MONITORING ENVIRONMENTAL STRESS IN FOREST TREES USING BIOCHEMICAL AND PHYSIOLOGICAL MARKERS[1]

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ABSTRACT

Our objective was to determine the usefulness of polyamines, particularly putrescine, and amino acids such as arginine, as foliar indicators of abiotic stress in visually asymptomatic trees. An evaluation of apparently healthy trees is essential in developing risk assessment and stress remediation strategies for forest trees prior to the onset of obvious decline. Previous research by our group established a positive correlation between putrescine in red spruce foliage and Al:Ca ratios in the forest soil. A positive correlation was also observed between foliar putrescine and nitrogen deposition in the soil in pine, maple, and oak at Harvard Forest, MA. Preliminary data further show that the free amino acids, especially arginine, increase several fold in response to high nitrogen input, indicating that foliar arginine could also be used as a useful marker of excess nitrogen inputs in otherwise nitrogen-limited forest soils. This research is a part of several multi-institutional, long-term interdisciplinary research projects that are aimed at assessing the current and future ramifications of exposure of several conifer and hardwood species to various stressors including soil nutritional imbalances, storm injury, pests, and pathogens. The study involves a cooperative effort among physiologists, ecologists, pathologists, and hydrologists, and provides links between tree function and environmental disturbances.

INTRODUCTION

Acidic deposition has been cited as a major cause of reduced forest productivity (forest decline) within northeastern United States. It has been postulated that this decline in productivity

[1] Scientific contribution number 2169 from the NH Agricultural Experiment station.

is associated with the amount of introduced acidity (NO_X and SO_X compounds) from atmospheric deposition and fertilizer usage that can far exceed the rates of acid buffering by the natural weathering of minerals in the soil. This, in turn, results in lowering of the soil pH (van Breemen 1985) and solubilization of aluminum which has a variety of deleterious effects on plant growth.

Aluminum, bound as oxides and complex aluminosilicates, is the most abundant metal in the earth's crust. Until recently, surface water concentrations of Al ion (Al^{3+}) have remained minimal due to the insolubility of aluminum hydroxide complexes at neutral pH (Macdonald and Martin 1988). The lowering of soil pH due to acidic deposition or excess fertilization leads to the solubilization of otherwise insoluble Al in the mineral soil horizon. Aluminum moves up from the mineral to the organic soil horizon through water movement and biocycling (Lawrence *et al.* 1995). Here it competes with Mg and Ca on soil particle exchange sites, leading to leaching of these nutrients into the mineral soil and eventually into surface waters. Besides causing indirect effects on plant metabolism through nutrient deficiencies, Al also has direct toxic effects on plant growth (Schier *et al.*, 1990).

In addition to increased Al solubility, there are other concerns about the impacts of increasing levels of N from acidic deposition and excessive fertilization in acidic soils. Elevated rates of N deposition can cause potentially adverse effects on water quality and the health of forested ecosystems. Since the United States Clean Air Act was revised in 1990 to target a 50% reduction in S deposition but only a 10% reduction in N deposition, these concerns have been heightened. Although most temperate soils are N limited, continuous deposition of N from the atmosphere can move them towards N saturation (Aber *et al.* 1989). Nitrogen saturation has been defined as the availability of ammonium (NH_4^+) and nitrate (NO_3^-) at rates in excess of total combined plant and microbial nutritional demand (Aber *et al.* 1989). If N deposition rates remain high, the excess N may lead to N saturation and reduced plant growth through major changes in C and N metabolism, including the sequestration of N compounds in leaves and lower photosynthetic rates. It is important to understand how chronic additions of N to agricultural and forested ecosystems may change their structure and function.

Forest trees are constantly exposed to many natural and anthropogenic stressors. Negative effects of acidic deposition on soil and forest productivity due to soluble Al, leaching of nutrients, or excess N may be subtle and slow to appear. This issue is of major concern to forestland managers because such processes may impact growth and productivity over large areas. A major long-term goal of our research is to develop a set of early physiological and biochemical markers of stress in trees before the appearance of visual symptoms. Our specific objective is to determine the usefulness of polyamines, particularly putrescine, and amino acids such as arginine, as foliar indicators of stress in visually asymptomatic trees. Specific stressors being investigated include perturbations in soil chemistry attributed to acidic deposition (N saturation, nutrient depletion, and Al mobilization), silvicultural management practices (such as thinning), and ice storm injury. The evaluation of apparently healthy trees is essential in developing risk assessment and stress remediation strategies for forest trees prior to the onset of obvious decline.

Aliphatic polyamines (putrescine, spermidine, and spermine) play an important role in the growth and development of all living organisms. They are metabolically derived from the amino acids arginine and ornithine, and at cellular pH they carry a net positive charge (Cohen 1998). Polyamine metabolism is an important link between N and C metabolism in plants. Abiotic stress conditions such as low pH, high SO_2, high salinity, osmotic shock, nutrient stress, low temperature, high Al, and chronic N deposition all result in increased in cellular putrescine levels (Flores 1991; Minocha *et al.* 1992, 1996, 1997, 2000; Bouchereau *et al.*, 1999). Polyamine concentrations have an inverse correlation with the cellular levels of inorganic cations such as Ca, Mg, Mn, and K in response to Al treatment (Minocha *et al.* 1992, 1996, Zhou *et al.* 1995).

Long-term elevated N deposition typically increases the concentration of total foliar N, with or without similar changes in the base elements such as Ca, Mg and K (Aber *et al.* 1995, Magill *et al.* 1997, 2000, van Dijk and Roelofs 1988, Rasmussen and Wright 1998). This increase in leaf N content also leads to significant shifts in the internal partitioning of N within the leaf. For example in conifers, N deposition significantly increases leaf N present in the form of free amino acids such as arginine (Ericsson *et al.* 1993, 1995, Näsholm *et al.* 1997).

MATERIALS AND METHODS

Field sites and treatment applications

Information about the various field sites, including treatments, foliar and soil sample collection protocols, land-use history, and species composition has been published previously (Minocha *et al.* 1997, 2000, Wargo *et al.* 2002). At the Hubbard Brook Experimental Forest site, NH, the treatments were: 160 kg Ca alone, or 100 kg N or 160 kg Ca + 100 Kg N ha^{-1} Yr^{-1}, hand broadcast three times during the growing season. Treatments began in 1992 and ended in 2000. There were three plots per treatment (total 12 plots) and ten trees per plot were sampled in 1997, 1999, and 2000.

Soluble foliar polyamines and inorganic elements

Foliage samples were removed from branches in the upper to mid-canopy of five to twenty dominant and co- dominant trees per plot depending upon the site. For hardwood samples, two or three healthy leaves were collected from each tree and were wiped with clean Kimwipes to remove surface contaminants. 60 to 75 discs (6 mm in diameter) of leaf tissue containing no major veins (about 200-300 mg fresh wt) were punched from leaves and placed in individual pre-weighed microfuge tubes containing 1 ml of 5% perchloric acid. For conifers, needles were finely chopped and placed in individual pre-weighed microfuge tubes containing 1 ml of 5% perchloric acid. Samples were kept on ice in the field and during transportation to the laboratory where they were stored at -20°C until processing. Samples were processed according to the procedures described by Minocha *et al.* (1990, 1997). Exchangeable foliar elements and free polyamines were extracted using the freeze-thawing method (Minocha and Shortle 1993, Minocha *et al.* 1994, 2000). Calcium was extracted with 1 M ammonium acetate and analyzed using ICP as described in Long *et al.* (1997).

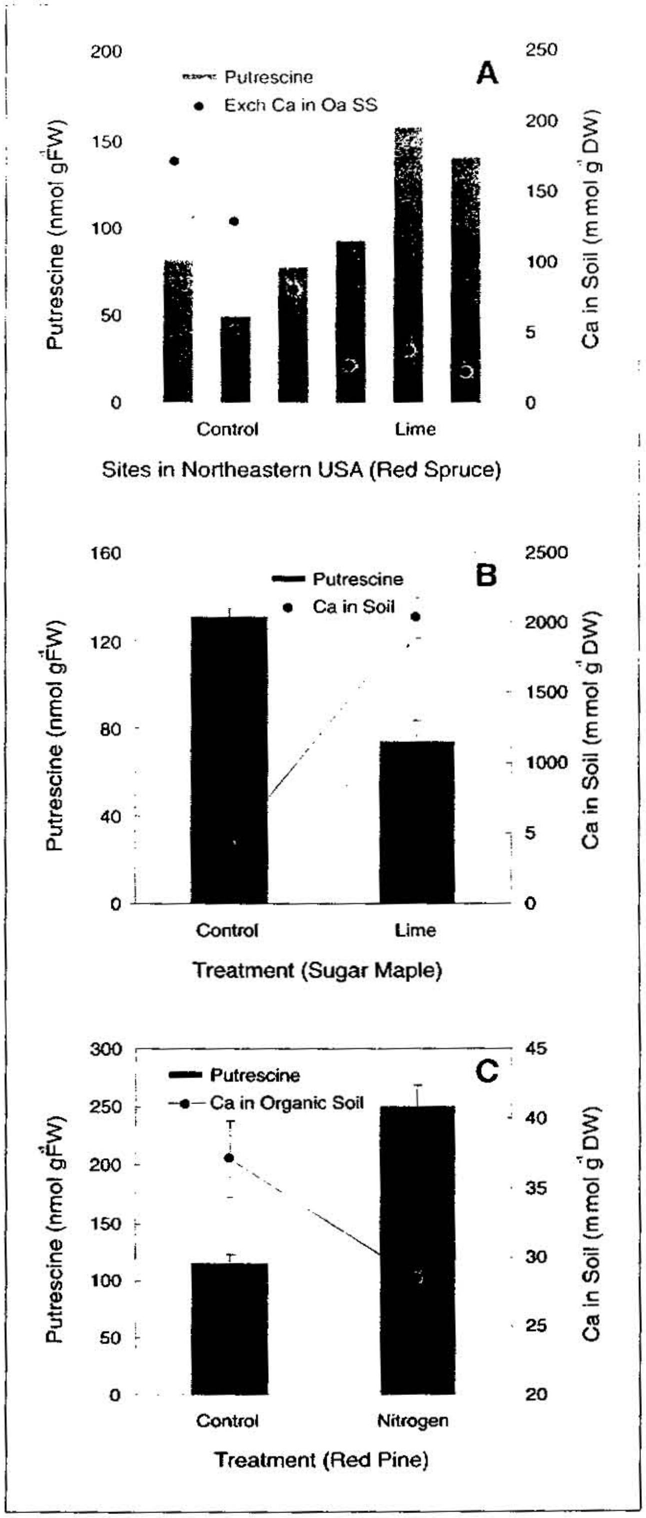

Fig. 1. A: Relationship between exchangeable Ca in soil solution of organic soil and foliar putrescine concentrations in red spruce trees growing at six sites across the Northeastern USA. R = 0.6375. **B:** Impact of soil liming treatment (one application of 22.4 Mg.Ha^{-1} of dolomitic lime in 1985, for details on study design see Long *et al.*, 1997) on exchangeable Ca in organic soil and foliar putrescine in declining sugar maple stands on the Allegheny Plateau, PA, USA. The treatments were significantly different at a = ≤0.05. **C:** Effects of chronic N addition (as ammonium nitrate) on exchangeable Ca in trees at Harvard Forest, MA, USA. The treatments were significantly different at a = ≤0.05. For details on these studies, see Minocha *et al.* (1997, 2000) and (Wargo *et al.* 2002). Note the inverse correlation between foliar stress indicator (putrescine) and calcium in soil in three different tree species under different growth conditions.

Results and Discussion

An inverse relationship between Ca levels in the soil solution from Oa horizon and foliar putrescine was observed in red spruce trees growing at various sites across the Northern New England (Fig. 1A, Minocha *et al.* 1997). This decrease in soil Ca was often accompanied by increasing soil Al levels at these sites. Putrescine has also been used successfully as a marker of stress remediation from nutrient imbalance by the addition of lime in sugar maple stands in Pennsylvania (PA), USA (Fig. 1B). Addition of N in the form of ammonium nitrate caused a significant increase in foliar putrescine accompanied by a decrease in Ca in the Oa horizon of soil in the red pine stand at Harvard Forest, MA (Fig. 1C).

A positive correlation between foliar putrescine and high N deposition in the form of ammonium nitrate was also seen in mature pine, spruce, maple, and oak trees at Harvard Forest, MA, Hubbard Brook Experimental Forest, NH, and Big Moose and Delaware River Basin, NY, USA (Minocha *et al.* 2000, unpublished data). Preliminary data show that free amino acids, especially arginine, in the foliage, increase several fold in response to high N input (Fig. 2), indicating

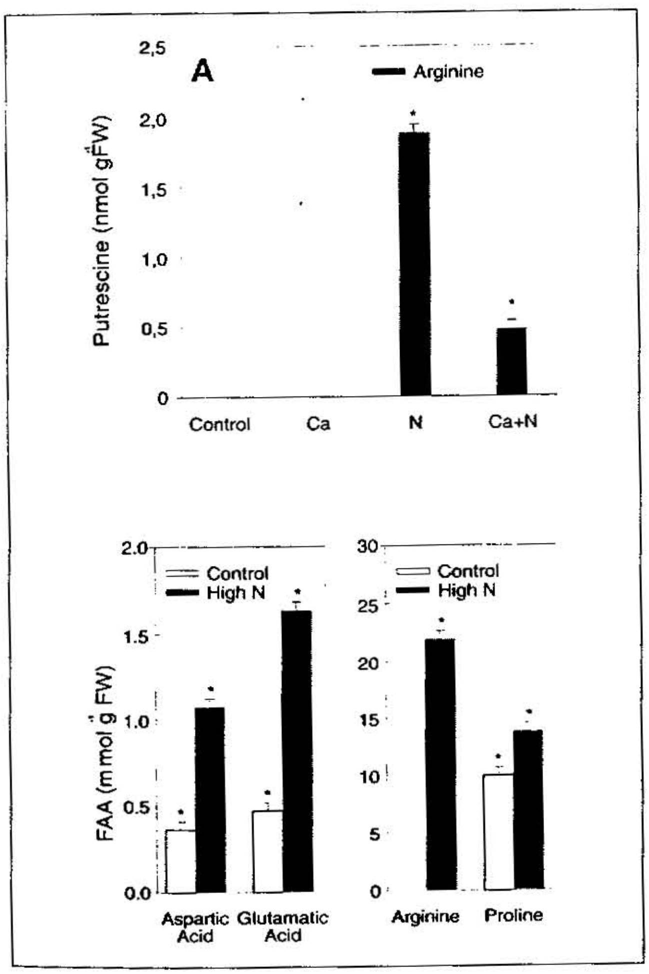

Figure 2. Effects of chronic nitrogen addition (as ammonium nitrate) on foliar arginine levels growing on soil supplemented with Ca and N. **A:** Red spruce trees at Hubbard Brook Experimental Forest, NH. **B:** red pine trees at Harvard Forest, MA, USA. a = ≤ 0.05.

that arginine could also be used as a potential marker of excess nitrogen inputs in otherwise nitrogen limited forest soils. Other significant effects of acidic deposition on the physiology of red spruce that are currently under investigation in our laboratory include changes in organic acid metabolism, membrane properties, chlorophyll and protein content.

In conclusion, based upon our extensive work using both cell cultures and mature conifer and hardwood trees in the field, we have proposed that putrescine could be used as a potential biochemical indicator of stress in forest trees. Our results show a strong correlation between soil nutrient deficiencies (e.g. Ca) and increased foliar putrescine levels, indicating that in conjunction with soil chemistry, foliar putrescine can potentially be used as a marker of general stress in visually healthy trees (Minocha *et al.* 1996, 1997, 2000, Wargo *et al.* 2002). We anticipate the development of an extensive database on biochemical changes (including levels of polyamines, amino acids, chlorophyll, proteins, etc.) in foliage and their relationship to environmental factors that could greatly enhance forest health monitoring programs and other forest management strategies. In recent years our ongoing research partnerships have expanded to include researchers from several Northeastern states, universities, and federal agencies in USA and other countries such as Korea, Japan, and India. This type of research depends heavily on the cooperation of physiologists, ecologists, pathologists, and hydrologists as well as ecosystem modelers who can integrate a wide variety of parameters into a working model of the environmental impact on forest trees. The research incorporates information about soil and soil solution chemistry, dendrochronology, dendrochemistry, and foliar physiology at species and forest stand levels.

Acknowledgments

The authors are thankful to all the collaborators, whose names are listed below in the reference list as coauthors in the joint publications, for providing their sites and/or soil data to compare with our foliar data on polyamines, amino acids and inorganic ions. Technical assistance of Benjamin Mayer, Kenneth Dudzik, and the field crew is also appreciated.

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