Determining Nitrogen Fixation Levels in previously nodulated *Alnus maritima* plants using a commercial slow release fertilizer as a nitrogen source

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ABSTRACT

Alnus maritim is an endangered species that shows potential for use in the landscape, because it has the ability to fix its own N from the air by way of *Frankia* bacteria in root nodules. This study was conducted to determine if a commercially available, 3-4 month slow release fertilizer would inhibit N-fixation and further nodulation in these plants. If the alders were able to do so, chances of survival when out-planted would increase and N fertilizer would not need to be applied to them, thus helping the environment. Plants were grown under greenhouse conditions and fertilized at rates ranging from 0.0-32.0 g fertilizer. The experiment lasted for 20 days and data was collected by measuring root and stem/leaf dry weights and counting nodules. There was a marked increase in nodule numbers per plant from the beginning to the end of the experiment. This increase may suggest that N-fixation was occurring. Further, plants grown at lower rates of fertilizer grew almost as much as those grown at higher rates, which may also suggest that N-fixation occurred due to the similar growth rates.

INTRODUCTION

Frankia, a type of bacteria known as actinomycetes, fix atmospheric N_2 in root nodules of plants classified in eight families of angiosperms (Clawson et al., 2004). These soil borne bacteria do not require a host plant to survive (Batzli et al., 2004) but will form relationships with actinorhizal plants (non-leguminous N_2 fixing plants) when present. The bacteria are especially common in newly formed soils and in soils of marginal quality (Clawson et al,. 1997). Plants that utilize this symbiotic relationship are also able to colonize these same soils making them valuable in soil restoration and improvement. These plants also add usable N to the natural environment (Ekblad, Huss-Danell, 1995: Pashke, 1997).

Actinorhizal plants should be used more in the home landscape. Many of these plants have characteristics that equal or exceed the beauty of non-actinorhizal plants, and have lower N requirements than non N-fixing plants. Most commercial plant growers intensively fertilize with N to maximize plant growth, but this can leach unused nitrate fertilizer into the environment. If more actinorhizal plants were produced, fertilizer use could be reduced, which helps the environment and hopefully will reduce costs to growers.

Alnus, an actinorhizal genus, includes species that are already commonly grown in the home landscape. They generally nodulate as well as or better than other actinorhizal species (Ekbald and Huss-Danell achieved a 96% nodulation rate) and have a fast growth rate. These qualities not only make them nice landscape plants, but also make them a model plant for use in actinorhizal research.
Many studies have been preformed with alders to test the effects of N on root nodualtion and N production in nodules. It is generally accepted that when sufficient

levels of N are present in the soil, nodulation is inhibited in actinorhizal species (Gentili and Huss-Danell, 2003: Gentili and Huss-Danell, 2002: Laws and Graves, 2005). Laws and Graves (2005) also tested the effects of different levels of N on an endangered tree species, *A. maritima*, using ammonium nitrate (NH_4 NO_3) ranging in concentration from 0.0 mM-8.0 mM. They concluded that 0.25mM-4.0mM solutions of NH_4 NO_3 were best suited for nodule production, even though more nodules were produced when no N was added. They stated that this treatment had weaker stems than others with added N and thus was not acceptable. All alders were given a modified Hoagland solution minus N to ensure nutrient deficiencies from other nutrients were eliminated.

To make the experiment more applicable to commercial growers, I conducted a similar study to that of Laws and Graves (2005), but changed the hypothesis to state that N from a complete, commercially available, slow release fertilizer will not inhibit N production by *Frankia* bacteria in previously nodulated *A. maritima* plants. Further, the slow release fertilizer will also not prevent further nodulation in these plants by the bacteria. The commercial fertilizer Osmocote® was used as the N source, because this fertilizer is widely used in the nursery industry.

MATERIALS AND METHODS

Fifty consistently sized alders growing in 1-gallon nursery pots were selected for this study. Each was grown in 1:1 peat moss/perlite planting medium and inoculated with soil containing *Frankia* bacteria. Liquid fertilizer was withheld for two weeks before the start of the experiment to encourage root nodulation.

Prior to treatment application, 5 plants were randomized using the S.A.S. program and placed into 8 treatments with 5 plants per treatment. The treatments received fertilizer at levels of 0.0, 0.5, 1.0, 2.0, 4.0, 8.0, 16.0, or 32.0 grams per pot. At the beginning of the experiment, 5 plants were randomly selected for destructive harvest to obtain stem/leaf and root dry weights, and an average nodule count per plant. Five unused plants were put back into stock population. To simulate the alders' naturally wet environment, plants were irrigated 4 times a day a with a drip system connected to tap water that delivered approximately 800 milliliters per application. To prevent deficiencies of other nutrients, all plants were irrigated weekly with 800 milliliters of modified, ¼ strength Hoagland solution containing no N.

Nitrate tests were performed on leachate from one plant in each treatment two weeks into the experiment and at the end to verify that the slow release fertilizer was working as expected. At the end of the experiment, 2 plants from each treatment were destructively harvested to obtain leaf/stem and root dry weights. Nodules were also separated, counted and dried from one harvested plant per repetition. Acetylene reduction a standard experimental practice for measuring N-fixation, was also attempted to determine if root nodules were fixing N, but could not be completed because both gas chromatographs available for use were not set up for acetylene detection. (Chisholm and Moulin, 2003.; Gauthier *et al*, 1981.)

RESULTS

A positive indication that plants were fixing N was the general increase in the average number of nodules. At the beginning of the experiment an average of 26 nodules were present and at the end the average was 146 (see Table 1). This also supports the second part of the hypothesis that fertilizer would not prevent further nodulation.

Dry weights of destructively harvested plants were also taken at the beginning and end of the experiment. All alders increased in root weights and leaf/stem weight from the beginning to the end of the experiment, but plants generally did not grow in proportion to how much fertilizer was applied at the beginning of the trial (Fig. 1). This fact may support the theory that N-fixation occurred, because of the plants that received no or low amounts of fertilizer grew almost as much as plants that had applied fertilizer at rates that far exceeded recommended levels.

Due to the many difficulties encountered during this experiment, not all desired data could be obtained. One example of this is that it could not be determined if N-fixation was prevented by excess fertilizer, because acetylene reduction could not be calculated due to technical difficulties with the G.C. If the procedure had worked, *Frankia* bacteria would have absorbed the acetylene gas and reduced it to ethylene, which could have been detected by the G.C.

Nitrate was released as expected by the slow release fertilizer, but it was surprising to see how much nitrate levels declined over a twenty-day period of time (see graph 2). This would suggest that nurseries may want to re-fertilize every 3 months instead of 4 with this particular fertilizer. Even though this decline happened, no plants experienced visible nutrient deficiencies including N. This is especially encouraging, because it may mean that the treatments that were well below the recommended fertilizer application rates were fixing N.

DISCUSSION

Many mishaps occurred during this study. At the beginning of the experiment the alders were not supposed to have nodulated but did so unexpectedly. Another problem that occurred was that leachate nitrate levels were consistently too high for the amount of N that should have been released from the different levels of fertilizer. After much consternation, it was found the drip system had been connected to the house nutrient solution instead of tap water. The experiment was restarted two weeks later to allow all residual N from the nutrient solution to leach out of the potting medium. Nitrate tests were repeated at this time, and the results were more consistent with where they should have been.

Even though there were many difficulties, some promising preliminary results were obtained that merits a follow-up experiment in the future, with some modifications. Much younger asexually propagated plants should be used to eliminate genetically variable in growth rates. G.C. methods should also be improved before repeating, and it is imperative not to assume that the alders have not nodulated before repeating.

Another reason to further research in this area includes environmental improvement. If more N-fixing plants were utilized, the use of chemical fertilizer could be reduced in commercial production and the landscape, which would reduce the likelihood of environmental contamination from fertilizers.

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TABLE 1	
Nodule counts from 1 plant per treatment destructively harvested at experiment end.	
0	160
0.5	160
1.0	182
2.0	110
4.0	90
8.0	205
16.0	180
32.0	80
Mean	145.88
Std Dev.	16.20
Nodule count for 5 plants destructively harvested at the beginning of the experiment.	
1	21
2	25
3	5
4	55
5	24
Mean	26
Std. Dev.	43.46

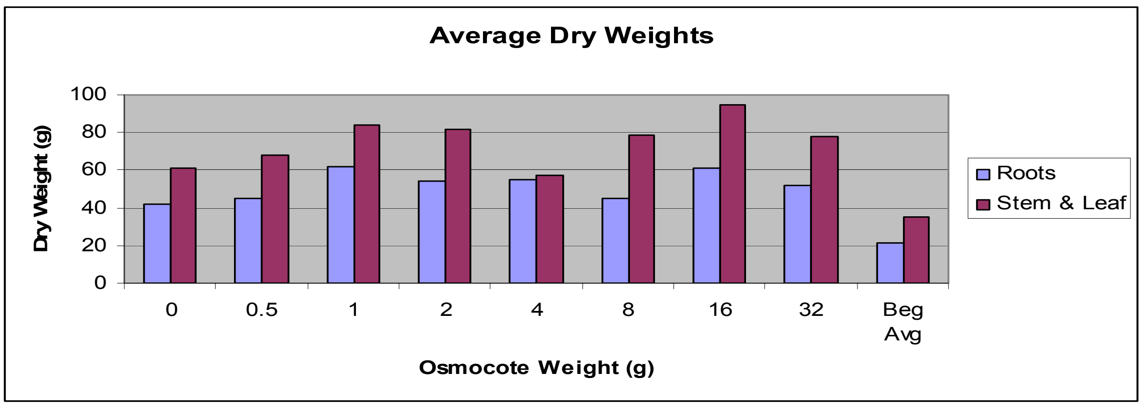

Figure 1

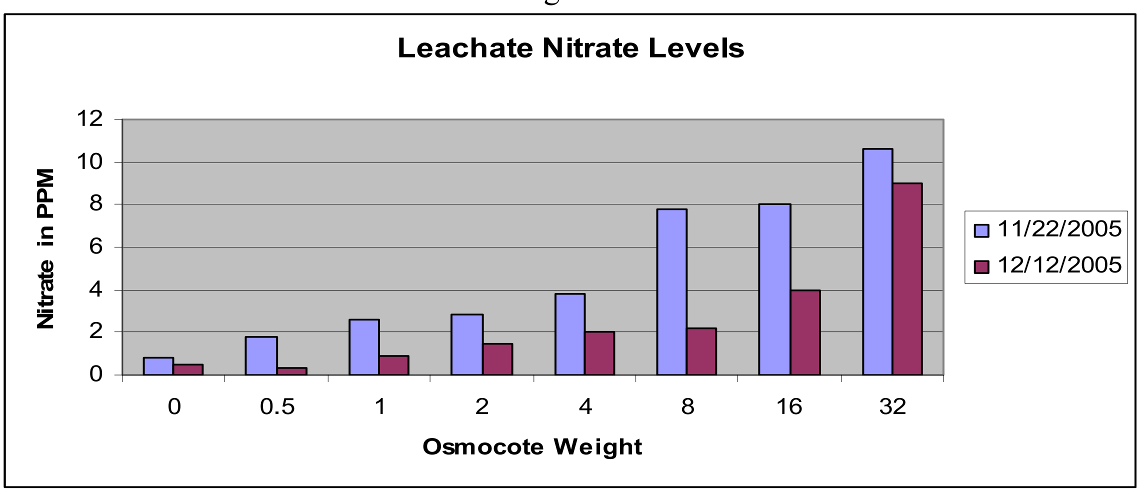

Figure 2