Phloroglucinol Root Staining: Testing the ability to age the roots of perennial pepperweed (*Lepidium latifolium)*.

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Background

Perennial pepperweed (*Lepidium latifolium),* a perennial herbaceous plant in the Brassicaceae family, is an invasive species known to create monocultures in a variety of habitats, including riparian areas (Renz 2002). The extensive underground root structures of this species can extend up to 3m per year from which pepperweed returns from dormancy each spring (Renz 2002, Blank & Young 1997). The age of perennial herbaceous plants, can often be determined based on the production of annual growth rings in stem and root structures (Dietz & Ullmann 1997).

For example, Dietz (2002) harvested roots occurring along transect lines from through the center of multiple patches of nonnative species to determine the age of a particular patch and ultimately the age distribution of a particular species. The age of a patch was determined by counting annual growth rings and the age distribution of the species was determined based on the position of the oldest and youngest individuals along each transect line from which a mode and rate of spread could be established (Dietz 2002).

Aging pepperweed through growth rings in underground root structures has not been tested. However, through the use of a root-staining procedure, five species in the Brassicaceae, including *Cardaria draba* and *Rorippa austriaca*, were found to have clear annual growth rings and could be aged effectively (Dietz & Ullmann 1997). In our attempt to understand the rate and mode of spread of pepperweed patches at the Cosumnes River Preserve we tested the reliability of ageing pepperweed roots using a phloroglucinol/HCL procedure and whether using stain-based methods to age pepperweed patches in the pepperweed control project test plots will be informative.

Methods

Four pepperweed root structures were collected from one patch at the Cosumnes River Preserve near the Visitors Center and processed two days later in lab facilities at UC Davis. A phloroglucinol/HCL root staining procedure (Forestry Suppliers) was used to age the roots. This procedure stains the lignin of the roots red and leaves other areas of the root, composed mainly of cellulose, unstained.

Collected roots were cut into five, 3-5mm thick sections, washed with water and dried. The root sections were then placed in a 1% phloroglucinol solution in 95% ethanol for about one minute. Samples were removed and excess phloroglucinol solution was allowed to drip off. Treated samples were then placed in 50% HCL until they began to turn red in color. Finally, samples were rinsed in water and dried (Forestry Suppliers).

Root rings of dried samples were examined using a hand lens and the determined age of each tested root was recorded.

Results

The effectiveness of the phloroglucinol procedure used to stain roots was inconclusive (Table 1). All samples were processed using the same protocol; however some root sections were dyed entirely red, while others did not retain the dye as the samples dried. Many samples were dyed red on their exterior woody stems, the most lignin rich, while the remaining parts of the sample remained unstained (Fig. 2 &3). Other samples dyed red in all places except for the pith of the root section (Fig. 1). These results were not linked to any particular root specimen but instead affected a proportion of samples from each root.

Regardless of the effectiveness of the HCL/phloroglucinol staining procedure, it was possible to delineate growth rings in the root samples, using a hand lens or the naked eye. Of the four plants sampled one was determined to be one year old, one two years old and two of the samples were determined to be three years old (Fig. 1, 2 & 3). The determined age of the patch, based on those four samples, is three years old (Table 1).

Figure 1. Root B: three years of age, stained red in all areas except pith of stem.

Figure 2. Root C: three years of age, stained slightly on outer woody section.

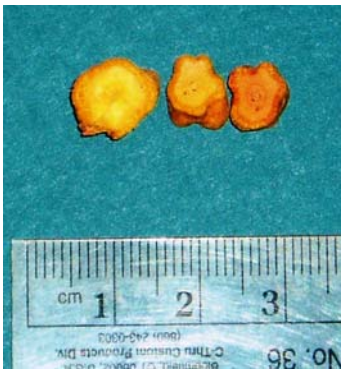

Figure 3. Root D: one year old, slightly stained on outer woody section

Table 1. All root sections with determined age and level of effectiveness of HCL/phloroglucinol stain (red pigment).

Root #	Determined Age	Level of red pigment
1a	**2**	**None**
2a	**2**	**None**
3a	**2**	**Outer lignin stained**
4a	**2**	**None**
5a	**2**	**Outer lignin stained**
1b	**3**	**None**
2b	**3**	**All areas stained excluding pith**
3b	**3**	**Outer lignin stained**
4b	**3**	**None**
5b	**3**	**None**
1c	**3**	**None**
2c	**3**	**All areas stained excluding pith**
3c	**3**	**All areas stained excluding pith**
4c	**3**	**Lightly stained in lignin rich areas**
5c	**3**	**None**
1d	**1**	**All areas stained**
2d	**1**	**Lightly stained in lignin rich areas**
3d	**1**	**Lightly stained in lignin rich areas**
4d	**1**	**None**
5d	**1**	**None**

Discussion

The root structures of the pepperweed stems tested were large enough and had clearly defined growth rings to enable us to accurately age the stems independently of the HCL/phloroglucinol procedure. While some root segments stained "correctly" and enhanced the visibility of individual growth rings, the variability of the results decreases

the likelihood that we would use or recommend usage of this procedure in the future. It is possible that either procedural error or inapplicability of the staining with our particular species is to blame for the ineffectiveness of this procedure. This procedure may also be unnecessary for larger perennial species with well developed root structures, especially when yearly growth rings are commonly visible with the naked eye.

Accurately determining the age and age-distribution of a pepperweed patch would enhance the understanding of how a patch expands (Dietz 2002). Our ability to age each individual root section gives us confidence that, if we were able to exhaustively sample a patch or weed occurrence, we would not only be able to age any pepperweed stem but determine both the age of a patch and the age distribution of the species.

The four root samples collected for this study were all from the same patch and while we determined that the oldest collected individual was three years of age, that individual's age does not accurately reflect the age of that patch. If each individual root structure were sampled from within this patch, we may have discovered that the true age of the patch was older than the oldest individual in our initial sampling. In order to accurately age a patch, extensive destructive sampling must take place along a transect line or through other sampling techniques (Dietz 2002).

To adequately sample pepperweed control project populations at the Cosumnes River Preserve would include at least ten samples from each of our six experimental sites. Sample populations would include small populations (less than 25 individual stems), medium populations (25-500 individual stems) as well as large populations (>500 individual stems). Sampling procedures of this nature are not practical while utilizing control methods and monitoring population growth of pepperweed at the preserve.

The scope of work requires that if we determine the aging process to be both accurate and useful, a sample of pepperweed control experiment plots should be aged by testing individuals "in the vicinity" of these plots. While the aging process itself is possible and useful, our success with the control project after one year of treatment inhibited determining the age of experimental plots as these plots are either devoid of pepperweed or have reduced populations. To accurately age a patch it would be necessary to remove a majority of the underground root structures. Aging the root structures "in the vicinity" of the test plot would not accurately reflect the age of the test plot or the whole patch and was therefore determined to neither be useful or accurate.

This pilot study suggests that root-based aging is likely to be less effective for our purposes than survey and marking methods to determine the age structure of pepperweed patches. In rapidly expanding populations, like those at the preserve, it may be more valuable to try to determine a minimum and maximum age of the largest and presumably oldest patches. This method, combined with an inventory of populations at the preserve could help reconstruct a history of the initial invasion as well as an age distribution of a patch.

Literature Cited

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