TITLE:

Root-knot nematodes parasitizing turfgrass in the Western United States.

JUSTIFICATION:

Turf grass, in all its aspects, is a major industry in the United States. Considered as a crop, and including commercial, residential, and recreational components, irrigated turf is the largest single crop in America. Three times more irrigated turf than irrigated corn is grown, covering a surface area of more than 128,000 square kilometers (Milesi, et al., 2005). Golf is a primary consumer/producer of turf. The 15,000 golf courses in the USA utilize an estimated 2,244, 512 acres of land, including 1,504210 acres of maintained turfgrass (Lyman et al., 2007) and the economic impact of the golf course industry is significant. In 2007, the golf industry in California, alone, directly contributed approximately $6,900,000,000 to the State's economy. When direct, indirect, and induced economic output are combined, the value exceeds $15 billion and it supports 160,000 jobs with $4.8 Billion in wage income (McCullough, 2008). There are an estimated 3 million golfers in the state, 10 percent of the nation's total. The desert regions of Arizona, and Nevada also have significant acreage in turfgrass. In Arizona there are more than 400 golf courses with a combined 46,00 acres of irrigated turf, twice the acreage of the State's commercial citrus.

Plant-parasitic nematodes have become a significant factor affecting the health, quality, production, and maintenance of turfgrass on golf courses. The nematode, *Anguina pacificae,* is currently the most devastating pest of *Poa annua* golf course greens in northern California. Professional golf associations and individual golf courses have spent hundreds of thousands of dollars in unsuccessful attempts to control this nematode. The Sting Nematode, *Belonolaimus longicaudatus* has long been associated with turfgrass decline in the Southern USA and is the subject of exclusionary quarantines in most Western states. More recently, root-knot nematodes have gained attention as a as a serious threat to both cool and warm-season turf grasses. Nematode problems were exacerbated in the West when the only pesticide (Nemacur), registered for nematode control on turf grass was withdrawn from the market in November 2008, leaving golf course superintendents without effective or economical management options. According to Crow, "*Problems with plant-parasitic nematodes can be expected to increase in coming years due to several factors. With modern globalization, the spread of damaging nematode species to new regions by means of contaminated soil and plant material is liable to continue. Also, current worldwide trends toward decreasing mowing heights and the use of sand-based putting greens tend to reduce tolerance to plant-parasitic nematodes. Finally, there is a lag between the removal of the historically used turfgrass nematicides and the identification and implementation of new nematode management strategies that are practical, environmentally safe, and effective.*" (Crow, 2005).

Nematodes frequently associated with turfgrass in the Western USA include Root-knot nematodes, *Meloidogyne* spp., Cyst nematodes, *Heterodera spp*.,the Pacific shoot gall nematode, *Anguina pacificae,* Ring nematodes, *Criconemella* spp., Spiral nematodes, *Helicotylenchus* and *Rotylenchus* spp., and Stubby root nematodes *Trichodorus* spp. Severe damage has been attributed to both the Root-knot nematodes and the Pacific shoot gall nematode. The Pacific

Shoot Gall nematode is a major problem in Northern California but its distribution is limited to a narrow strip along the Pacific Coast. Root-knot nematodes, on the other hand, are wide-spread and high populations have been implicated in turf decline in both California and Arizona. But, our knowledge of their impact on turf health is largely anecdotal. Furthermore, the species of *Meloidogyne* found on infested golf courses and their host range, in most cases, is unknown.

PREVIOUS WORK AND PRESENT OUTLOOK:

Over the past 30 years, numerous research papers have been published on plant-parasitic nematodes attacking turfgrass. Most of them deal with problems that occur in the Eastern and Southern USA. Few deal specifically with nematodes on Western turf where soils, climate, turf varieties, and cultural practices are distinct. In the South and Southeast, Sting, Lance, and Root-knot nematodes are the primary pathogens. Sting Nematode is not indigenous in the West and Lance Nematodes, although found occasionally in California golf greens, seldom occur in high numbers and have never been associated with turf decline. Consequently, little that has been learned about turfgrass nematodes has direct application to Arizona and California. Most of the previous work in these two states has been published in industry newsletters and on-line communications rather than peer-reviewed journals. Twenty-two Southern California golf courses were surveyed in 2002 to describe the range of genera and populations of plant-parasitic nematodes. Nematode counts were used in an attempt to determine the relationship between green quality and nematode populations. No significant correlation was found (Stowell and McClure, 2003). In contrast, a nematode analysis of "Good" and "Poor" greens in Washington state, found significantly higher populations of Ring and Spiral nematodes in "Poor" greens (Chastagner and McElroy, 1985). Root-knot nematodes were also higher in some "Poor" greens, but mean values were not significantly different from those in "Good" greens . A more recent study in Washington found 348 infective Meloidogyne juveniles per 100 cc of soil taken from damaged turf and none from healthy areas (Cook, 2008). A preliminary survey of nematodes in western golf greens included 17 courses in Arizona and 2 in California. Twelve of the 17 courses were infested with Root-knot nematodes, three of them above the "damage threshold" (Howard, 2005). Unfortunately, the "damage threshold" was based on Florida data and the published information did not include the volume of soil sampled, an omission that rendered the data useless. Nor were the species of *Meloidogyne* identified. Not surprisingly, the author was unable to draw any conclusions from his work. In his own words, "*I am not sure precisely to make of the data.*" But, he did note that " *In a couple of the instances with severe infestation, I know that the unfortunate superintendents have really struggled with greens turf.*" A survey of golf 14 golf courses in Northern California found Root-knot nematodes in 64 percent of the greens sampled, but no attempt was made to determine the nematode species or relate population levels to turf quality (Westerdahl, 2005). The best study to date compared levels of Root-knot nematodes with green performance on 18 bentgrass greens at the La Jolla Country Club in San Diego County, CA. No correlation was found between "good" and "poor" performing greens and nematode population levels, including those of an unidentified *Meloidogyne* species (Stowell and Gelernter, 2008).

An increasing number of sod samples from distressed golf course greens are being submitted to the Nematode Diagnostic Laboratory at the University of Arizona. In some cases the damage is so severe that the greens are unsightly and nearly unplayable. Many of these contain very high

populations of Root-knot nematodes, indeed, much higher than the "damage threshold" reported in the Southeastern States and elsewhere. Therefore, the need to assess the role of Root-knot nematodes in turf decline is clear. Previous studies, cited above, have had one major fault. None have identified the species of *Meloidogyne* associated with the turf being investigated. Consequently, it is not known if the observed damage is caused by a single species or several species. It is even conceivable, that the species differ from green to green in a given golf course, depending on edaphic factors, sun vs. shade, drainage, etc. And, unless the species have been identified, is it not possible to assess the host range of the nematodes, an important consideration when new or replanted greens are contemplated. Because identification of plant parasitic nematodes is the bedrock foundation of all regulatory issues and is essential for efficient, economic control measures (cultural and biological as well as chemical), the overall goal of this project is to identify the *Meloidogyne* species affecting Western golf courses and to examine their role in the decline of turf grasses that are commonly grown on golf course greens in the Western states.

OBJECTIVES:

1. **Surveys:** Determine the breadth and incidence of Root-knot nematode infestations in the Western states, primarily Arizona and California. New Mexico, Nevada, and Southern Oregon will be included if funding from those states can be obtained.
2. **Species Identification:** Characterize and identify the species of *Meloidogyne* found in the surveys.
3. **Host Range:** Identify the host range of *Meloidogyne* species on turf grasses commonly found on Western golf course greens, including invasive grasses.
4. **Impact on Turf Quality:** Once the species and their host ranges are known, determine the ability of those species to damage turf.

PROCEDURES:

1. **Surveys:** Turf/soil samples will be examined from golf courses across a broad range of climatic zones, soil types, greens construction, turf varieties, water sources, and disease problems. Samples will include plugs from healthy areas of greens as well as damaged areas. Sod from golf courses in Arizona will be obtained from a variety of sources, including samples submitted to the Nematode and Disease Diagnostic Laboratories at the University of Arizona, samples collected first-hand by the principal investigator, and samples collected by golf course superintendents who are interested in participating in the survey. Samples from golf courses in California will be obtained from individual golf course superintendents, industry consultants (PACE Turf, Inc., M. Mahady & Associates) and the Northern California Golf Association. Nematodes will be extracted from the soil in a mist chamber and roots from those samples containing *Meloidogyne* infective juveniles (J2) in the soil extracts will be examined for the presence of galls containing adult females and adhering egg masses.

2. **Species Identification:** Root-knot nematodes from soil extracts and roots will be identified by a combination of classical morphological means and molecular methods. *Meloidogyne* J2 and adult males will be hand-picked from soil extracts and fixed in buffered

aldehyde fixatives for morphological examination. Adult females will be dissected from roots and fixed in a similar fashion. Alternatively, roots containing adult females will be heated in lactophenol and, after cooling, the females will be dissected for preparation of perineal patterns. Specimens for DNA analysis will be hand-picked into a preservative solution of EDTA-DMSO saturated with sodium chloride that preserves morphological features as well as DNA. Mitochondrial DNA from individual J2 will be characterized by PCR (Powers and Harris, 1993; Powers et al., 2005) and the amplification products sequenced. Nematode populations with sufficiently distinct sequences will be examined morphologically by light and electron microscopy. Based on these procedures, any populations that are deemed to represent new species will be described as such. Dr. Jon Eisenback (Department of Plant Pathology, Physiology, and Weed Science, Virginia Tech), a trained taxonomist, has agreed to collaborate on the morphological examination and species descriptions.

3. **Host Range of *Meloidogyne* species:** The host range of all species found in the surveys will be determined in growth chambers utilizing methodology developed in my laboratory for *Anguina pacificae* (McClure et al., 2008). Individual seedlings are grown in one ml disposable pipette tips filled with quartz sand. These "mini pots" are placed in the original racks and boxes in which they were supplied, and the boxes filled with sufficient nutrient solution to submerge the bottom 7 mm of the tubes, wetting the sand to the top of the tube by capillarity. With the lid of the boxes closed, the cultures of the common turf grasses can be maintained for 3 months without replenishing the nutrient solution. Growth chamber parameters can be programed to simulate the conditions in which the nematodes thrive best. Under such conditions, most *Meloidogyne* species complete their life cycle in 30 days or less.

4. **Nematode Pathogenicity:** Growth chamber methodology will also be used to measure the pathogenicity of existing and newly describe species of *Meloidogyne* on selected turf grass cultivars. Individual seedlings in the "mini pots" described above will be inoculated with defined dosages of J2 to determine the minimum number of J2 required to measurably reduce the growth of the turf being tested. Because the cultural conditions are not monoxenic, these tests will not be a strict application of Koch's postulates. Rather, they will serve as a starting point for developing damage thresholds under field conditions, information that is now lacking completely.

All studies will be designed to insure that, when appropriate, acquired data can be analyzed by established statistical procedures. No animal subjects will be required (aside from the nematodes) and no special permissions will be necessary. Results of these studies will be published in peer-reviewed journals such as Plant Disease, published by The American Phytopathological Society, and the *Journal of Nematology*, published by the Society of Nematologists.

PROBABLE DURATION:

February 1, 2009 to June 30, 2012

FINANCIAL SUPPORT:

No funds are requested. Funds are currently available in my Revenue Account, FRS 246170, for a 3-year project, including salary and employee benefits for Mark E. Schmitt.

PERSONNEL:

Michael A. McClure - Project leader

Mark E. Schmitt - Research Technician

INSTITUTIONAL UNITS INVOLVED:

The University of Arizona, Tucson: Providing facilities to conduct portions of the proposed research.

COOPERATION:

Dr. Jon Eisenback (Department of Plant Pathology, Physiology, and Weed Science, Virginia Tech), a trained taxonomist, has agreed to collaborate on the morphological examination and species descriptions.

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CRIS SEARCH

A CRIS search on the key words “nematodes” and “turf” returned 17 records. Only one project relates to the one proposed here: Dr. Eisenback is investigating only nematodes on turf in the East and Southeastern USA. He will collaborate with me on my project and provide the morphological and taxonomic descriptions of any new species discovered in my surveys.

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PROJ NO: VA-135860 AGENCY: CSREES VA.
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START: 01 JAN 2009 TERM: 31 DEC 2013
INVESTIGATOR: Eisenback, J. D.
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TAXONOMIC CHARACTERIZATIONS OF ROOT-KNOT AND CYST NEMATODES PARASITIZING TURFGRASS