CLEMSON UNIVERSITY
Turfgrass Program
(www.clemson.edu/turfornamental/)

Biology and molecular analysis of dinitroaniline-resistant *Poa annua* L.

David B. Lowe, Ginger A. Swire-Clark, Lambert B. McCarty,

Ted Whitwell and Wm.Vance Baird

Clemson University

Abstract

Annual bluegrass is a problem weed in intensively managed turf. Recent reports indicate that cultural practices resulted in the selection of resistance to the anti-microtubule dinitroaniline herbicides. Plants, either collected on site from Georgia and South Carolina or grown from seed purchased commercially or collected from North Carolina plants, were evaluated to identify resistant populations and compare relative levels of resistance; as well as vegetative and reproductive growth potential between populations. To accomplish this, plants were grown in greenhouses and a rooting bioassay using stem segments was developed. In addition, investigations into the molecular mechanism of resistance were initiated and focused on beta-tubulin. These experiments were accomplished using Southern blots, reverse transcriptase-polymerase chain reaction, and DNA sequence analysis. Over a 39-week period, vegetative growth of susceptible plants out-paced that of resistant plants. This was particularly true for canopy development (shoot numbers). Over the same time period, although resistant plants initially produced more inflorescences, there was not a consistent difference in reproductive growth between the two biotypes. Also, in general, there was not a significant difference in seed germination between the biotypes. A gene family of five to eight members encodes beta-tubulin. A number of missense point mutations were identified. In one beta-tubulin sequence, isolated from a resistant biotype, a mutation at amino acid 241 (converting arginine to lysine) is homologous to a mutation that confers resistance to anti-microtubule fungicides, in a yeast beta-tubulin.

Introduction

Annual bluegrass, *Poa annua* L., is a cool season grass with both annual and perennial biotypes. Because of traits such as vegetative vigor, shade tolerance, prolific seed production, etc., turfgrass scientists are trying to develop improved varieties of *P. annua* with desirable traits primarily suited for the golf course industry. In the southeastern U.S., annual bluegrass is considered a very troublesome weed to control in managed turfgrass situations. In general, herbicide resistant weeds continue to be problems in many agricultural situations. Resistance to triazine has been reported for *P. annua*. Recently, at least one dinitroaniline herbicide (DNAH-) resistant biotype of annual bluegrass was reported from North Carolina.

The DNAHs selectively inhibit growth of annual grasses and exert their phytotoxic effect by disrupting microtubule polymerization. Microtubules are proteinaceous structures principally composed of tubulin heterodimers, which are themselves made up of an alpha- and a beta-tubulin protein subunit. The objectives of this study were to characterize populations of annual bluegrass with respect to DNAH-resistance, as well as their general growth parameters. This was accomplished by monitoring root initiation and morphology in the presence of the DNAHs and measuring shoot number, shoot weight, root weight, number of inflorescences, seed weight and germination. These parameters were compared between our unknown collections and those of wildtype susceptible and previously reported resistant plants [Isgrigg III and Yelverton, 1999]. This information was used to determine if the competitive advantage of herbicide resistance results in a disadvantage in another trait. In addition, a preliminary molecular biological analysis of the ß-tubulin genes was carried out on resistant and susceptible biotypes to identify a lesion correlated for DNAH resistance. Here we report our findings regarding the existence of two resistant biotypes, their levels of resistance and the organization of the ß-tubulin gene family.

Results and Discussion

Assumption: for every competitive advantage in one situation (e.g., simazine resistance) there is a corresponding disadvantage (e.g., lower photosynthetic efficiency) under non-selective conditions. We compared and contrasted vegetative-growth parameters between two presumptive susceptible biotypes (CAs and SCs) and two presumptive resistant biotypes (SCr and NCr). CAs shoot and root mass was greatest, as early as 21 weeks after seed sowing, but always by 39 weeks. Although presumptive DNAH-resistant plants initially produced shoots more quickly than susceptible plants by study termination the resistant plants had significantly less canopy. Results from the shoot and root weight measurements showed a similar trend – by midway through the experiment biomass accumulation of susceptible plants was catching up to and surpassing that of the resistant plants. At termination, the growth parameters of the CAs biotype were significantly greater than the other three genotypes, and that of the NCr plants was significantly lower.

Measurements of reproductive capacity also were compared between populations. On average, the presumptive resistant plants formed inflorescences more quickly and to a greater extent than did the presumptive susceptible plants, but by study termination the South Carolina plants were significantly out-producing the CAs and the NCr plants.

The DNAHs affect root growth. Compared to untreated tillers those from presumptive susceptible plants (SCs) formed very short, discolored, bulbous or nodule-like roots in each of the three concentration of prodiamine. In contrast, tillers from South Carolina presumptive resistant plants (SCr) produced slender, white roots in the low concentration, but shorter than roots initiated in untreated tillers. In the medium and high prodiamine concentrations, SCr roots were discolored, short and club-shaped. Tillers from resistant North Carolina plants (NCr) formed white, normal looking roots in each prodiamine concentration, but their length was affected by the herbicide. The NCr tillers treated with the low prodiamine produced roots noticeably shorter than those on untreated NCr tillers. The two higher concentrations decreased root length further. The resistant plants from Georgia (GAr) were the least affected by prodiamine. GAr roots in low prodiamine showed normal color and morphology. Roots initiated in medium and high prodiamine were white in color, relatively slender, and compared favorably in length to roots on untreated tillers.

Reported in the Clemson Turf Field Day Program