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Ecological Relationships between *Meloidogyne spartinae* and Salt Marsh Grasses in Connecticut

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Abstract: Healthy specimens of selected grasses were collected from salt marshes and grown in the greenhouse. Plants were inoculated with *Meloidogyne spartinae* to determine the host range of this nematode. After 12 weeks, *Spartina alterniflora* plants formed root galls in response to infection and increased *M. spartinae* populations. *Spartina patens*, *Spartina cynosuroides, Juncus gerardii* and *Distichlis spicata* were non-hosts. In order to determine the natural distribution of *M. spartinae* in dieback areas, *S. alterniflora* plants were sampled from transects adjacent to dieback areas in Madison, CT, at low tide. Plants were sampled at the top or the creek and at 1-m intervals to the lowest area of plant growth at the low tide water's edge. Five samples were taken over an elevation drop of 90 cm. Two transects were taken each day on 21 June and 5 July 2007, and one transect was taken on 31 October 2007. *Meloidogyne spartinae* galls per gram root were higher at the higher elevations. In late June and early July 2007, *M. spartinae* developed more quickly in the higher elevations, perhaps because peat and sediments were drier and warmer away from low tide water levels. The effects of *M. spartinae* on *S. alterniflora* and the role of the nematode in marsh decline and dieback in the northeast United States remain to be determined.

Key words: Distichlis spicata, *Juncus gerardii*, *Meloidogyne spartinae*, root-knot nematode, salt marsh decline, *Spartina alterniflora*, *Spartina cynosuroides*, *Spartina patens*

Meloidogyne spartinae Rau and Fassuliotis, 1965 was first observed on roots of smooth cordgrass (*Spartina alterniflora* Loiselius) in Florida in 1958 and described as *Hypsoperine spartinae* from South Carolina in 1965 (Rau and Fassuliotis, 1965). To date, the primary interest in this nematode has been taxonomic, and it has been variously placed in the genera *Hypsoperine, Spartonema* (Siddiqi, 1986) and subsequently *Meloidogyne* (Whitehead, 1968; Handoo et al., 1993; Eisenback and Hirschmann, 2001; Plantard et al., 2007). *Meloidogyne spartinae* has been collected from Florida, Georgia, South Carolina, North Carolina, New Jersey and New York (Rau and Fassuliotis, 1965; Eisenback and Hirschmann, 2001). Recently, we have collected *M. spartinae* from *S. alterniflora* in salt marshes that have exhibited decline in Connecticut, Massachusetts (LaMondia and Elmer, 2007) and Maine.

This decline of *S. alterniflora* was first reported in 2002, when wetland ecologists noticed that large areas of salt marshes had areas of dieback in the low and high marshes (Alber et al., 2008). Low marsh areas are flooded by tides twice daily and dominated by the tall form of *S. alterniflora*. The high marsh is slightly elevated, flooded only by the highest tides, and contains a short form of *S. alterniflora* as well as other salt-tolerant plant species (Lippson and Lippson, 2006). Studies have shown that the tall and short forms are genetically identical and that their phenotypes (tall vs. short) can easily convert to the other type when environmental changes are imposed (Valiela et al., 1978). In almost all areas where decline has occurred, only S. *alterniflora* and *S. patens* were reported to be affected. Most areas of decline do not recover and become reduced to mudflats within a few years. This decline has been termed Sudden Vegetation Dieback (SVD). The pattern associated with SVD is erratic and could result in small patches of dead plants scattered over a marsh or could result in the loss of large areas of marsh. In many instances in Connecticut, SVD was restricted to high marsh creek banks of intertidal marshes, whereas other times SVD was specifically localized away from the tidal creeks (Elmer, unpublished). The nature of SVD appeared distinctly different from other important factors that can impact salt marshes, such as wrack buildup, hurricanes, herbivory from geese, crabs or snails, fire, pollutants and erosion (ice rafting). However, in specific areas, some of these factors may have also played roles in salt marsh decline (Silliman et al., 2005).

Examination of aerial photography suggested that the New England SVD began in 1999 and was coincident with the massive dieback of salt marshes along Louisiana's coast in which over 150,000 ha of *S. alterniflora* suddenly died. This phenomenon was termed brown marsh (McKee et al., 2004). Since then, SVD has been reported in Delaware, Georgia, Maine, New Hampshire, New York's Long Island, Rhode Island, South Carolina and Virginia (Mackinnon and Huntington, 2005; Alber et al., 2008). Although the etiology of the dieback is unclear, drought has been associated with many of the SVD events (McKee et al., 2004). In addition, other abiotic stresses, such as soil acidity and heavy metal toxicity, have been implicated (Silliman et al., 2005). Schneider and Useman (2005) reported that declining *S. alterniflora* plants in Louisiana had symptoms of black leaf spots and internal stem rots. They isolated an undescribed species of *Fusarium* associated with the decline and demonstrated Koch's postulates. Elmer (2007) also found pathogenic species of *Fusarium* from *S. alterniflora*

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in SVD sites, but the species were not the same as in Louisiana.

Because *M. spartinae* has often been found in marshes affected by SVD, sometimes in high densities, this research was initiated in order to examine a possible relationship of this nematode with SVD. At present, it is not known if *M. spartinae* plays any role in SVD. In order to address this possible role, our objectives were to determine where *M. spartinae* was present in the marsh and whether other marsh grasses were hosts of the nematode.

Materials and Methods

To determine the host status of marsh grasses to *Meloidogyne spartinae*, we collected plants from high marsh areas of Hammonassett State Park in Madison, CT. Salt marsh grasses collected were: *Spartina alterniflora* (short form of smooth cordgrass), *Spartina patens* (Ait.) Muhl. (saltmeadow cordgrass), *Spartina cynosuroides* (L.) Roth (big cordgrass), *Juncus gerardii* (Loisel.) (saltmeadow rush) and *Distichlis spicata* (L.) Greene (spike grass).

Field-collected plant roots were washed free of peat and tidal sediments, and roots examined under a dissecting microscope (×30 magnification). All plants were found to be free of galls and were transplanted into plastic pots containing 800 cm^3 pasteurized Merrimac fine sandy loam field soil (73.4% sand, 21.4% silt, 5.2% clay, pH 6.4). Twenty-four days after transplanting, a suspension of 125 eggs and second-stage juveniles (J2) of *M. spartinae* obtained from *S. alterniflora* roots collected from Hammonassett State Park in Madison, CT, was equally distributed into 4 holes (1 cm diam. × 2 cm deep) per pot approximately 1 cm from the plant. There were 10 replicate pots of each plant species infested.

Plants were grown in the greenhouse on a plastic-lined bed and flooded weekly. After 12 wk, roots of test plants were washed free of soil, and the number of galls counted. Root galls were dissected, and the developmental stages of nematodes present recorded. Gall numbers were subjected to the nonparametric Kruskal-Wallis test (NCSS, Kaysville, UT).

In order to determine the natural distribution of *M. spartinae* in dieback areas, *S. alterniflora* plants were sampled from transects adjacent to dieback areas from the Tom's Creek tributary in Hammonassett State Park, Madison, CT, at low tide. Plants were sampled at the border of the high and low marsh (the border between high and low forms of *S. alterniflora*) and at 1-m intervals to the lowest area of plant growth at the low tide water's edge. Each 1-m interval represented a change of approximately 0.25 m in elevation. Five samples were taken per transect, and the elevation drop over this transect was approximately 90 cm. Two transects were sampled on 21 June 2007, two on 5 July 2007 and an additional transect was sampled on 31 October 2007. Roots of *S. alterniflora* plants were washed free of peat and tidal sediments, and the number of galls counted. Root galls were dissected, and the developmental stages of *M. spartinae* present recorded. Gall numbers were subjected to the nonparametric Kruskal-Wallis test. Means were separated by the Kruskal-Wallis multiple comparison Z-value test.

Results

Of the five common salt marsh plant species examined, only *S. alterniflora* had visible galls and *M. spartinae* present within roots, both on samples taken from certain locations in the salt marsh (data not shown) and in the greenhouse experiment (Table 1). The distribution of *M. spartinae* in *S. alterniflora* was sporadic and clumped in the salt marsh. The samples that we took in the high marsh of the short form of *S. alterniflora* were free of nematodes, but those same plants were hosts of *M. spartinae* when inoculated in the greenhouse experiment. Tall forms of *S. alterniflora* collected in different locations in the low marsh areas ranged from free of nematodes to high densities of *M. spartinae* in roots (data not shown).

When *S. alterniflora* plants were sampled from *M. spartinae*-infested areas in transects across the low marsh, the numbers of *M. spartinae* galls per gram root were higher at or near the border of the low and high marsh (Table 2). In addition, for samples taken in late June and early July 2007, there were marked differences in the percentage of galls containing fully mature females with eggs. In October nearly all the galls collected contained all stages of the nematode, including gravid females.

Discussion

The salt water marsh in Hammonassett State Park in Madison, CT, is a deep coastal marsh with peat and tidal sediments typically more than 2 m deep (Hill and Shearin, 1970). SVD in the Tom's Creek tributary is characterized by the death or sudden loss of *Spartina alterniflora* in the low marsh and subsequent erosion of

Table 1. Host status of salt marsh grasses to *Meloidogyne spartinae*.

Salt marsh grass	Galls per plant[a]	Nematode stages in galls
Spartina alterniflora	30.8[b]	all stages present
Spartina patens	0.0	[c]n.d.
Spartina cynosuroides	0.0	n.d.
Juncus gerardii	0.0	n.d.
Distichlis spicata	0.0	n.d.
P =	0.0001	

[a]Galls counted 12 weeks after inoculation
[b]Data are means of 10 plants. Data were analyzed by the Kruskal-Wallis test.
[c]n.d. = not done. No galls were detected.

TABLE 2. Distribution of *Meloidogyne spartinae* within the low marsh on *Spartina alterniflora* plants sampled from transects adjacent to dieback areas in the Thames Creek tributary of Hammonassett State Park in Madison, CT.

Location[a]	*M. spartinae* galls per g root [b]	Percent of galls containing females with eggs [c]
0 (border with high marsh)	13.16 a [d]	50.0 a
1	10.08ab	40.8 a
2	0.32 bc	14.6 ab
3	0.15 c	12.5 ab
4 (low tide water's edge)	0.03 c	0.0 b
$P =$	0.03	0.02

[a]Plants were sampled at the border of the high and low marsh and at 1-m intervals to the lowest area of plant growth at the water's edge. Five samples were taken per transect, and the elevation drop was approximately 90 cm.

[b]Data from five transects: two sampled on 21 June 2007, two on 5 July 2007 and one on 31 October 2007.

[c]Data included only from four transects sampled on 21 June 2007 or 5 July 2007, as nearly all galls collected in October contained all stages of the nematode.

[d]Means within the same column that are followed by the same letter are not significantly different ($P = 0.05$), based on analysis by the Kruskal-Wallis Z-test.

creek banks to create wider areas of open water or mud flats.

We have collected *M. spartinae* from *S. alterniflora* plants in the low marsh adjacent to dieback areas, often at what we consider to be high densities comparable to populations of *M. hapla* in cultivated vegetable crops. In our limited sampling, we have not found *M. spartinae* from short form *S. alterniflora*, *S. patens*, *S. cynosuroides*, *Juncus gerardii* or *Distichlus spicata* in the high marsh, which may be free of tidal inundation for a week or more. None of the high marsh sites sampled was in decline, with low marsh creekbank decline being the common form of decline in Hammonassett State Park. *Spartina alterniflora* (tall form) dominates the low marsh, which is submerged by most high tides, and, of the salt-tolerant marsh grasses examined, seems to be the only host of *M. spartinae* that we have found.

Spartina alterniflora and *M. spartinae* survive harsh conditions in the low marsh. We have measured salt concentrations as high as 35,000 ppm in the water in the sediments, similar to sea water, and the peat and tidal sediments are often flooded, waterlogged and anaerobic.

In areas where *M. spartinae* has been identified, we have determined that densities in roots are higher at the upper levels of the low marsh and lower closer to mean low tide. Higher elevations in the low marsh had drier peat and tidal sediments than locations closer to the water. The nematodes develop to maturity and produce eggs faster in the peat and sediments that are not continuously waterlogged (and therefore presumably warmer) than areas at or near the low tide water levels. This border area between the low and high marsh is dynamic, and plant death or damage by pathogens, pests or herbivory may restrict the ability of *S. alterniflora* plants to rapidly adapt to changes such as rising water level. Other nematodes have been associated with declines and die-out of dune grasses (Seliskar and Huettel, 1993; De Rooij-Van der Goes, 1995). The effects of *M. spartinae* on *S. alterniflora* growth, vigor and rhizome production are currently unknown; however, *M. spartinae* galls were reported to differ in number and size on different geographical populations of *S. alterniflora* (Seneca, 1974). The effects of *M. spartinae* on *S. alterniflora* and the role of the nematode in marsh decline and dieback in the northeast should be the subject of further inquiry. Furthermore, given that pathogenic species of *Fusarium* have also been implicated in SVD (Elmer, 2007; Schneider and Useman, 2005) and that numerous disease complexes on terrestrial crops have been documented with root-knot nematodes and *Fusarium* (Mai and Abawi, 1987), it becomes essential to determine if these organisms are capable of causing SVD in a stressed marsh ecosystem.

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