

## FIRST REPORT OF *CHRYSORHIZA ZEA* (VOORHES) ANDERSEN AND STALPERS CAUSING NECROTIC LESIONS ON *CYNODON DACTYLON* (L.) PERS. IN CUBA

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**B**rown patches were observed in golf courses situated in Matanzas, a province of western Cuba, planted with *Cynodon dactylon* (Bermuda grass). Weed samples from eleven golf courses showing necrotic lesions in stems and leaves were collected. Vegetal tissues were disinfected with sodium hypochlorite (3%) for five minutes and placed in Petri dishes containing water agar and incubated at  $28 \pm 2^\circ\text{C}$ . The ITS1-5.8S-ITS2 region was amplified, sequenced and compared with sequences in GenBank database. A phylogenetic tree was constructed using the program MEGA3.1 by the neighbor joining method, strength of the tree branches was tested by 1000 bootstrap trials. Seven out of eleven samples had a fast growing rate and showed a white mycelium in first four days which became orange later. Main hyphae had  $7.3 \mu\text{m}$  wide on average. Colonies also presented small, spherical, orange colored sclerotia of 0.3 to 0.7 mm diameter not differentiated in ring and medulla. The sclerotia were homogeneous both in size and shape and hyphae were multinucleated with four to six nuclei per cell. Sequencing of ITS region showed more than 95% of homology with *Waitea circinata* sequences in GenBank. According to the new concept of *Rhizoctonia* complex, the name *Chrysorhiza zea* is proposed to designate multinucleate *Rhizoctonia* like anamorph of *Waitea circinata* Warcup and Talbot [Roberts, 1999]. *W. cir-*

*cinata* has been split into three varieties: var. *circinata*, var. *zea* and var. *oryzae* [Leiner and Carling, 1994; Toda *et al.*, 2005, 2007]. Phylogenetic tree showed that all isolates obtained in this study (ERR1 to ERR7) clustered together with isolates of *W. circinata* var. *zea* with 99% bootstrap values (Fig. 1). By using both cultural morphology and molecular data ERR isolates were classified as *Chrysorhiza zea* (teleomorph: *W. circinata* var. *zea*).

Pathogenicity tests were carried out using *C. dactylon* 21 days old seedlings. The inoculum used consisted of corn meal and sand colonized with ERR1. This inoculum was homogeneously mixed with sterilized soil, dampened and seedlings were transplanted on it and incubated at room temperature. Necrotic lesions were observed in first leaves and stems about two weeks later unlike negative controls which remained healthy (Fig. 2). The isolate ERR1, used in pathogenicity test also damaged others species of poaceae such as *Panicum maximum* Jacq, *Sorghum bicolor* (L.) Moench and *Zea mays* L. There are records of nine species of susceptible poaceae to this fungus [Poltrorieri *et al.*, 2002; McCall, 2006]. This is the first report of *Chrysorhiza zea* (teleomorph: *W. circinata* var. *zea*) causing necrotic lesion and brown patches on *Bermuda grass* in Cuba.

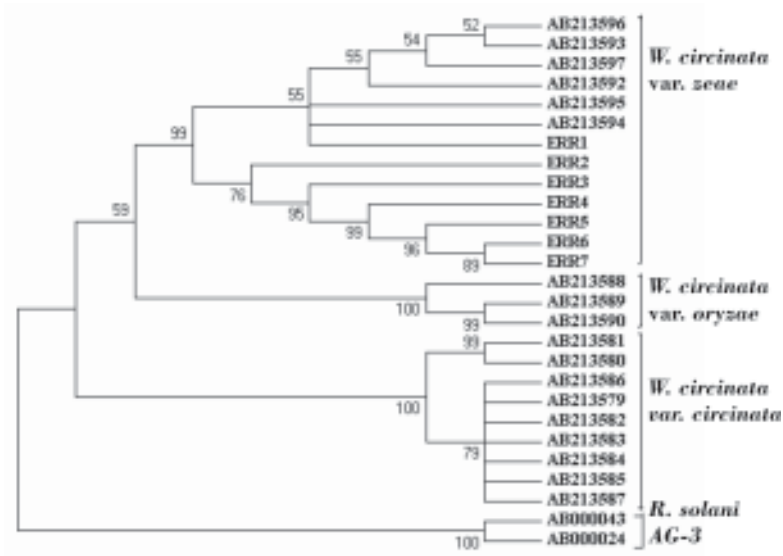


Figure 1. Phylogenetics tree construted by the neighbor-joining method, to compare ITS-rDNA sequences of ERR isolates with sequences of three varieties of *W. circinata* [Toda *et al.*, 2007]. Two sequences of *R. solani* AG-3 (taken from GenBank) were used as the out group. The accession numbers in the GenBank data base are given (Ex: AB213594). The numbers on the branches are confidence values obtained for 1000 bootstrap replicates.



Figure 2. Pathogenicity tests: Right side control and left side necrotic seedlings

## REFERENCES

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## Sequences of ITS1-5.8S-ITS2 of nuclear DNAr from the seven isolates obtained in this study

### ERR1

ACTGTGGAAGCGGAGGATATTATGATTGGTGGCTGTTGCTGACTAGTGTTCCTAGTATGTGCACGCCACACCTTCA  
ATCCCACTTACACCTGTGCACCTTCTGTAATAGATCTATGTGGATACGGAGATGTGAAAGTTTGCGCTCTCCTACT  
CTGTTGAAGCAGTCTCACTACGTTTTTTTACACACACACACAATAGTCATTGAATGTATTTTTTATTCTTATGATAAA  
AACAACTTTCAACAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGTAGCGAATTACGATATGTAATGTGAATT  
GCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCTTTGGTATTCCGAAGAGCATGCCTGTTTGAG  
TGTCATGAATCTCTCAAATACAATGATTTTTATTAATTGTTGTGTTTGGACTTGGAAGCTTGTCGGCGCAAGTCGAC  
TCTTCTTAAATGCATTAGCTGGGGTTTATATAGTTGGATCCTTGGTGTGATAATTATCTACGCCTTGAAGTCCCTGT  
AGACTCTGCTTCAAATCGTCTCCTAGAGACAACATTTGAATCATCTGACCTCAAATCAGGTAGGACTACCCGCTGA  
ACTTAAGCATATCATAANCGGGAGGAAAA

### ERR2

AGTGTGGAACGGAGCATACTATGATTGGTGGCTGTAGCTGACTAGTGTTCCTAGTATGTGCACGCCACACCTTCA  
ATCCCACTTACACCTGTGCACCTTCTGTAATAGATCTATGTGGATACGGAGATGTGAAAGTTTGCGCTCTCCTACT  
CTGTTGAAGCAGTCTCACTACGTTTTTTTACACACACACACAATAGTCATTGAATGTATTTTTTATTCTTATCATAAAA  
ACAACCTTTCAACAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGTAGCGAATTACGATATGTAATGTGAATTG  
CAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCTTTGGTATTCCGAAGAGCATGCCTGTTTGAGT  
GTCATGAATCTCTCAAATACAATGATTTTTATTAATTGTTGTGTTTGGACTTGGAAGCTTGTCGGCGCAAGTCGACT  
CTTCTTAAATGCATTAGCTGGGGTTTATATAGTTGGATCCTTGGTGTGATAATTATCTACGCCTTGAAGTCCCTGTA  
GACTCTGCTTCAAATCGTCTCCTAGAGACAACATTTGAATCATCTGACCTCAAATCAGGTAGGACTACCCGCTGAA  
CTTAAGCATATCATAANCGGGAGGAAAA

### ERR3

ACAGTGGAACGGAGCATACTATGAATGGTGGCTGTAGCTGACTAGTGTTCCTAGTATGTGCACGCCACACCTTCA  
ATCCCACTTACACCTGAGCACCTTCTGTAATAGATCTATGTGGATACGGAGATGAGAAAGTTTGCGCTCTCCTACT  
CTGTTGAAGCAGTCTCACTACGTTTTTTTACACACACACACAATAGTCATTGAATGTAAATTTTTATTCTTATCATAAA  
AACAACTTTCAACAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGTAGCGAATTACGATATGTAATGTGAATT  
GCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCTTTGGTATTCCGAAGAGCATGCCTGTTTGAG  
TGTCATGAATCTCTCAAATACAATGATTTTTATTAATTGTTGTGTTTGGACTTGGAAGCTTGTCGGCGCAAGTCGAC  
TCTTCTTAAATGCATTAGCTGGGGTTTATATAGTTGGATCCTTGGTGTGATAATTATCTACGCCTTGAAGTCCCTGT  
AGACTCTGCTTCAAATCGTCTCCTAGAGACAACATTTGAATCATCTGACCTCAAATCAGGTAGGACTACCCGCTGA  
ACTTAAGCATATCATAAACGGGACCAAAA

### ERR4

TCTGTGGAACGGAGCATACTATGATTGGTGGCTGTAGCTGACTAGTGTTCCTAGTATGTGCACGCCACACCTTCA  
ATGGCACTTACACCTGAGCACCTTCTGTAATAGATCTATGTGGATACGGAGATGAGAAAGTTTGCGCTCTCCTACT  
CTGTTGAAGCAGTCTCACTACGTTTTTTTACACACACACACAATAGTCATTGAATGTAAATTTTTATTCTTATCATAAA  
AACAACTTTCAACAACGGATCTCTTGCCTCTCGCATCGATGAAGAACGTAGCGAATTACGATATGTAATGTGAATT  
GCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCTTTGGTATTCCGAAGAGCATGCCTGTTTGAG  
TGTCATGAATCTCTCAAATACAATGATTTTTATTAATTGTTGTGTTTGGACTTGGAAGCTTGTCGGCGCAAGTCGAC  
TCTTCTTAAATGCATTAGCTGGGGTTTATATAGTTGGATCCTTGGTGTGATAATTATCTACGCCTTGAAGTCCCTGT  
AGACTCTGCTTCAAATCGTCTCCTAGAGACTTCATTTGAATCATCTGACCTCAAATCAGGTAGGACTACCCGCTGA  
ACTTAAGCATATCATAAACGGGACCAAAA

### ERR5

AGAGTGGAACGGAGCATACTATGATTGGTGGCTGTAGCTGACTAGTGTTCCTAGTATGTGCACGGGACACCTTC  
AATGGCACTTACACCTGAGCACCTTCTGTAATAGATCTATGTGGATACGGAGATGAGAAAGTTTGCGCTCTCCTAC  
TCTGTTGAAGCAGTCTCACTACGTTTTTTTACACACACACACAATAGTCATTGAATGTAAATTTTTATTCTTATCATAA  
AAACAACCTTTCAACAACGGATCTCTTGCCTCACGCATCGATGAAGAACGTAGCGAATTACGATATGTAATGTGAATT  
GCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCTTTGGTATTCCGAAGAGCATGCCTGTTTGAG  
TGTCATGAATCTCTCAAATACAATGATTTTTATTAATTGTTGTGTTTGGACTTGGAAGCTTGTCGGCGCAAGTCGAC  
TCTTCTTAAATGCATTAGCTGGGGTTTATATAGTTGGATCCTTGGTGTGATAATTATCTACGCCTTGAAGTCCCTGT  
AGACTCTGCTTCAAATCGTCTCCTAGAGACTTCATTTGAATCATCTGACCTCAAATCAGGTAGGACTACCCGCTGA  
ACTTAAGCATATCATTTCGGGACCAAAA

**ERR6**

AGAGTGGTTTCGGAGCATACTATGATTGGTGGCTGTAGCTGACTAGTGTTCCTAGTATGTGCACGGGACACCTTCA  
ATGGCACTTACACCTGAGCACCTTCAGTAATAGATCTATGTGGATACCCAGATGAGAAAGTTTGCGCTCTCCTACT  
CTGTTGAAGCAGTCTCACTACGTTTTTTTTACACACACACACAATAGTCATTGAATGTAAAAAAATTCTTATCATAAA  
AACAACTTTCAACAACGGATCTCTTGCCTCACGCATCGATGAAGAACGTAGCGAATTACGATATGTAATGTGAATT  
GCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCTTTGGTATTCCGAAGAGCATGCCTGTTTGAG  
TGTCATGAATCTCTCAAATACAATGATTTTTATTAATTGTTGTGTTTGGACTTGGAAGCTTGTCGGCGCAAGTCGAC  
TCTTCTTAAATGCATTAGCTGGGGTTTATATAGTTGGATCCTTGGTGTGATAATTATCTACGCCTTGAAGTCCCTGT  
AGACTCTGCTTCAAATCGTCTCCTAGAGACTTCATTTGAATCATCTGACCTCAAATCAGGTAGGACTACCCGCTGA  
ACTTAAGCATATCATTTTCGGGACCTTTT