

## Annual Report Summary

**Period Covered by the Report:** 1/1/2001-12/31/2002

**Date of Report:** 2/27/2003

**Title:** DMSP and its role as an antioxidant in the salt marsh macrophyte *Spartina alterniflora*.

**Investigators:** Ronald P. Kiene, and Joseph D. Husband

**Institution:** The University of South Alabama

**Research Category:** Estuarine Studies

**Project Period:** 2 years

**Objective(s) of the Research Project:** To test the hypothesis that the osmolyte dimethylsulfoniopropionate (DMSP) and its degradation products (dimethylsulfide (DMS) and dimethylsulfoxide (DMSO)) function as antioxidants in the smooth cordgrass *Spartina alterniflora*. Field and nursery-grown plants will be used to examine natural and experimentally-imposed stresses. We focus on the responses of *S. alterniflora* to natural environmental stress factors, including elevated salinity, high solar radiation, nitrogen limitation and iron limitation. Additionally, we will impose oxidative stress selectively by administration of the herbicides paraquat and DCMU. Plant responses to be monitored will include DMSP dynamics (along with its degradation products, DMS, DMSO and methanesulfonic acid), reactive oxygen scavenging enzymes (peroxidase and superoxide dismutase) and the quantum yield of photosystem II. The data gathered will be used to assess whether DMSP production confers advantages to *S. alterniflora* living in salt marsh habitats.

**Progress Summary/Accomplishments:** The production of DMSP by *S. alterniflora* was well known, but no previous studies had looked for potential oxidation products of DMSP in the tissues of this important salt marsh macrophyte. Our initial efforts focused on adapting assays to measure DMSO in plant tissues. The method proved to work well and for the first time we have demonstrated that DMSO is present in the leaves of field-collected *S. alterniflora*. Not only is DMSO present, but its intracellular concentrations were relatively high, in the low millimolar range and equivalent to 5-20% of the DMSP concentrations. At these concentrations DMSO could indeed function as an important radical scavenger and antioxidant. This is an important and very exciting finding. In plants from the natural environment, the ratio of DMSO: DMSP was higher in segments of necrotic tissue (0.16) vs. healthy tissue (0.06) from the same leaf. In addition, we found higher concentrations of DMSO in the spring and summer when insolation and photosynthetic activity are at a maximum. These periods may correspond to the highest oxidative stress. Furthermore, we found that intracellular DMSP concentrations decreased along the length of leaves from the ligule to the tip. DMSO, in contrast, showed the opposite trend, being highest near the leaf tip. As *S. alterniflora* leaves tend to senesce from the tip, this further argues that DMSP oxidation may be promoted in stressed tissues.

Direct evidence that DMSP may be involved in oxidative stress response was obtained from experiments in which selected leaves of nursery-grown plants were treated with the herbicide paraquat (methylviologen). Paraquat is known to promote excessive production of reactive oxygen species (ROS) in plants exposed to light. Application of paraquat to leaves held in sunlight caused visible leaf chlorosis within 24 h. Intracellular DMSP and DMSO concentrations were significantly higher in leaves treated with the paraquat (34.3  $\mu\text{mol}/\text{ml}$  leaf

water, and 3.25  $\mu\text{mol}/\text{ml}$  leaf water respectively), compared to untreated control leaves (14.4  $\mu\text{mol}/\text{ml}$  leaf water, and 2.54  $\mu\text{mol}/\text{ml}$  leaf water) from the same plant. These findings suggest that *S. alterniflora* may be able to rapidly increase synthesis of DMSP in response to oxidative stress. Our findings further suggest that DMSP is oxidized during times of stress, such as that caused by natural tissue necrosis or by administration of an oxidative stressor such as paraquat. However, it remains to be demonstrated whether translocation of DMSP (or DMSO) into stressed areas is responsible for the localized increases that we observed.

We have carried out some tests showing that DMS release from chlorotic or necrotic (i.e. stressed) tissues of *S. alterniflora* leaves is significantly higher than from healthy green leaves. DMS is a cleavage product of DMSP and can be produced via the enzyme DMSP lyase. These findings suggest that the stressed leaves are consuming more DMSP to produce DMS. Some of this DMS is volatilized from the leaves, but some undoubtedly is further oxidized to yield DMSO, explaining why we found higher DMSO in chlorotic (yellow) leaf sections and near the leaf tips. At the present time we do not know whether the *S. alterniflora* plants are the source of the DMSP lyase enzyme that is apparently cleaving DMSP into DMS, or whether microbes (bacteria or fungi) are involved. This will be a subject of further study in the year to come. Preliminary tests for DMSP lyase activity in healthy green leaf sections of *S. alterniflora* failed to show significant activity. However, we did find that leaf extracts prepared for these lyase assays rapidly consumed DMS in some circumstances. Thus, we demonstrated that *S. alterniflora* tissues have the capability to consume DMS (presumably by oxidation). More tests on potential lyase activity and DMS consumption are planned.

*S. alterniflora* is native to the east and gulf coasts of North America, but it has recently invaded various estuaries on the west coast. While valued on the east coast for its ability to stabilize shorelines and as a major source of organic matter and habitat, on the west coast *S. alterniflora* is considered an unwelcome invader. There is also growing evidence that *S. alterniflora* is hybridizing with the native west coast species *S. foliosa* providing a further threat to the ecological balance in west coast marshes. On the European side of the Atlantic, *S. alterniflora* has crossed with the native species, *S. maritima*, producing the successful hybrid *S. anglica*. Given the ability of *S. alterniflora* and its con-generics to dominate some ecosystems, and its apparent ability to invade new areas and cross with closely related species, we need to understand what makes this species so successful in colonizing euryhaline coastal margins. What specific adaptation(s) allows *S. alterniflora* to dominate under the harsh conditions prevailing in estuaries where the stressors include variable salinity, aerial desiccation, high light exposure, soil/sediment anoxia, sulfide, and anthropogenic pollutants? We believe the high concentration of DMSP in *S. alterniflora* may help explain this dominance.

### **Publications/Presentations:**

Husband, J. D., and R. P. Kiene. Role of DMSP in oxidative stress protection in the salt marsh macrophyte *Spartina alterniflora*. Third International symposium on DMSP and related sulfonium compounds, Rimouski, Quebec, September 25-29, 2002.

**Future Activities:** We will continue to measure seasonal patterns of DMSP and its oxidation products in natural populations of *S. alterniflora* throughout the 2002-2003 project period. We will carry out tests using antibiotics and antifungal agents to evaluate whether leaf microbes

might be involved in DMSP transformations. Further work on assaying the potential DMSP lyase activity in *S. alterniflora* tissues will be conducted. To aid in this work, we have initiated a side project involving isolation and purification of DMSP lyase enzyme(s) from the green macroalga *Enteromorpha intestinalis*. This macroalga has very high DMSP lyase activity and offers us good potential for obtaining a purified, functional protein. If we can get such a pure DMSP lyase, we can obtain the N-terminal amino acid sequence and thereby infer the DNA sequence encoding this enzyme. We would then be able to use this information to generate DNA probes that would allow us to test *S. alterniflora* (and other organisms) for this important enzyme.

Methods are under development to measure methanesulfonic acid (MSA) in leaf tissue samples. MSA is a potential oxidation product of DMSP via DMS and DMSO and it is a difficult compound to measure in a saline matrix. If we can measure MSA and observe its production *in vivo*, it would provide unambiguous confirmation that DMSP and its degradation products are scavenging ROS in *S. alterniflora*.

Other methods development will include assays for ROS scavenging enzymes including peroxidase and superoxide dismutase. Together with pulsed amplitude modulated fluorometry we will use these assays to assess the level of oxidative stress experienced by plants.

More experimental work is planned to examine the effects of direct oxidative stressors on DMSP production and dynamics in the *S. alterniflora* leaves. We will repeat experiments with Paraquat and also test DCMU, another herbicide that should promote oxidative stress in the plants.

Nursery-grown plants will be used for a series of experiments examining how common stresses including elevated salinity, N-limitation and iron limitation affect oxidative stress and DMSP dynamics in the *S. alterniflora* plants.

In addition we will investigate the hypothesis that the high concentrations of DMSP found in *S. alterniflora* confer increased resistance to oxidative stress for this species. To test whether DMSP provides *S. alterniflora* with greater resistance to oxidative stress (relative to the non-DMSP containing congener *S. patens*) time series measurements will be made of oxidative stress in paraquat treated *S. alterniflora* and *S. patens* in parallel with measurements of the concentration of DMSP and its oxidation products in *S. alterniflora*. A PAM-Fluorometer will be used to assess *in situ* oxidative stress in the paraquat treated plants.

**Supplemental Keywords:** Dimethyl sulfide, Dimethyl sulfoxide, DMS, DMSO, invasive species, Methane sulfonic acid, MSA, OH radical, Radical oxygen species, ROS, Salt marsh

**Relevant Web Sites:** A project web site will be created this year.

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