**TITLE:** Improving the interpretation of lichen biomonitoring for nitric acid and ozone air pollution in the detection monitoring program  
*Continuing Proposal – 3rd year*

**DURATION:** Yr 3 of 3-year project  
**FUNDING SOURCE:** Base

**PROJECT LEADER:** Dr. Pamela Padgett, PSW, Atmospheric Deposition Unit, 951/680-1584, ppadgett@fs.fed.us

**COOPERATORS:** Dr. Thomas Nash, Ms. Jennifer Riddell (Ph.D. Student), Arizona State University, Dr. Sarah Jovan, Portland Forestry Sciences Lab.  
**Sponsor:** Andi Koonce, Southern California Shared Services Area.

**PROJECT OBJECTIVES:**  
1.) Investigate the effects on the air pollutant, nitric acid, on lichen biology.  
2.) Improve the understanding of the difference between the effects of ozone and nitric acid on lichen biology  
3.) Identify the mechanisms for lichen decline in the presence of ozone and nitric acid.

**JUSTIFICATION:** Monitoring forest health requires that we not only assess the current status of the trees, but gauge the health of ecological processes and functions. The presence, absence and community composition of lichens are currently used by FHM as an indicator of air pollution impacts in forests. In the west where sulfur air pollutants are generally low, ozone (O₃) has been considered the primary deleterious pollutant. Therefore changes in lichen populations have been generally attributed to O₃, particularly in the semi-arid forests. However, this conclusion is not universally accepted. Advances in pollution monitoring have shown that O₃ and nitric acid (HNO₃) are co-contaminants. Recent research results have demonstrated that dry deposition of HNO₃ to vascular plants directly attacks the cuticle resulting in surface lesions. The effects of HNO₃ on lichen biology are largely unknown. Nitrogen (N) deposition in general is thought to affect the biological activities of all photosynthesizing organisms, but the acidity and oxidation behavior of HNO₃ may exacerbate damage due to O₃. Deposition of ammonia is also thought to contribute to changes in lichen populations, but as the source of ammonia is different from O₃ and HNO₃, we will address ammonia in a subsequent proposal.

**DESCRIPTION:**  
**a. Background:** Our overarching question is whether gaseous HNO₃ deposition predisposes lichens to be sensitive to O₃ or whether either HNO₃ or O₃ alone is sufficiently toxic to cause the well-documented decline in epiphytic lichens in the western US. In the early 1980’s oxidants, with an emphasis on O₃, were widely regarded as the probable cause of both lichen and ponderosa pine decline in southern California. Subsequently, very high N-deposition was documented in semi-arid environments across western US. In forests adjacent to urban centers the oxide forms of N gases predominate (HNO₃, HNO₂, NO, NO₂, and PAN) as automobiles are the primary source. Of the 5 gases listed, NO and NO₂ are precursors to the formation of the others and are only known to become toxic at concentrations much higher than typically found. Also, at fairly high concentrations PAN is known to be phytotoxic, but the effects on lichens are minimal. Both HNO₃ and HNO₂ are strong acids and may affect lichens in a manner similar to H₂SO₄, as derived from SO₂. Of the two N-gases, HNO₃ occurs in the highest concentrations. The occurrence in this region of high levels of HNO₃, a gas with a very high deposition velocity, has only recently been appreciated, and its effects on lichens are, as far as we know, completely unknown. Recent studies of vascular plant leaves have demonstrated that HNO₃ causes cuticular lesions, which, if also occurring in lichens, may allow greater penetration of O₃. Thus, our emphasis is on documenting the unknown effects of HNO₃ on lichens as well as poorly investigated O₃ effects, where the gases are examined individually and in combination under controlled and ambient conditions.
Both O₃ and HNO₃ are secondary pollutants created by the same photochemical processes from the same precursors, NO/NO₂ and volatile organic carbon. Although O₃ and HNO₃ have natural as well as anthropogenic sources, human activity has doubled the amount of N entering the global N cycle. Ambient concentrations of O₃ have increased globally from estimated background levels of 15 ppb, to more than 30 ppb. Highly polluted areas may have concentrations as high as 120 ppb. For HNO₃, average 12-h atmospheric concentrations greater than 2 µg m⁻³ are considered significantly elevated above background. Average 12-h concentrations of 45-50 µg m⁻³ have been recorded in our study area. The toxicity of O₃ to photosynthesizing organisms is well known; less is known about the toxicity of HNO₃, although some data suggest that HNO₃ may be even more deleterious than O₃ under some conditions. There are virtually no data on the interaction between O₃ and HNO₃.

Lichens as Biomonitor of Air Pollution: Lichen monitoring is an integral part of the Forest Health Monitoring Program. Just as canaries provide warnings of toxic gases to coal miners, so can the investigation of lichen communities provide information on potential deterioration of ecosystems stressed by air pollutants. Lichen species are differentially sensitive to air pollutants. The most sensitive species may become locally extirpated in urban areas or near industrial facilities, while a few very tolerant species will survive and even flourish. Except for SO₂, the mechanisms underlying this differential sensitivity are poorly understood.

In order to successfully understand lichen responses to a mixture of pollutants, it is critical to know to which factors lichens may be responding. In the case of southern California, we know that approximately half the epiphytic lichen species known to occur in 1913 have subsequently disappeared. Interpreting probable cause(s) is a complex exercise because different factors may be important in different locations. Are the lichens known to exhibit community variation along oxidant gradients truly responding to O₃ or are they responding to some covariate, such as the HNO₃ gas we know now to co-occur? Furthermore, local extirpation may simply occur with urbanization as habitats become destroyed or other factors come into play. We can never provide a complete explanation but we can make substantial progress by investigating factors, for which a major influence can be reasonably postulated. Given its extreme acidity, its very high deposition velocity relative to other air pollutants, and its comparatively high concentrations in southern California atmospheres, we believe that investigating the effects of HNO₃ and its potential interaction with O₃ is a logical next step in understanding the dynamics of lichen responses to air pollutants in southern California and elsewhere in the west.

b. Methods (please refer to year 1 for details): The project has 2 components; a controlled experimental laboratory fumigation study and a field transplant study in the San Gabriel Mountains east of the Los Angeles basin. The study will focus on two species, Ramalina menziesii and Hypogymnia imshaugii. A

c. Products: The main product will be an updated manual on physiological responses of lichens to O₃ and HNO₃. We expect the two pollutants to manifest themselves differently in the two lichen species, but if they do not – that in and of itself will be worth noting. We also expect the transplant study to guide future work on the difficulty of finding appropriate monitoring methods in extremely polluted areas such as the LA Basin. We anticipate 2 or 3 peer reviewed journal articles and presentations at professional meetings.

d. Schedule of Activities: Year 1: Fumigation studies, Year 2 Field transplant studies, Year 3: Data analysis, write-up and presentations.

e. Progress and accomplishments:

Year 2: Fumigations studies with O₃ alone were conducted in the winter and spring of 2007-2008, at very low, moderate (60 ppb) and high (120 ppb) levels, which are representative of chronic and acute levels experience in southern California. Experiments ran for eight weeks at a time, with O₃ levels rising in the morning around 9:30 am, and tapering off after 4:00pm in the afternoon. We found no differences
between control and O₃ treated *R. menziesii* chlorophyll and phaeophytin pigment contents, chlorophyll fluorescence, and membrane integrity. There was a slight difference between controls and high O₃ fumigation levels in respiration and photosynthesis, after 8 weeks of treatment. The results of these experiments suggest that O₃ alone may not have a strong effect on *R. menziesii*. We hope to fumigate more species with both O₃ and HN O₃ alone and in combination in the fall and winter of 2008 to achieve a broader understanding of how lichens respond to these pollutants.

In the summer of 2008, we conducted a field study resurveying 22 of the sites formerly surveyed in 1976 and 1977 (Sigal and Nash 1981) for lichen diversity and abundance on California black oak (*Quercus kelloggi* Newb.), ponderosa and Jeffrey pine (*Pinus ponderosa* Dougl., and *P. jeffreyi* Grev. & Balf.), and white fir (*Abies concolor* (Gord. & Glend.) Lindl. ex Hildebr) boles in three mountain ranges surrounding the Los Angeles basin. Our sites were co-located with Forest Inventory and Analysis style lichen surveys in order to glean more information from each site. The goal of this field work is to track changes in lichen communities over time in the Los Angeles (South Coast) air basin. We also collected bark and twig pH data for oaks, and bark pH data for conifers, and are attempting to determine if bark and twig pH are altered by the pollution regimes in the basin, and if changes in bark pH are driving lichen community composition. Data and samples are still being processed, thus we cannot report results at present.

A paper on the first fumigation experiments that took place in the winter of 2006 and spring of 2007 was published in the January 2008 issue of Flora (listed below). Posters of results to date including the summer 2008 field work was presented at the Graduates in Earth, Life, and Social Sciences symposium (January 2008), the FHM symposium in San Antonio (February 2008), at the 40th Annual Air Pollution Workshop and Symposium Series, in Raleigh NC (April 2008), and an oral presentation was given at the International Association of Lichenology in Asilomar, CA (July of 2008).

COSTS (the budget remains the same as the original proposal):

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**Specific budget information:** This project is the basis of a Ph.D. dissertation for Jen Riddell at Arizona State University under the guidance of Dr. Tom Nash. Ms. Riddell has received a Science To Achieve Results (STAR) fellowship from the EPA to support 2 years of her salary and funding for travel to meetings and supplies. We are requesting 9 months of her salary in the final year. The additional funding requested here will be used to enhance the project to insure that the results meet the needs of the FHM program. The Forest Service PI, Pam Padgett is providing the fumigation and laboratory facilities in Riverside and assisting with access and use of the San Dimas Experimental Forest in Southern CA. We are asking for summer salary support for Dr. Nash ($6000) and salary support for a technician to calibrate and maintain the air quality instrumentation ($6000). The project will also require substantial travel both back and forth between ASU and Riverside, and to field sites for specimen collection and managing the field experiments, which is not covered by the STAR fellowship.