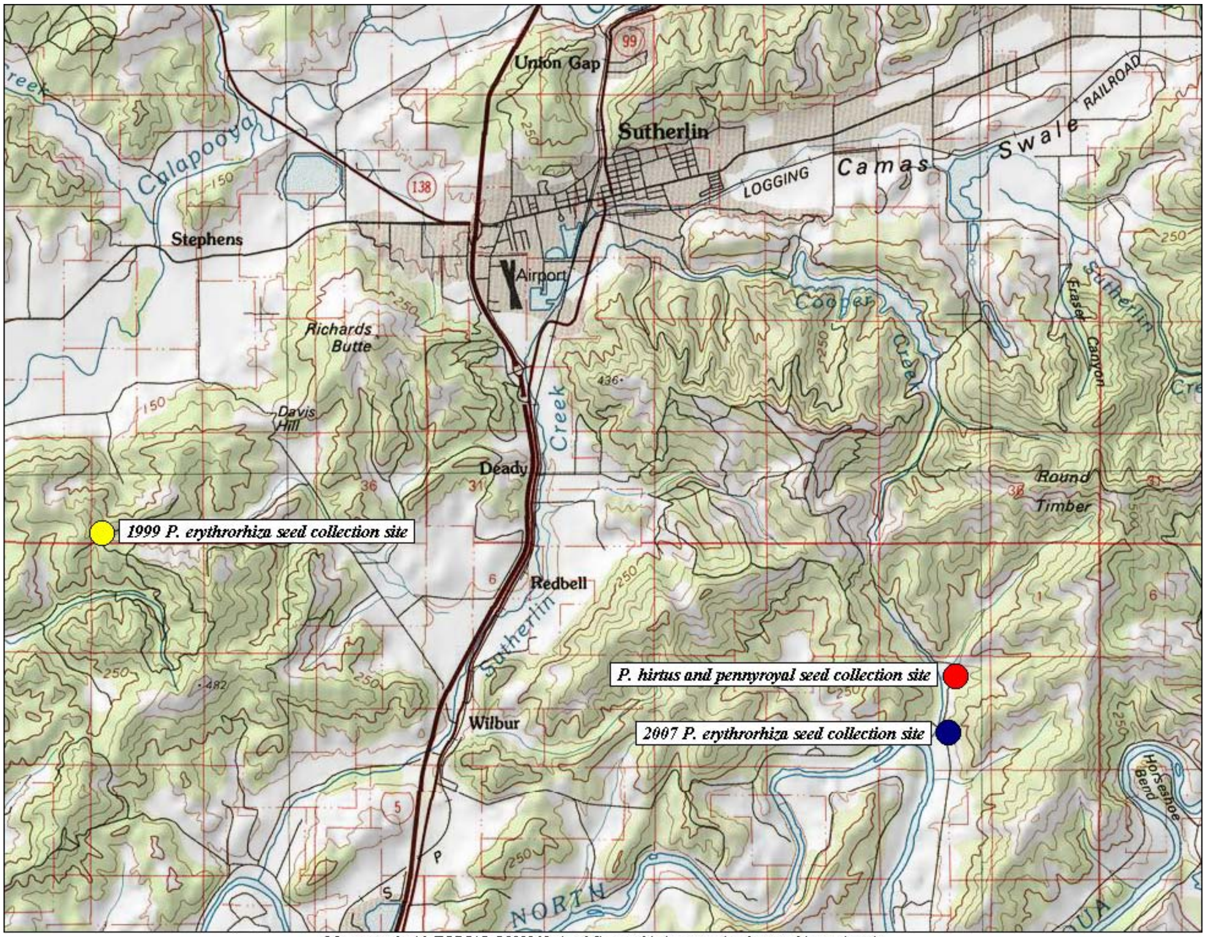
Appendix



Seed collection sites in Douglas County, Oregon, for seeds used in this study.