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Evaluation of *Poa annua* L. Resistance to Mitotic Inhibiting Herbicides

A Thesis Presented for the Master of Science degree.

The University of Tennessee, Knoxville.

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THESIS ABSTRACT

Four bioassays were conducted to evaluate an ecotype of *Poa annua* L. from Chattanooga for resistance to prodiamine, pendimethalin, and dithiopyr herbicides. MS-media, filter-paper, and soil-based bioassays all evaluated root growth response in mature seed from the Chattanooga ecotype and a sensitive control. The hydroponics bioassay evaluated mature tiller response. All the bioassays were able to diagnose the Chattanooga population as resistant to prodiamine and pendimethalin. The soil-based bioassay was able to detect minimal resistance to dithiopyr. Additionally, the Chattanooga population exhibited variability of resistance to dinitroanilines.

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I. LITERATURE REVIEW

Biology of *Poa annua*

Annual bluegrass (*Poa annua* L.) is a problem turfgrass weed on golf courses, sports turf, and ornamental lawns (Hutto et al., 2004). Annual bluegrass is a native of Europe. The origin is believed to be in the northern Mediterranean (Gibeault, 1967) though it is now widely distributed throughout the world and is found in diverse regions such as New Foundland, Florida, California, and Alaska in North America (Beard et al., 1978). The geographical range of annual bluegrass emphasizes the importance of elucidating the current methods for managing annual bluegrass.

Annual bluegrass is easily identified by morphological characteristics. The seedhead has an “open pyramidal panicle 3-7 cm long, 4 mm long crowded spikelets; first bloom 1.5 to 2 mm, the second 2 to 2.5 mm long; lemma not webbed at base, distinctively 5-nerved, pubescent on the lower part of the keel and anthers 0.5 to 1 mm long” (Hitchcock et al., 1950). When mowed the size of the panicle is proportionally reduced. The leaf is folded with a keeled tip, glabrous, white at the base, with a small (1-3 mm) membranous ligule (Beard et al., 1978). Under closely mowed and well irrigated situations, such as those found in managed turf areas, annual bluegrass may form a dense uniform quality turf of intermediate leaf texture (Beard et al., 1978). Additionally, the height of the turf is inversely proportional to the fineness and density of the annual bluegrass until it is mowed to a length shorter than 6 mm (Beard et al., 1978).

While classically known as an annual, both annual (*Poa annua* ssp. *annua*) and perennial (*Poa annua* ssp. *reptans*) biotypes of *Poa annua* exist (Tutin, 1957). A perennial or annual biotype may inhabit the same geographical area but have unique ecological niches in that area. The perennial biotype is adaptive to closely mowed,

highly irrigated, frequently fertilized golf greens which is why it is considered a golf course weed (Breuninger, 1993). However, there is extensive biological and morphological variability in annual bluegrass even among different annual and perennial subspecies of annual bluegrass (McElroy et al., 2002). Many of these differences may be attributed to the varying ecological and turf management practices (McElroy et al., 2004).

Annual bluegrass lacks the aesthetic and utility characteristics of traditional turfgrass species such as bermudagrass (*Cynodon dactylon*) and creeping bentgrass (*Agrostis stolonifera* L.). Additionally, annual bluegrass is more prone to injury or death from heat stress than other turfgrasses (Beard et al., 1978). In the southeast, where persistent temperatures over 32 C with high humidity are common in the summer, annual bluegrass dies, which results in unsightly bare soil (Beard et al., 1978). During these intense summer conditions annual bluegrass is more susceptible to disease. A particular fungus active during the summer months *Colletotrichum graminicola* or foliar anthracnose is devastating to annual bluegrass (Turgeon et al., 2004). Moisture stress in the summer may lead to the development of this disease which is characterized by a bronze appearance and an overall thinning of turf in greens and fairways. In more arid climates annual bluegrass is not favored for cultivation due to its inability to tolerate drought conditions when compared to some other grasses such as Kentucky bluegrass (*Poa pratensis* L.) (Hitchcock et al., 1950).

Though easily injured under certain conditions, annual bluegrass is considered an opportunistic grass which often invades turfgrass stands weakened by management errors such as over-watering, or out competes a non-aggressive bentgrass cultivar (Beard et al., 1978). Areas which have been mechanically damaged, such as greens with unattended

ball marks and heavily divoted tee boxes, are optimal places for annual bluegrass.

Another management practice contributing to annual bluegrass infestation is mowing in the late fall on or around fairways and greens, this will spread seed to new locations.

Annual Bluegrass Control

One of the most effective methods for controlling annual bluegrass is the utilization of preemergence herbicides in established turf (Callahan et al., 1992). A preemergence herbicide has minimal effect on established turfgrass. Therefore, the application of a preemergence herbicide in mature turfgrass will preserve the grass's ornamental and playability features while controlling weeds which germinate in the turfgrass area. Effective preemergence herbicides include oxadiazon (oxadiazole family), dithiopyr (pyridine family), prodiamine, pendimethalin (dinitroaniline family) and simazine (Kelly, 1998). Oxadiazon has been found to provide the most control of annual bluegrass; however, it is more expensive than other control strategies and causes significant damage to several host turfgrasses (Gibson et al., 1998). Dinitroanilines and other mitotic inhibiting herbicides are the most common type of preemergence herbicides for controlling annual bluegrass and other weed infestation in turf and ornamental lawn settings. Common dinitroaniline herbicides used in turf include prodiamine (Barricade¹), pendimethalin (Pendulum Aquacap²), Oryzalin (Surflan³), and benefin (Balan⁴). The chemical structure of a dinitroaniline consists of a benzene ring with an aniline group

¹ Barricade TM 4FL, Syngenta Crop Protection, Inc., Greensboro North Carolina 27409

² Pendulum 1 PT, BASF Agricultural Products, 26 Davis Drive, Research Triangle Park, NC 27709

³ Oryzalin 4 PRO, Dow Agrosciences, LLC, P.O. Box 21365, Seattle, WA 98111

⁴ Balan L, Elanco Products Co., 740 South Alabama St. Indianapolis, IN 46285

substituted between two nitro groups on the ring (Senseman, 2007). Preemergence applications of dinitroanilines and dithiopyr are typically less expensive when compared to other control strategies. These herbicides all inhibit microtubule formation.

Microtubule Formation in Plants

Plant microtubules are involved in regulation of multiple cellular processes such as cell division, intracellular transport, organization, and motility. The four basic microtubule structures in plants are the cortical interphase array, the preprophase band, the phragmoplast, and the mitotic spindle (Vassileva et al., 2005). The functions of the aforementioned structures vary in accordance to the stage of the cell cycle (Williamson, 1991). Microtubules are made up of long polymers of the protein tubulin. Alpha and Beta tubulin form heterodimers at the microtubule-organizing center (MTOC) in a process called nucleation. These α - β dimers polymerize away from the MTOC to form microtubules. The β -tubulin has a binding site for the active guanine tri-phosphate (GTP), though the α -tubulin has a bound GTP as well. When a tubulin dimer is added to the microtubule, the β -GTP is hydrolyzed (Downing et al., 1998). The hydrolysis of GTP causes a kink in the microtubule structure that subsequently has been shown to initiate depolymerization of a tubulin molecule (Fygenson, 1997). When tubulin dimers are added faster than the GTP is hydrolyzed, a stable GTP cap is formed on the positive end and depolymerization does not occur. Microtubules involved in cell division undergo phenomena called dynamic instability (Walker et al., 1988). Dynamic instability has two phases, catastrophe and rescue. The first phase is the transition from growth to disassembly or depolymerization and is labeled catastrophe. The subsequent phase of

reassembly and polymerization is termed rescue. Due to the polarity of the α/β heterodimers, the rate at which catastrophe and rescue occur varies between either ends of the microtubule. Typically the positive end gains and loses tubulin heterodimers faster than the negative end, which is anchored at the MTOC. If the negative end is not anchored to the MTOC the negative end loses dimers while the positive end gains, this phenomena is termed treadmilling (Margolis and Wilson, 1978). Microtubules able to treadmill are potentially very mobile. Other important proteins involved in microtubule dynamics are microtubule associated proteins (MAPS). Some MAPS bind to the microtubule lattice and increase stability and polymerization of the microtubule by allowing for bundling and cross-linking interactions between adjacent microtubules (Chan et al., 1999; Whittington et al., 2001). Cross-linking of microtubules also impedes events which lead to catastrophe of the tubulin polymer. Regulatory kinases control the activity of MAPS. If a MAP is phosphorylated by a kinase, then it cannot bind to tubulin and is unable to stabilize the growing tubulin polymer. Additionally, factors such as the cellular concentration of GTP and calcium are important for gauging the potential of tubulin polymerization and microtubule turnover in plant cells (Vaughn et al., 1991).

Mode of Action of Mitotic Inhibiting Herbicides

Dinitroanilines and dithiopyr have similar modes of action in that they inhibit microtubule formation (Senseman, 2007). Polymerization of microtubules is prevented when dinitroanilines bind to tubulin (Vaughn et al., 1991). When the dinitroanilines are bound to tubulin it is unable to polymerize into a microtubule (Vaughn et al., 1991). Additionally, depolymerization of the microtubule is not inhibited; thus, a net loss in

microtubules is observed (Anthony et al., 1999). It is believed that the most dynamic microtubules, such as the spindle kinetochore, and phragmoplast, are the most affected by dinitroanilines (Vaughn et al., 1991). Without microtubules the spindle apparatus is unable to form during mitosis; subsequently, the sister chromatids are not able to segregate during anaphase, and cell division is halted. A cell wall does not form at telophase. Instead of multiple cell formation, a square polyploidy cell forms (Anthony et al., 1999). Dithiopyr directly binds to a MAP and inhibits the binding interaction between the MAP and tubulin. The growing tubulin polymer then becomes destabilized and a microtubule is unable to form. At the morphological level, germinating plants coming in contact with dinitroanilines or dithiopyr have stunted root growth as well as shoot growth inhibition (Senseman, 2007). The root tip is usually swollen and club shaped indicating the cells inability to differentiate and divide (Anthony et al., 1999).

Dinitroanilines bind to tubulins from different organisms with varying degrees of affinity (Anthony et al., 1999). Tests have revealed that mammalian cell tubulin and dinitroanilines have a low degree of interaction (Hughdall et al., 1993). An additional benefit of dinitroanilines is their ability to bind tightly to the soil limiting runoff and subsequent surface water and ground water contamination when applied in non-rainy conditions.

Dinitroaniline Resistant Weeds

The benefits of dinitroaniline herbicides have lead to continual applications in crop fields and turf. Repeated herbicide use can select for resistant weeds, especially at suboptimal application area annually or biannually selection pressure will develop

favoring resistant weeds in the targeted area (Gressel et al., 1978). Therefore, the probability of herbicide resistant type weed infestation increases every year the same herbicide is sprayed. In turfgrass areas, to maximize the aesthetic value of the turf, a spray rate of 100% kill of the target weed should be obtained. The desired kill rate was not achieved in North Carolina where prolonged spraying of dinitroanilines on golf courses selected for dinitroaniline resistant annual bluegrass (Isrigg et al., 2002). Though dinitroanilines have been known to induce somatic mutations in bermudagrass cultivars, (Capo-chichi et al., 2005) the resistance seen in the North Carolina annual bluegrass is most likely from herbicidal selection pressure.

Effect of Soils on Herbicide Persistence

Soil conditions have a large impact on the effectiveness of herbicides. Persistency of herbicides is influenced by organic matter, soil temperature, soil moisture, and microbial activity. Under normal moisture conditions Kulshrestha and Singh (1992) found there was 14% more degradation of pendimethalin in non-sterile than sterile soil over 90 days. However, combinations of the aforementioned factors can have a profound effect on herbicide stability. In flooded non-sterile soil pendimethalin has been shown to completely degrade in 4 days (Kulshrestha and Singh, 1992). One of the reasons for the rapid degradation may be attributed to chemical reactions which occur in anaerobic conditions created by the flooded soil (Kulshrestha and Singh, 1992). Elimination of a dinitroaniline from the soil in 4 days greatly reduces control of a target weed. When reduced control not attributed to management error is observed, soil analysis should be performed to help clarify whether the weed is resistant or the herbicide was not effective in the soil.

Molecular Biology of Dinitroaniline Resistance

Resistance to tubulin drugs in multiple organisms has been attributed to mutations in tubulin, which either affect the binding site of the chemical or increase the stability of the microtubule. Mutation sites which exhibited the most resistance occurred in the α -helix 7 (Morrisette et al., 2004). The only mutation seen in plants on α -helix 7 is a substitution of isoleucine for threonine at amino acid residue 239 which was present in both goosegrass (*Eleusine indica*) and green foxtail (*Setaria viridis*). Transformation of maize with gene constructs containing the isoleucine substitution and subsequent herbicide assays proved that the mutation was responsible for herbicide resistance (Anthony et al., 1998). The threonine at position 239 is positioned at the end of the central helix 7 located near a conserved tyrosine 24 residue. A dinitroaniline resistant *Chlamydomonas reinhardtii* mutant contained a histidine residue at position 24 (Anthony et al., 1999). Therefore, it has been hypothesized that both threonine 239 and tyrosine 24 are part of the herbicide binding site (Anthony et al., 1999). Alternatively because of the exterior location of residue 239 the mutation may affect the stability of the microtubule (Anthony et al., 1999). The substitution of a hydrophobic branched chain amino acid for a hydrophilic amino acid effects the folding of the α/β dimer. Differential folding of the dimer will affect dynamic instability, microtubule turnover, and overall stability of an active microtubule.

Analysis of annual bluegrass α -tubulin gene and protein in suspected dinitroaniline resistant ecotypes will help clarify the mechanism of resistance. Due to the economic importance of annual bluegrass control with dinitroaniline herbicides an effective monitoring system should be established; subsequently, development of a rapid

screen such as a bioassay or DNA assay for resistant biotypes should be the next step for weed scientists.

Screen for Herbicide Resistance

Useful resistance assays would be beneficial for monitoring dinitroaniline resistance in annual bluegrass is the development of an efficient screening process (Kelly et al., 1998). Currently, a field assessment of dinitroaniline resistance requires a six to eight months should be considered to obtain ripened seed, harvest field plants in spring, allow panicles to develop, harvest seed, allow seed to ripen and evaluate resistance in a greenhouse or laboratory environment. Due to the long time period before a diagnosis that occurs when mature seed is needed for analysis, a complete assessment of resistance will not be ready by the time the turf manager is ready to apply fall preemergence herbicides. Therefore, the turf manager may switch to a more expensive control program when there is no resistant annual bluegrass or may spray a dinitroaniline herbicide when the annual bluegrass is resistant, spending time and money on an ineffective process.

An initial characterization of a potentially herbicide-resistant weed biotype requires a scrutinized dose response (Beckie et al., 2000). A dose response should be generated across a range of herbicide concentrations, and plant response should be compared to a known herbicide sensitive biotype of the weed (Beckie et al., 2000). An ideal herbicide resistance screen should take a short amount of time and minimize confounding variables. Greenhouse screens utilizing soil growth medium have been used for screening herbicide resistance. Factors such as leaching and microbial degradation

are impossible to control. In such studies variation in soil medium will not allow direct comparison of dose response between locations.

A more efficient technique used to screen for dinitroaniline resistance is a Petri dish bioassay in which blackgrass seeds were placed in a dish containing three layers of filter paper and herbicide covered by a single layer of glass fiber paper (Beckie et al., 2000). Dishes were placed in polyethylene bags and incubated for 21 days at 18 C. Potential confounding variables include evaporation and condensation inside the bag which can change the concentrations of herbicide potentially confounding the results.

An improved dinitroaniline resistance screening process is needed to provide a more scrutinized dose-response curve. Utilizing sterile growth media such as MS media (Murshaige and Skoog, 1962) for a dinitroaniline resistant in vitro assay has not been evaluated. No published method for screening dinitroaniline resistance in annual bluegrass has utilized a sterile environment for seed germination and growth, while providing a way to accurately control the amount of herbicide in the environment. The growth media screen will be compared to the Petri dish bioassay and a greenhouse screen. A second potential screen will utilize hydroponics supplemented with dinitroaniline herbicide to analyze root and tiller morphology from annual bluegrass taken from the field when seed is not available. This screen will take 10 days and provide an alternative to seed germination screens. Due to the dynamic nature of a liquid substrate hydroponics has substantial confounding variables. Induction of callus from shoots/roots in tissue culture media (Artunduaga et al., 1988) supplemented with herbicides could be a more definitive vegetative screen. However, utilizing hydroponics to obtain a diagnosis is much faster than tissue culture. A PCR based assay focusing on

identifying mutations conferring resistance in the tubulin gene may provide the most rapid assessment of resistance (Deleye et al., 2005).

REFERENCES

- Anthony, R.G., and P.J. Hussey. 1997. Generation of dinitroaniline and phosphorothioamidate resistant transgenic plants using a mutant α -tubulin. *Cell Biol. Intl.* 21:37-41.
- Anthony, R.G., and P.J. Hussey. 1999. Dinitroaniline herbicide resistance and the microtubule cytoskeleton. *Trends Plant Sci.* 3:112-116.
- Anthony, R.G., and P.J. Hussey. 1999. Double mutation in *Eleusine indica* α -tubulin increases the resistance of transgenic maize calli to dinitroaniline and phosphorothioamidate herbicides. *Plant J.* 18:669-674.
- Anthony, R.G., T.R. Walden., J.A. Ray., S.W.J. Bright., and P.J. Hussey. 1998. Herbicide resistance caused by spontaneous mutation of the cytoskeleton protein tubulin. *Nature* 393:260-263.
- Artunduaga, I. R., C.M. Taliaferro., and B.B. Johnson. 1988. Effects of auxin concentrations on embryogenic callus induction from cultured young inflorescences of Old World bluestem (*Bothriochloa spp.*) and bermudagrasses (*Cynodon spp.*) *Plant Cell Tiss. Org Cult.* 12:13 -19.
- Breuninger, J. 1993. *Poa annua* control in bentgrass greens. *Golf Course Mngt.* 61: 68-73.
- Baird, W.V., L. Zeng., and E. Yamamoto. 1998. A tubulin missense mutation correlate with anti-microtubule drug resistance in *Eleusine indica*. *Plant Cell.* 10:297-308.

- Beard J.B., P.E. Rieke., A.J. Turgeon., and J.M. Vargas Jr. 1978. Annual Bluegrass (*Poa annua* L.) Description, Adaptation, Culture and Control. Res. Rep. 352. Michigan State Univ. Agric. Exp. Stn.
- Beckie, H.J., I.M. Heap., R.J. Smeda., and L.M. Hall. 2000. Screening for herbicide resistance in weeds. *Weed Technol.* 14:428-445.
- Callahan, L. M., and E.R. McDondald. 1992. Effectiveness of bensulide in controlling two annual bluegrass (*Poa annua*) subspecies, *Weed Technol.* 6:97-103.
- Capo-chichi, L., J. M. Goatly Jr., W. Philley., J. Krans., D. Davis., A. Kato., and E. Van Santen. 2005. Dinitroaniline -induced genetic changes in bermudagrass. *Crop Sci* 45:1504-1510.
- Chan, J., C.G. Jensen, L.C.W. Jensen, M. Bush, and C.W. Loyld. 1999. The 65 kDa microtubule associated protein forms regularly arranged filamentous cross-bridges between microtubules. *Proc Natl Acad Sci USA.* 96: 14931-14936.
- Cho, M.J., P.G. Lemeauz, and C.D. Ha. 2001. Stable transformation of a recalcitrant Kentucky bluegrass (*Poa Pratensis* L.) cultivar using mature seed-derived highly regenerate tissue. *In Vitro Cell. Dev. Biol.-Plant* 37:6-11.
- Darmency, H., C. Delye., Y. Menchari. 2004. Molecular basis for sensitivity to tubulin-binding herbicides in green foxtail. *Plant Physiol.* 136:3920-3932.
- Delye, C., Y. Menchari., and S. Michel. 2005. A single polymerase chain reaction-based assay for simultaneous detection of two mutations conferring resistance to tubulin-binding herbicides in *Setaria viridis*. *Weed Research* 45:228-235.
- Downing, K.H., E. Nogales, and S.G. Wolf. 1998. Structure of the α - β tubulin dimer by electron crystallography. *Nature* 391: 199-202.

- Drewes, G., A. Ebner, and E-M. Mandelkow. 1998. MAPs, MARKs and microtubule dynamics. TIBS 23-August 307-311.
- Fygenson, D. K., 1997. A unifying hypothesis for the conformational change of tubulin. PhD dissertation. Department of Physics, University of California, Santa Barbara.
- Gibeault, V.A. 1967. Investigations on the control of annual meadow-grass. J. Sport Turf Research Institute 42:17-40.
- Gibson, E.S., L.B. McCarty, J.K. Higgingbottom, and F.C. Waltz. 1998. *Poa annua* control in overseeded bermudagrass Turf. Proc. South. Weed Sci. Soc. 51: 69-70.
- Gressel, J. 2002. Molecular Biology of Weed Control. Taylor and Francis. New York, New York. p.576.
- Gressel, J., and L.A. Segel. 1978. The paucity of plants evolving genetic resistance to herbicides: possible reasons and implications. J. Theor Biol. 75:349-371.
- Hitchcock, A. S. and A. Chase. 1950. Manual of the grasses of the United States. USDA Miscellaneous Publication No. 200. pp 1-1051.
- Hugdahl, J.D., and L.C. Morejohn. 1993. Rapid and reversible high-affinity binding of the dinitroaniline herbicide oryzalin to tubulin from *Zea mays* L. Plant Physiol. 102:725-740.
- Hutto, K.C., G.E. Coats, and J.M. Taylor. 2004. Annual bluegrass (*Poa annua*) resistance to simazine in Mississippi. Weed Technol. 18:846-849.
- Isgrigg, J. III., F.H. Yelverton., C. Brownie., and L.S. Warren Jr. 2002. Dinitroaniline resistant annual bluegrass in North Carolina. Weed Sci. 50:86-90.

- Kelly, S. T., G. E. Coats, and D.S. Luthe. 1998. Identification and assessment of simazine resistance in annual bluegrass (*Poa annua* L.) Ph.D. dissertation. Mississippi State University, MS. 41.
- Matsuo, H. and K.U. Kobayashi. 1996. Variation in reproductive allocation and propagule output of *Poa annua* L. in Golf Course. Grassland Sci. 42:193- 201.
- Margolis, R.L., and L. Wilson. 1978. Opposite end assembly and disassembly of microtubules at steady state *in vitro*. Cell. 13:1-8.
- McElroy, S.J., R.H. Walker, and E. Santen. 2002. Patterns of variation in *Poa annua* populations as revealed by canonical discriminant analysis of life history traits. Crop Sci. 42:513-517.
- McElroy, S.J., R.H. Walker and G.R. Wehtje. 2004. Annual bluegrass (*Poa annua*) populations exhibit variation in germination response to temperature, photoperiod, and fenarimol. Weed Sci. 52:47-52.
- Murashige, T., and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum. 15:473-497.
- Park, B.S., and P.J. Landschoot. 2003. Effect of dazomet on annual bluegrass emergence and creeping bentgrass establishment in turf maintained as a golf course fairway. Crop Sci. 43:1387-1394.
- Senseman, S.A. 2007. Herbicide Handbook Weed Science Society of America. Ninth Edition. p.14.
- Sibely, L.D., N.S. Morissette., D. Sept., and A. Mitra. 2004. Dinitroanilines bind α tubulin to disrupt microtubules. MBC. 15:1960-1968.

- Turgeon, A.J., and J.M. Vargas, Jr. 2004. *Poa annua* Physiology, Culture, and Control of Annual Bluegrass. John Wiley & Sons, Inc., Hoboken, New Jersey pp. 4-16.
- Tutin, T.G. 1957. A contribution to the experimental taxonomy of *Poa annua* L. *Watsonia*. 4:1-10.
- Vassileva, V.N., Y. Fujii, R.W. Ridge. 2005. Microtubule dynamics in plants. *Plant Biotechnology*. 22: 171-178.
- Vaughn, K.C. and L. P. Lehn. 1991. Mitotic disruptor herbicides. *Weed Sci.* 39:450-457.
- Walker, R.A., E.T. O'Brien, N.K. Pryer, M.F. Soboleiro, W.A. Voter, H.P. Erickson, and E.D. Salmon. 1988. Dynamic instability of individual microtubules analyzed by video light microscopy: Rate constants and transition frequencies. *J. Cell Biol.* 107: 1437-1448.
- Whittington, A.T., O. Vugreck, K.J. Wei, N.G. Hassenbein, K. Sugimoto, M.C. Rashbrooke, and G.O. Wasteneys. 2001. MOR1 is essential for organizing cortical microtubules in plants. *Nature*. 411: 610-613.
- Williamson, R.E. 1991. Orientation of cortical microtubules in interphase plant cells. *Int Rev Cytol.* 129: 135-206.

II. COMPARISON OF FOUR BIOASSAY METHODS FOR DETECTION OF MITOTIC-INHIBITING HERBICIDE RESISTANT ANNUAL BLUEGRASS.

ABSTRACT

Mitotic- inhibiting herbicides such as dinitroanilines are commonly used to control annual bluegrass (*Poa annua* L.) in established turf settings. Dinitroaniline resistant annual bluegrass has been reported in several states; however, there are no standardized screening methods for detecting resistance. Research was conducted to evaluate screening techniques for detection of dinitroaniline resistance in annual bluegrass. Four bioassays, Murashige and Skoog (MS) media, filter-paper, hydroponics, and soil-based were utilized to evaluate the responses of a suspected resistant ecotype of annual bluegrass from Chattanooga, TN (Chattanooga) and a sensitive ecotype purchased from Fresno, CA (Control) to dithiopyr, prodiamine, and pendimethalin. All the bioassays were able to diagnose the ecotype from Chattanooga as resistant to prodiamine and pendimethalin. Minor dithiopyr resistance from the Chattanooga ecotype was detected by the hydroponic and soil-based bioassays. Hydroponics provided the quickest diagnosis of resistance. The MS-media bioassay had the least confounding variables. These findings highlight the potential variation in results that can occur in mitotic inhibiting herbicide resistance detection simply based on how plant samples are assayed.

INTRODUCTION

Annual bluegrass (*Poa annua* L.) is a common and troublesome weed throughout the world in turfgrass environments (Hall and Carey, 1992). Numerous ecotypes of annual bluegrass exist that are adaptive to diverse areas from golf course putting greens to turfgrass seed production fields (McElroy et al., 2002). The light-green color and abundant seedhead yield of annual bluegrass, even at golf putting green heights, disrupt uniformity and causes unsightly and uneven turfgrass appearance. Annual bluegrass is very susceptible to disease and drought; thus, subsequent death occurs during the summer months resulting in aesthetically displeasing spots in turfgrass stands (Beard et al., 1978).

Preemergence herbicide applications are a common method for controlling annual bluegrass (Callahan et al., 1992). Preemergence herbicides preserve the ornamental and playability features in mature turfgrass while controlling annual bluegrass which germinates in the herbicide layer. Effective preemergence herbicides include oxadiazon (oxadiazole family), dithiopyr (pyridine family), prodiamine and pendimethalin (dinitroaniline family).

Because of their low costs, dinitroanilines and dithiopyr, compared to oxadiazon are commonly used for control. They also have the ability to bind tightly to the soil and have relatively low toxicity to humans (Hughdall et al., 1993). Dinitroanilines and dithiopyr have the same mode of action in that they inhibit microtubule formation (Senseman, 2007). At the morphological level, when germinating plants come in contact with dinitroanilines or dithiopyr, they have stunted root growth as well as shoot growth inhibition (Senseman, 2007). The root tip is usually swollen and club shaped indicating the cells are unable to differentiate and divide (Anthony et al., 1999). However, at the

protein level, the target sites differ. Dinitroanilines directly bind to the eukaryotic protein tubulin preventing the polymerization of tubulin into microtubules (Vaughn et al., 1991). Without microtubules the spindle apparatus is unable to form during mitosis; subsequently, the sister chromatids are not able to segregate during anaphase and cell division is halted. The target protein for dithiopyr is a microtubule associated protein (MAP) which assists in stabilizing microtubules involved in cell division (Vencill, 2002). When dithiopyr is bound to the MAP, polymerization of microtubules does not occur.

Multiple weeds have evolved resistance to dinitroanilines, however no documented cases of resistance to dithiopyr have occurred (Heap, 2007). One of the first economically important weeds to evolve resistance to dinitroanilines was goosegrass (*Elusine indica*; Mudge et al., 1984). Mudge et al. (1984) reported decreased control of a resistant biotype of goosegrass with trifluralin when compared to a susceptible biotype. Dinitroaniline resistant goosegrass now infests thousands of cotton fields in the Carolinas, Tennessee, Georgia, and Alabama (Heap, 1997). In North Dakota green foxtail (*Setaria viridis*) evolved resistance to trifluralin after 15 years of application in oilseed crop fields (Heap, 1997). The first case of annual bluegrass resistance to dinitroanilines was reported by Isrigg et al. (2002) in North Carolina. Prolonged spraying of prodiamine on golf course fairways putatively has selected for this resistant ecotype.

The most important criteria in developing a program to screen, evaluate, and monitor herbicide resistance is the selection of an herbicide bioassay. The first step is to develop a dose response curve to determine the statistical differences between the growth of the resistant and susceptible biotypes (Beckie et al., 2000). There are two bioassay techniques that have been utilized to test for suspected resistance to mitotic inhibiting

herbicides: soil-based and filter-paper bioassays. The soil-based bioassay utilized containers in a climate controlled growth chamber or greenhouse (Mudge et al., 1984). A known sensitive population of goosegrass and a suspected resistant population were planted in the containers and sprayed with seven different dinitroaniline herbicides (Mudge et al., 1984). After four weeks the plants were excised from the containers and dry-weight data were recorded. Isrigg et al. (2002) utilized a filter paper bioassay in a growth chamber to evaluate annual bluegrass shoot and root response to dinitroanilines. Seeds from a sensitive population and suspected resistant population were placed in petri-dishes with filter paper. Aliquots of dinitroanilines were transferred to filter paper and root and shoot data were recorded after three weeks.

Both the soil-based and filter-paper bioassays were effective in diagnosing weed resistance to dinitroanilines; however, both methods have potential confounding variables that could limit the bioassay techniques. The soil-based bioassay is limited by variation in soil type, microbial degradation of the herbicide in pot culture, and potential herbicide leaching. While Mudge et al. (1984) collected roots from seedlings, it is difficult to remove roots from soil without confounding damage. Thus, the soil-based bioassay is best limited to the collection of seedling emergence data. The filter-paper bioassay is limited by the difficulty of sterilizing the environment and volatilization problems, i.e., filter paper can dry out and alter actual herbicide concentrations. Root and shoot length can be measured in the filter paper bioassay; however, roots often grow horizontally or are imbedded into the filter-paper, which confounds accurate root-length measurements. Additionally, both the soil-based and filter-paper bioassays need mature seeds, causing delays of several months in evaluation results.

Currently, there is no standardized screening method for detecting dinitroaniline resistant annual bluegrass. If annual bluegrass seeds suspected of being resistant are evaluated in a traditional greenhouse or growth chamber assay, the researcher must allow the plants to senesce, set seed, allow the seed to ripen, and finally evaluate resistance in a greenhouse setting. The time period for this experiment may be over 6 months and the results are not available for the turf manager until after they have sprayed their fall preemergence herbicides (Table 2.1)⁵. Therefore, applying dinitroanilines on resistant annual bluegrass is costly and may enhance a resistance problem. A useful herbicide resistance screen will be: rapid, minimize confounding variables, and able to characterize the degree of resistance (Beckie et al., 2000). The most important aspect in evaluating resistance to preemergence herbicides such as dinitroanilines is obtaining a rapid diagnosis. Usually resistance assays require mature ripened seed for analysis. There is a need to explore mature plant response bioassays to preemergence herbicides and adopt a standardized screening method for detection of dinitroaniline resistance.

MATERIALS AND METHODS

Bioassay methods included two previously utilized methods- filter-paper and soil-based; as well as two additional methods, MS-media and hydroponic. In these evaluations, two populations were utilized. Population one (Control) was purchased from Valley Seed Service, Fresno, Ca. The Control population has no known resistance to any herbicide mode-of action and originated from production fields in Oregon (McElroy et al., 2002). A second population (Chattanooga) was collected at Eagle Bluff Golf Course

⁵ All figures and tables are located in the Appendix

near Chattanooga, TN. Chattanooga has suspected resistance to mitotic inhibiting herbicides from repeated prodiamine usage. Plants were collected from Eagle Bluff in May 2004. Plants were propagated in a greenhouse environment ($28\text{ C} \pm 4\text{ C}$) and seeds were collected for future evaluations.

Mitotic-inhibiting herbicides dithiopyr (Dimension Ultra, Dow AgroSciences LLC, Indianapolis, IN), prodiamine (Barricade 4FL, Syngenta Crop Protection, Inc., Greensboro NC) and pendimethalin (Pendulum AquaCap, BASF Agricultural Products, Research Triangle Park, NC) were utilized in each of the four bioassays. The hydroponic, MS-media, and filter-paper bioassays incorporated rates at the mM level. For the soil-based screen the herbicides were spray applied to the pot. The greenhouse, MS-media, and filter-paper screen were preemergence bioassays requiring mature seed. Annual bluegrass subjected to hydroponics was grown in a greenhouse and subsequently transferred to hydroponics. All bioassays were conducted as a completely randomized design with factorial arrangement of treatments. For all bioassays, factorial arrangement included three herbicides types by five herbicide concentrations by two annual bluegrass ecotypes.

In the germination bioassays the seeds were sterilized in 10% sodium hypochlorite (commercial bleach) supplemented with 0.1% enzyme grade Tween (Fisher Scientific, Pittsburg, PA) surfactant for 20 minutes. Subsequently, three ethanol washes and two water washes were performed for 1 minute durations each. The seeds were left under a laminar flow hood to dry for 24 hours. In order to maximize germination rates, seeds were imbibed in filter sterilized calcium sulfate ($20\text{ }\mu\text{M}$) at 4 C for 7 days prior to

utilizing in each bioassay. Petri-dishes were sealed with paraffin wrap (Nesco Film, Cottonwood, AZ) to prevent evaporation and contamination.

MS-Media Bioassay

Murashige and Skoog (MS) media supplemented with vitamins, pH 5.8, was utilized as the growth medium. (Murashige and Skoog 1965; Gamborg et al., 1968). The media contained pendimethalin, dithiopyr or prodiamine at 0, 0.01, 0.1, 1.0, and 10.0 mM concentrations. The plates were set vertically on racks and incubated at 26 C, 24 h photoperiod, and a photon flux density of $21 \mu\text{mol m}^{-2} \text{s}^{-1}$. Six seeds were placed in each media plate and replicated nine times for each herbicide by concentration interaction. After two weeks root length was measured.

Filter-Paper Bioassay

The filter paper bioassay utilized sterilized seed, petri-dishes (10 cm diameter, 1.5 cm depth; Thermo Fisher Scientific, Waltham, MA), and filter paper (9 cm diameter, 520 mg; Whatman[®] Filter papers, Whatman International Ltd, Maidstone, England). Dithiopyr, prodiamine, and pendimethalin concentrations of 0, 0.01, 0.1, 1.0, and 10.0 mM were prepared utilizing formulated product combined with tap water. Annual bluegrass seeds were placed between two filter paper sheets within a petri-dish and 4 ml of a given herbicide concentration were transferred into each dish to completely saturate the filter paper. Six seeds were placed in each petri-dish and replicated nine times for each herbicide by concentration interaction. Petri-dishes were incubated for 2 weeks at 26 C, 24 h photoperiod, and a photon flux density of $21 \mu\text{mol m}^{-2} \text{s}^{-1}$. After 2 weeks root length was measured.

Hydroponic Bioassay

The hydroponics bioassay utilized mature tillers that were excised from both populations from greenhouse pot-grown plants. Shoots and roots were trimmed to 3 cm. A 25% strength Hoagland solution (Hoagland and Arnon, 1950) with additions of pendimethalin, prodiamine, or dithiopyr (0, 0.01, 0.1, 1.0, and 10.0 mM) was used. A total of 800 ml was poured into 950 ml volume plastic tub containers (Takealongs, Rubbermaid[®], Fairlawn, OH). One cm diameter holes were drilled into the lid covering the tub. The tub was sprayed with silver spray paint in order to prevent algae growth. Air pumps (60 gallon Whisper Air Pump, Tetra[®], Blacksburg, VA) connected to aquarium bubble stones (Aqua culture, Wal-Mart Stores, Inc. Bentonville, AR) were utilized to agitate the Hoaglands solution. Ten tillers were added to each herbicide concentration with two replications. After 2 weeks root length was measured.

Soil-Based Bioassay

Ten seeds of each population were placed in 10 cm diameter greenhouse pots containing Sequatchie loam soil (fine-loamy siliceous, semi-active, thermic Humic Hapludults) with 2.1% organic matter and a pH of 5.8 . Pendimethalin and prodiamine were applied at 0.28, 0.56, 1.12, and 2.24 kg ha⁻¹. Dithiopyr was applied at 0.14, 0.28, 0.56 and 1.12 kg ha⁻¹. A CO₂ pressurized sprayer was utilized for application at a water carrier volume of 280 L/ha. Applications were made at the University of Tennessee Plant Biotechnology Building, Knoxville TN. Three pots were used per concentration per population including three for an untreated check. Percent germination (visual count of emerged shoots) was recorded for each pot 2 and 3 weeks after application.

Data Analysis

Data were subjected to ANOVA ($\alpha = 0.05$) using mixed model methodology (Statistical Analysis Software v. 9.1, Cary, NC). ANOVA was conducted separately for each individual bioassay. All bioassays were analyzed according to their factorial arranged completely random design, main plot factors of herbicide and herbicide concentration were considered fixed and replication was considered random. For MS media, filter paper, and hydroponic individual seed or tillers were analyzed as subsamples with each treatment. For the soil-based bioassay, number of emerged seedlings was pooled over each pot. Concentrations were natural log-transformed and the non-treated was adjusted to maintain equal spacing among treatments. Regressed equations were calculated based on statistical significance and biological meaningful interpretation. The lethal dose (LD) to reduce root and shoot growth by 50 (LD_{50}) and 90% (LD_{90}) was calculated as a means of comparing among bioassays.

RESULTS AND DISCUSSION

All herbicides had a significant effect on root growth ($p < 0.05$) for both populations in the petri-dish, MS-media, and hydroponic screens. Herbicides had a significant effect ($p < 0.05$) on percent emergence for both population in the greenhouse screen. Ecotype response to the individual bioassays will be discussed first, followed by comparison of bioassay methods.

MS Media

The Chattanooga population exhibited more root growth in prodiamine and pendimethalin than the control population in the MS-Media bioassay (Fig. 2.1 and 2.2).

Chattanooga is less tolerant to pendimethalin than prodiamine with total root length growth inhibition occurring at 1.0 mM pendimethalin. However, Chattanooga root growth was 22 mm at 0.01 mM pendimethalin, which was significantly greater than Control root growth of 2.0 mm. Dithiopyr completely inhibited root growth for both ecotypes at 0.01 mM (Fig. 2.3).

Filter paper

Similar results were seen in the filter-paper as in the MS media. Chattanooga root growth decreased as prodiamine concentrations increased from 0.01 to 0.1 mM; however, root length was >10 mm from 0.1 to 10.0 mM. A decline in Control root growth was observed between 0 and 1.0 mM prodiamine with complete inhibition occurring at 10.0 mM prodiamine (Fig. 2.4). Both ecotypes followed similar trends in their response to pendimethalin (Fig 2.5). Root growth of both ecotypes decreased from 0.01 to 10.0 mM but Chattanooga root growth was greater than Control at each concentration except 10 mM. Both ecotypes exhibited root growth at 0.01 mM dithiopyr, but root growth was inhibited at 0.1 mM and greater (Fig 2.6).

Hydroponics

Chattanooga exhibited higher root growth in prodiamine and pendimethalin than Control in hydroponics (Fig 2.7 and 2.8). Complete inhibition was not observed in Chattanooga, root growth occurred at 10.0 mM in both prodiamine and pendimethalin. Chattanooga exhibited more tolerance to prodiamine than pendimethalin. Root growth for Control was completely inhibited at 10.0 mM. Apparent greater root growth was observed for both population in hydroponics than filter paper or MS media. Neither

ecotype was completely inhibited at 10.0 mM dithiopyr, where- Chattanooga root growth was >15 mm, Control was < 10 mm (Fig 2.9).

Soil-Based

Chattanooga exhibited higher percent emergence than Control at rates of 0.28 kg ha⁻¹ and higher of pendimethalin and prodiamine 2 and 3 weeks post application (Fig. 2.10 and 2.11). Low emergence rates were recorded for both populations for dithiopyr. However, Chattanooga did exhibit greater emergence at 0.14 kg ha⁻¹ dithiopyr than Control (Fig. 2.12). No emergence was recorded for either ecotype at applications of dithiopyr higher than 0.14 kg ha⁻¹. No increase was observed in percent emergence between 2 and 3 weeks for either population.

Regression Analysis

An effective way to assess the effect of a herbicide on a potential resistant ecotype is through a dose response curve generated from regression analysis (Beckie et al., 2000). Calculation of a predictive herbicide dose which inhibits a growth response by either 50 (LD₅₀) or 90 (LD₉₀) percent allows for relevant comparison between a known sensitive ecotype and suspected resistant ecotype. The Chattanooga population exhibited much greater root growth in the non –treated check in MS-media and filter-paper than the sensitive Control. Therefore, comparing LD₅₀ and LD₉₀ of the two ecotypes characterizes the degree of resistance by accounting for the mean growth response in the non-treated check.

Variability of Resistance to Mitotic Inhibiting Herbicides

Bioassays indicate that the Chattanooga population exhibits variable resistance to the two dinitroanilines. More resistance to prodiamine than pendimethalin was exhibited

by Chattanooga, particularly in the MS-media bioassay. LD₅₀ generated from regression analysis revealed that Chattanooga was about 80X more resistant to prodiamine than the sensitive control in MS-media (Table 2.2). Chattanooga was only 8.5X more resistant to pendimethalin than Control in MS media. The MS-media bioassay has the least amount of confounding variables of all the bioassays: therefore, the LD₅₀ generated from the media screens may be the most accurate description of the herbicides effect on root growth. Variability of resistance to dinitroanilines was also evident in the filter-paper bioassay.

Isrigg et al., (2002) reported variability in response to different dinitroanilines for their resistant bio-type. Other species have exhibited variable response to multiple dinitroanilines. In South Carolina a Palmer amaranth (*Amaranthus palmeria* S. Wats) resistant ecotype varied in response to six dinitroaniline herbicides (Smeda et al., 1994). In the Chattanooga ecotype multiple years of spraying prodiamine may have increased selection pressure for a mutation that optimizes tolerance against prodiamine. The low amount of resistance observed to dithiopyr does not suggest a mutation in a MAP is likely responsible for resistance, indicating that there might be non-target site resistance (Yuan et al., 2007). Regardless of resistance type, the Chattanooga *Poa annua* is the first reported case of increased tolerance to dithiopyr.

Comparison of Bioassay Methods

Based on our experience with all four bioassay methods, the MS-media bioassay had the fewest confounding variables and greatest ease of measurement. The vertical set up of media plates simplified root and shoot measurements. The even distribution of the herbicide throughout the media allowed for uniform contact with all seeds. The media

and seeds were sterile, which minimized fungal contaminants. Problems occurred in the MS- media bioassay when the medium collapses in the vertically positioned plates. In our assays we avoided this by utilizing a 2X rate of gelling agent, which improved solidification. The filter-paper bioassay was similar to the MS media in that it had uniform light distribution and sterilized seeds. The plates were grown horizontally, and roots grew in such a way into the filter paper, that root length measurements were more difficult to make than in the MS-media bioassay. Both the MS- media and filter- paper bioassay required mature seed, which prolongs time for definitive results compared with assays utilizing vegetative parts of suspected resistant plants.

The hydroponic diagnostic screen allowed for an assessment of resistance within ten days of harvesting the mature plants. Rapidity of this diagnostic technique gives a researcher a more immediate diagnostic test. Negative characteristics of the hydroponic screen included the degree of agitation needed to create uniform distribution of the herbicide, i.e., if the agitation is not appropriate a false result might be obtained. Relative to the other diagnostic tests the difference between Chattanooga and Control population's root length was not as great. Hydroponics would not be as valuable a tool for determining the degree of resistance as the other screens. Solubility of the active ingredient and the formulation utilized should be considered before performing the hydroponics bioassay. The solubility of prodiamine, pendimethalin, and dithiopyr in water was 0.013 mg/L, 0.275 mg/L, and 1.38 mg/L respectively (Vencill, 2002). Solubility of these herbicides is low compared across all herbicides. Dithiopyr is more soluble than the two dinitroanilines, but we had the most problems with respect to the hydroponics bioassay. The formulation type seemed to affect degree of agitation and

amount of contact with the root zone. Wettable powders (WP) such as the dithiopyr formulation incorporated into this hydroponic screen may require substantially more agitation than liquid concentrates. The dinitroaniline formulations utilized in these bioassays were liquid concentrates and appeared to agitate better in hydroponics than dithiopyr WP.

The soil-based bioassay utilized native soil as the medium, which made it the most realistic screening assay. This assay allows the researcher to determine whether factors such as organic matter and soil pH have an effect on resistance. The soil-based bioassay lacked the ability to characterize the degree of resistance; however, increasing the concentrations to a level at which control is achieved could overcome this problem. Additionally, confounding variables such as microbial degradation, soil type variation among regions, and leaching of the herbicide may have an effect on the evaluation.

CONCLUSION

Based on these data across all four bioassays methods, Chattanooga exhibits greater tolerance to proflaminate and pendimethalin compared to the Control population. Filter-paper, hydroponic, and soil-based were able to detect greater tolerance of Chattanooga to dithiopyr; however, potential agitation issues may have confounded results of the hydroponic method. None of the bioassays had the desired utility of a complete screen. Furthermore, the screens either required mature seed or had multiple confounding variables. The ideal screening assay might focus on the molecular target of resistance. Elucidation of the mutation(s) in the Chattanooga population α -tubulin gene or protein could be used to produce a very rapid assay (Delye et al., 2005). However, if many

mutations in α -tubulin are responsible for the resistance to mitotic inhibiting herbicides seen in annual bluegrass, a bioassay would be the only reliable way to diagnose resistance.

REFERENCES

- Beard, J.P., P.E. Rieke, A.J. Turgeon, and J.M. Vargas Jr. 1978. Annual bluegrass (*Poa annua* L.) description, adaptation, culture and control. Res. Rep. 352. Michigan State Univ. Agric. Expn. Stn.
- Beckie, H.J., I.M. Heap, R.J. Smeda, and L.M. Hall. 2000. Screening for herbicide resistance in weeds. *Weed Technol.* 14:428-445.
- Callahan, L.M., and E.R. McDonald. 1992. Effectiveness of bensulide in controlling two annual bluegrass (*Poa annua*) subspecies. *Weed Technol.* 6:97-103.
- Deleye, C., Y. Menchari, and S. Michael. 2005. A single polymerase chain reaction-based assay for simultaneous detection of two mutations conferring resistance to tubulin-binding herbicides in *Setaria viridis*. *Weed Research.* 45:228-235.
- Gamborg, O.L., R.A. Miller, and K. Ojima. 1968. Nutrient requirements of suspension cultures of soybean root cells. *Expl. Cell. Res.* 50:150-158.
- Hall, J.C., and C.K. Carey. 1992. Control of annual bluegrass (*Poa annua*) in Kentucky bluegrass (*Poa pratensis*) turf with linuron. *Weed Technol.* 6:852-857.
- Heap, I.M. 2007. The international survey of herbicide resistant weeds. Web Page: <http://www.weedscience.org/in.asp>.
- Heap, I.M. 1997. The occurrence of herbicide resistant weeds world wide. *Pestic Sci.* 51:235-243.
- Hoagland, D. R., and D. I. Arnon. 1950. The water culture method for growing plants without soil. Circ. 347. Calif Agric. Exp. Stn. Berkeley, CA.

- Hughdahl, J.D., and L.C. Morejohn. 1993. Rapid and reversible high-affinity binding of the dinitroaniline herbicide oryzalin to *Zea mays* L. *Plant Physiol.* 102:725-740.
- Hutto, K.C., G.E. Coats, and J.M. Taylor. 2004. Annual bluegrass (*Poa annua*) resistance to simazine in Mississippi. *Weed Technol.* 18:846-849.
- Isrigg, J. III., F.H. Yelverton, C. Brownie, and L.S. Warren Jr. 2002. Dinitroaniline Resistant annual bluegrass in North Carolina. *Weed Sci.* 50:86-90.
- McElroy, J.S., R.H. Walker, and E.V. Santen. 2002. Patterns of variation in *Poa annua* as revealed by canonical discriminant analysis of life history traits. *Crop Sci.* 42:513-517.
- Mudge, L.C., B.J. Gossett, and T.R. Murphy. Resistance of goosegrass (*Eleusine indica*) to dinitroaniline herbicides. *Weed Sci.* 32:591-594.
- Murashige, T., and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.* 15:473-497.
- Senseman, S.A. 2007. *Herbicide Handbook* Weed Science Society of America. Ninth Edition. p.14.
- Smeda, R.J., and K.C. Vaughn. 1994. Resistance to dinitroaniline herbicides. pp. 215-228 in S.B. Powles and J.A.M. Holtrum, *Herbicide Resistance in Plants*. Boca Raton, FL: CRC Press.
- Vaughn, K.C. and L. P. Lehn. 1991. Mitotic Disruptor Herbicides. *Weed Sci.* 39:450-457.
- Yuan, J.S., P.J. Tranel, and C.N. Stewart, Jr. 2007. Non-target herbicide resistance: a family business. *Trends Plant Sci.* 12:6-13.

APPENDIX

Table 2.1 Typical timeline for annual bluegrass plant and seed collection in order to conduct soil-based and petri-dish bioassays.

Key Tasks	February	March	April	May	June	July	August	September
Harvest field plants								
Panicles develop								
Harvest seed								
Allow seed to ripen								
Evaluate resistance								
Initial preemergence application for annual bluegrass control								

Table 2.2 Regression equations and calculations of LD₅₀⁶ and LD₉₀ for root growth reduction of Chattanooga and Control populations.

Bioassay	Herbicide	Population	Equation	r ²	LD ₅₀	LD ₉₀
					———— mM ————	
MS-media	Prodiamine	Chattanooga	$y=28.1+(-6.8)x^2+2.6x^3+(-6.9)e^x$	0.99	1.99	8.01
		Control	$\ln y=2.6+(-4.6)e^x$	0.99	0.025	0.25
	Pendimethalin	Chattanooga	$\ln y=3.8+(-5.7)ce^x$	0.99	0.017	0.16
		Control	$y^{-1}=-0.1+3.8e^x$	0.99	0.002	0.05
	Dithiopyr	Chattanooga	$y^{-1}=2.8+0.9x$	0.99	0.002	0.002
		Control	$y^{-1}=2.8+0.9x$	0.99	0.001	0.008
Filter-paper	Prodiamine	Chattanooga	$y=27.7+-7.1x$	0.70	0.89	175
		Control	$y^{0.5}=1.8+-1.1x$	0.99	0.021	1.44
	Pendimethalin	Chattanooga	$\ln y=3.9+-1.3e^x$	0.98	0.33	4.25
		Control	$y^{0.5}=5.7+-2.0e^x$	0.78	1.1	5.2
	Dithiopyr	Chattanooga	$y^{0.5}=2.5+-1.4x$	0.89	0.028	2.02
		Control	$y^{0.5}=1.5+-1.2x$	0.93	0.017	0.72
Hydroponics	Prodiamine	Chattanooga	$y=28.3+-7.6x$	0.96	3.12	1153

⁶ LD₅₀ and LD₉₀ : Dose of herbicide required to inhibit growth by 50 or 90 percent.

Table 2.2, continued

Bioassay	Herbicide	Population	Equation	r ²	LD ₅₀	LD ₉₀
———— mM ————						
	Pendimethalin	Control	y ⁻¹ =0.02+0.1x ^{.05}	0.98	0.12	9.46
		Chattanooga	y= 47.0+-13.8e ^x	0.98	4.89	11.5
	Dithiopyr	Control	y= 50.9+ -21.1e ^x	0.81	1.84	7.75
		Chattanooga	y=66.3+-17.6e ^x	0.81	8.38	21.6
		Control	y= 26.3+ -15.9x	0.96	0.28	16.5
——— kg ai ha ⁻¹ ——						
Soil-based	Prodiamine	Chattanooga	NS			
		Control	y ⁻¹ = 0.01 +.6x	0.99	0.016	0.15
	Pendimethalin	Chattanooga	NS			
		Control	lny= 4.5+2.9x ^{0.5}	0.96	0.056	0.63
	Dithiopyr	Chattanooga	lny= 4.5+ -35.1x ^{2.5}	0.94	0.21	0.33
		Control	y ⁻¹ =0.01+8.01x ³	1	1.07 X 10-9	0.22

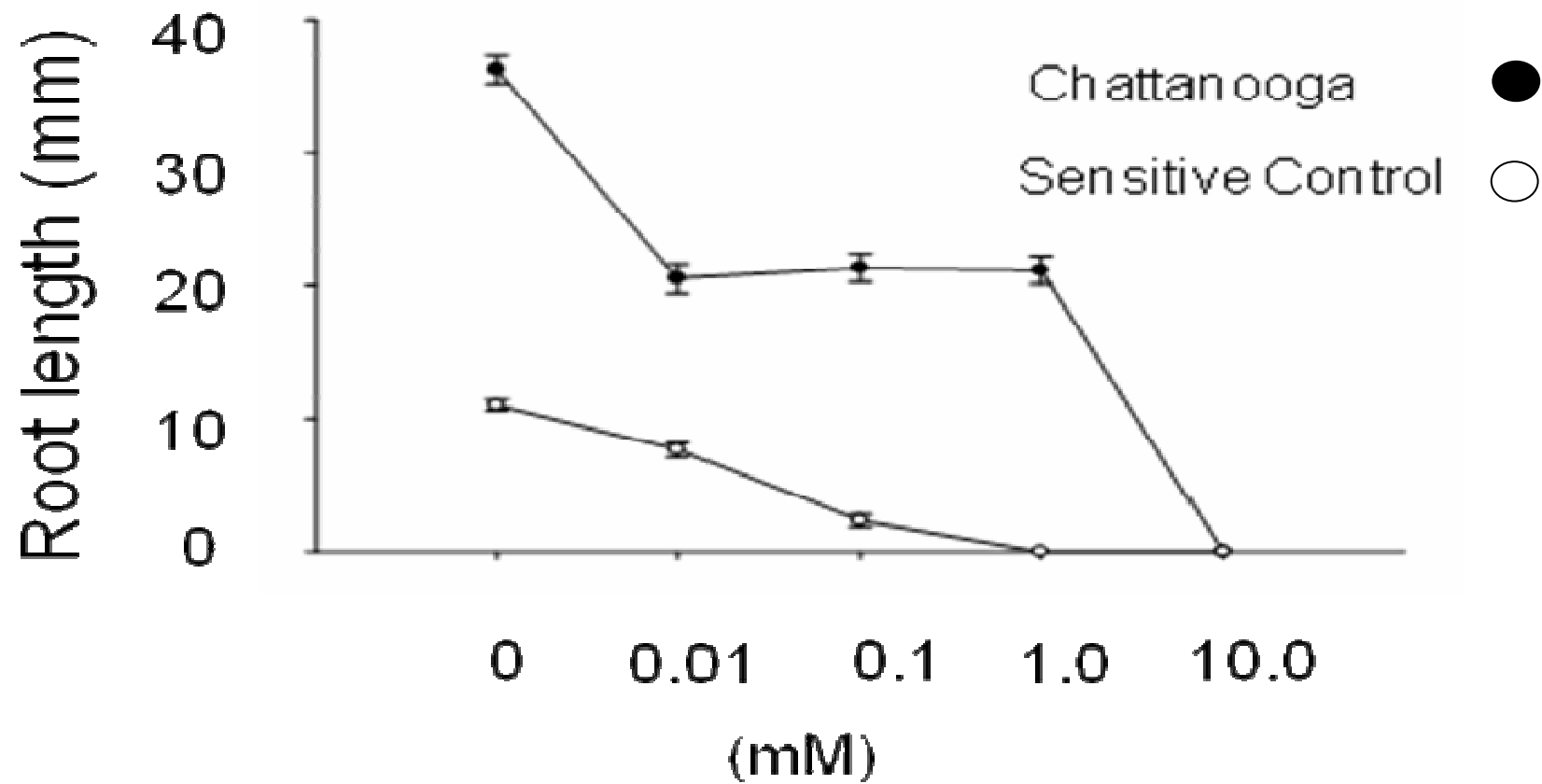


Fig 2.1 Effect of prodiamine concentration (mM) on root length (mm) of Chattanooga and Sensitive control ecotypes of *Poa annua* in MS-media.

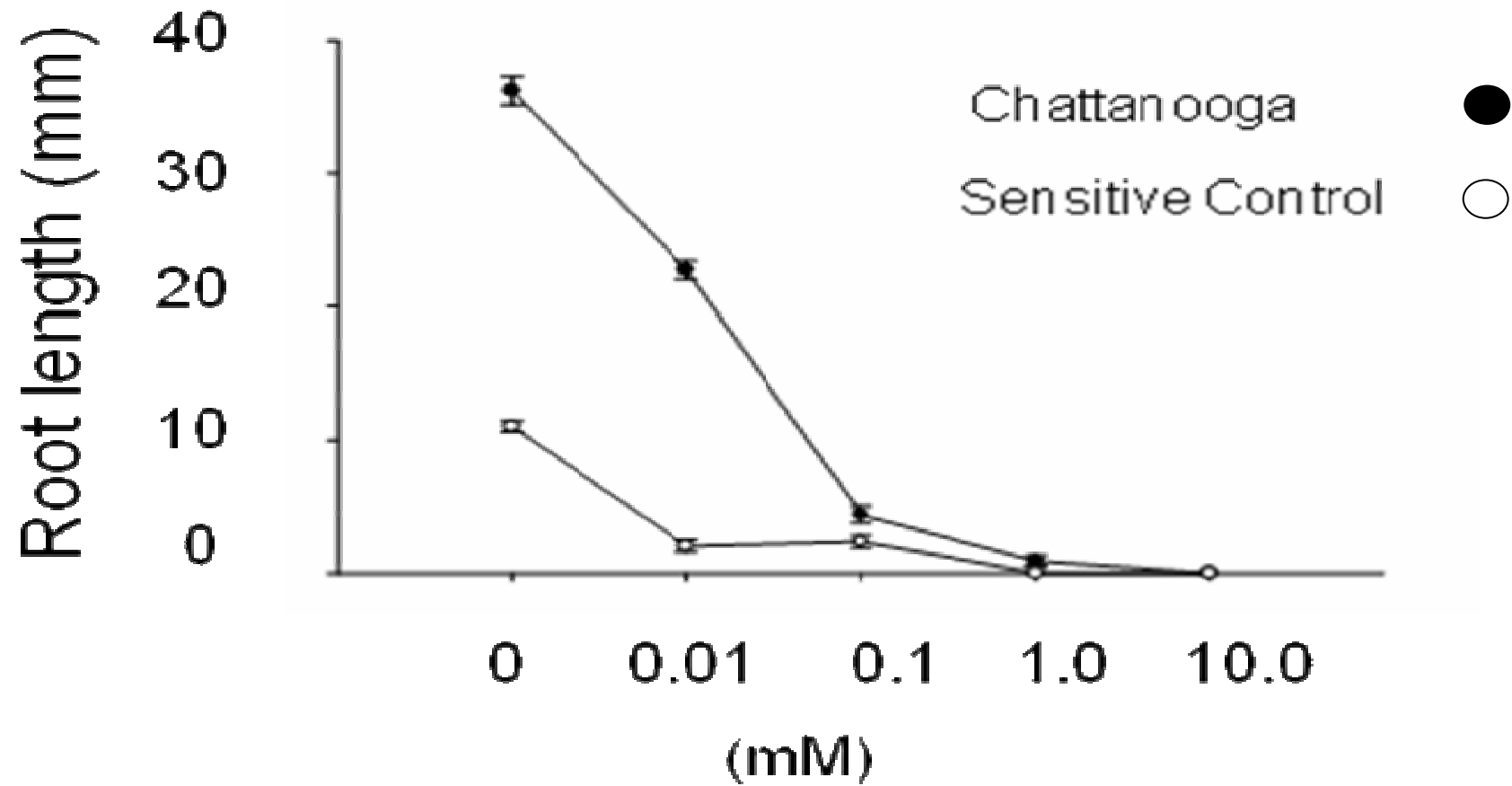


Fig 2.2 Effect of pendimethalin concentration (mM) on root length (mm) of Chattanooga and Sensitive control ecotypes of *Poa annua* in MS- media.

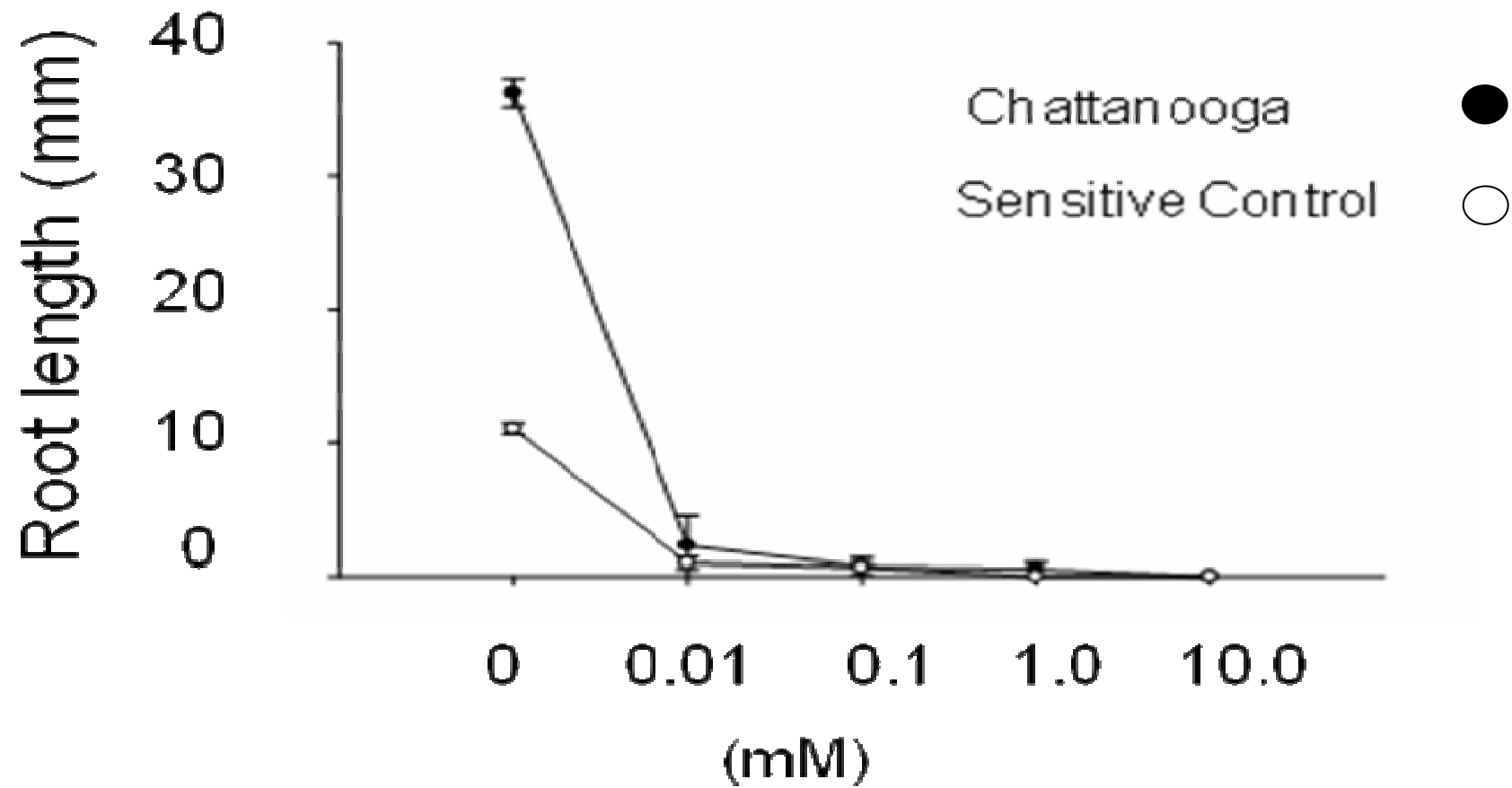


Fig 2.3 Effect of dithiopyr concentration (mM) on root length (mm) of Chattanooga and Sensitive control ecotypes of *Poa annua* on MS-media.

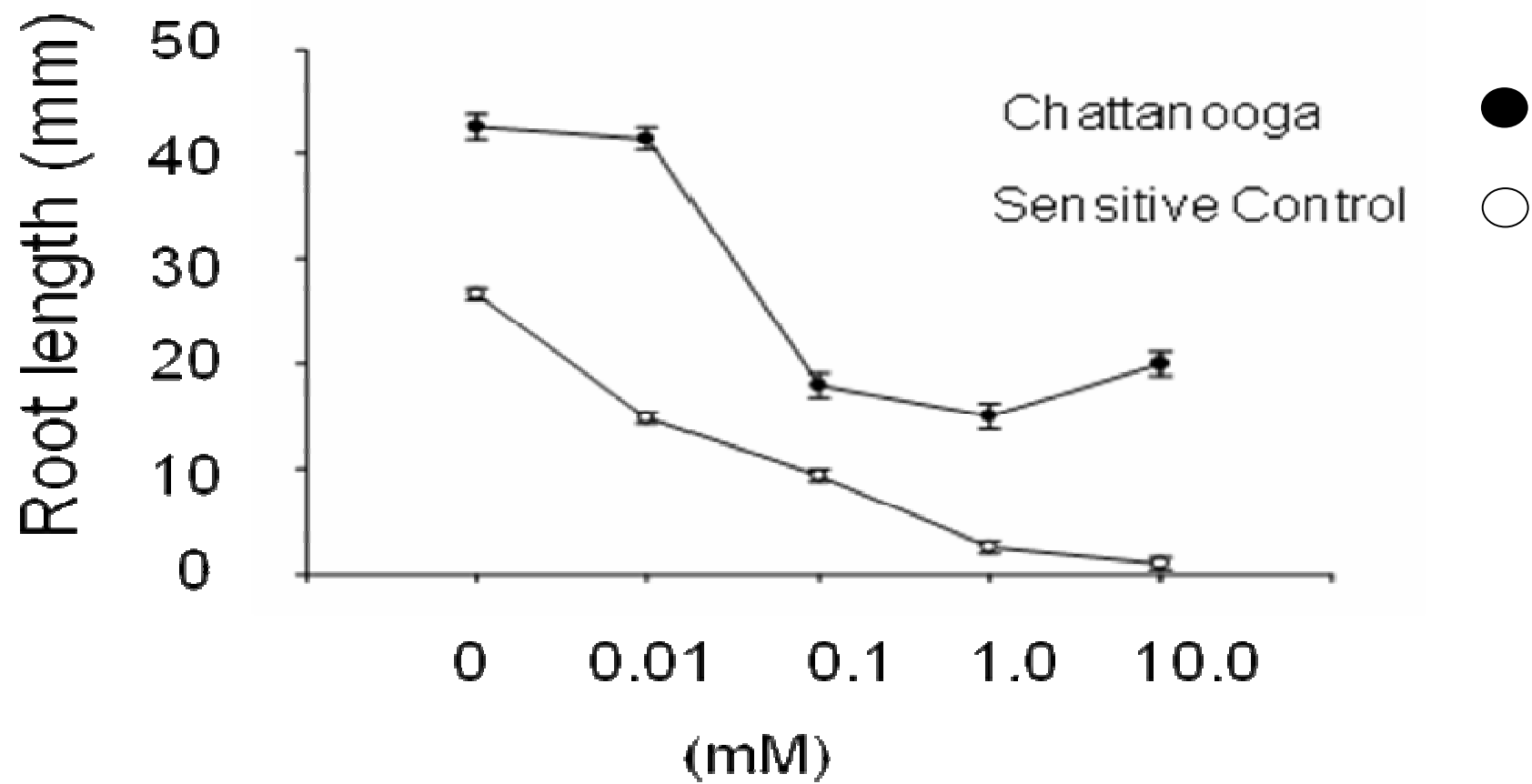


Fig 2.4 Effect of prodiamine concentration (mM) on root length (mm) of Chattanooga and Sensitive control ecotypes of *Poa annua* on filter-paper.

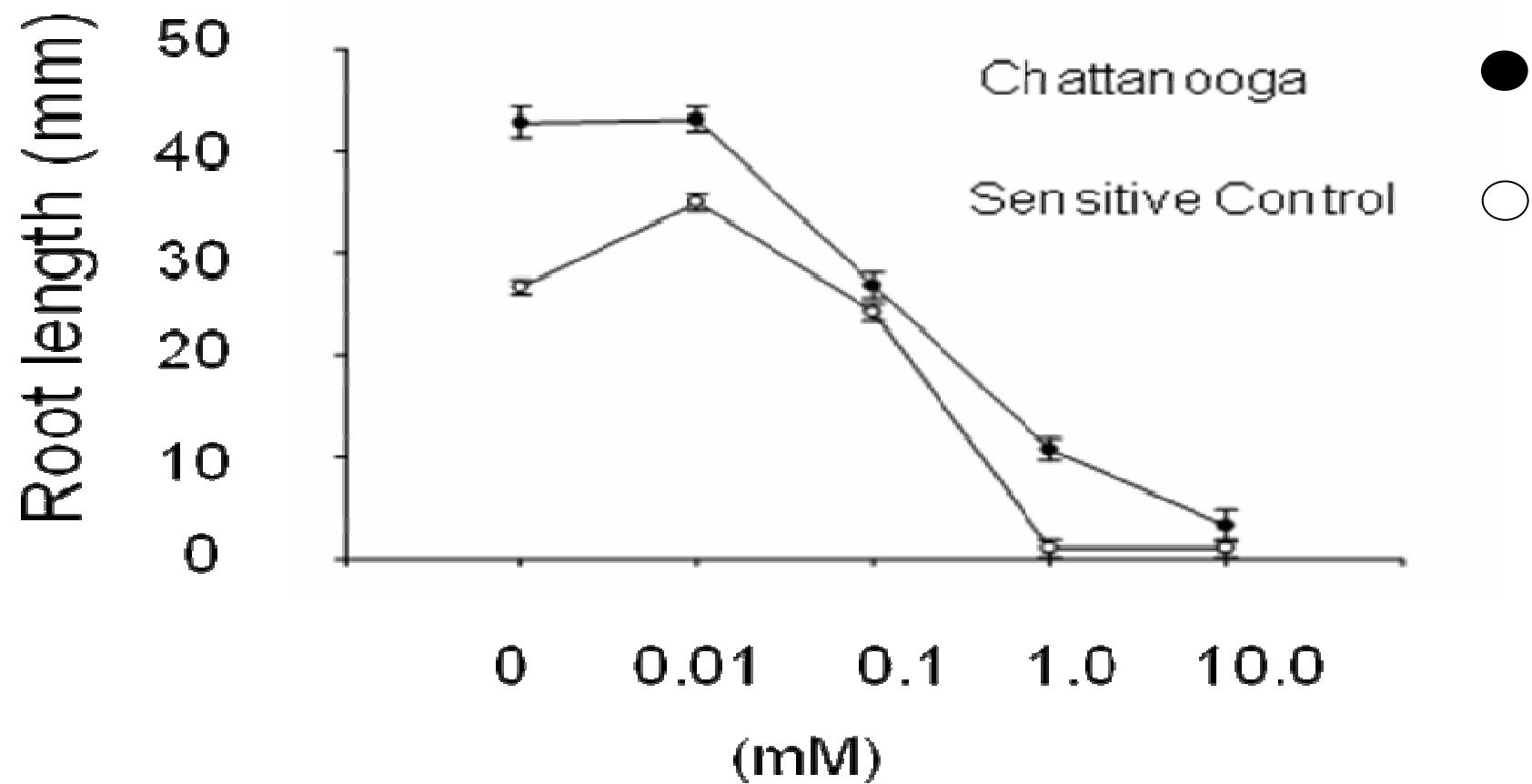


Fig 2.5 Effect of pendimethalin concentration (mM) on root length (mm) of Chattanooga and Sensitive control ecotypes of *Poa annua* on filter-paper.

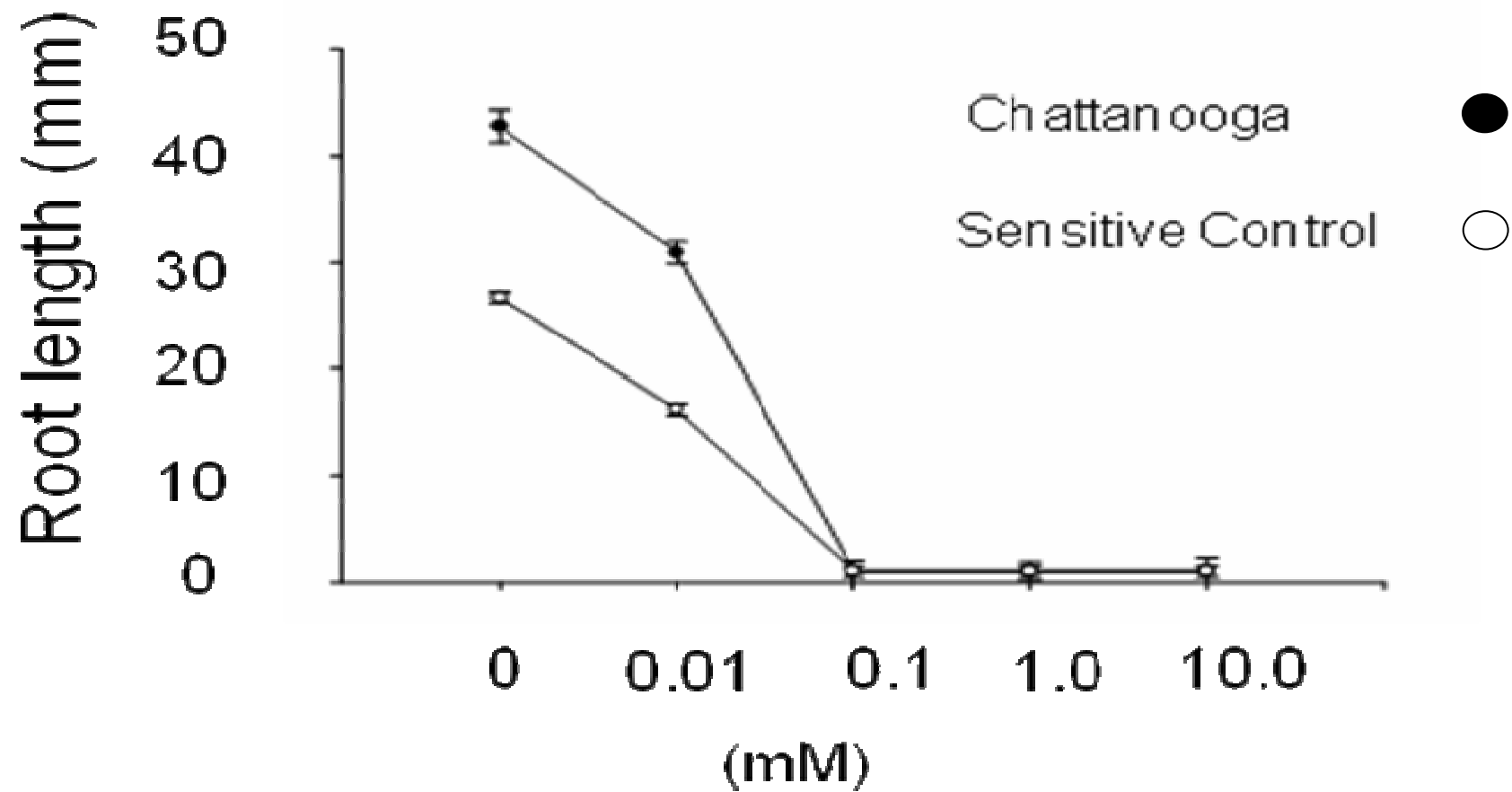


Fig 2.6 Effect of dithiopyr concentration (mM) on root length (mm) of Chattanooga and Sensitive control ecotypes of *Poa annua* in filter-paper.

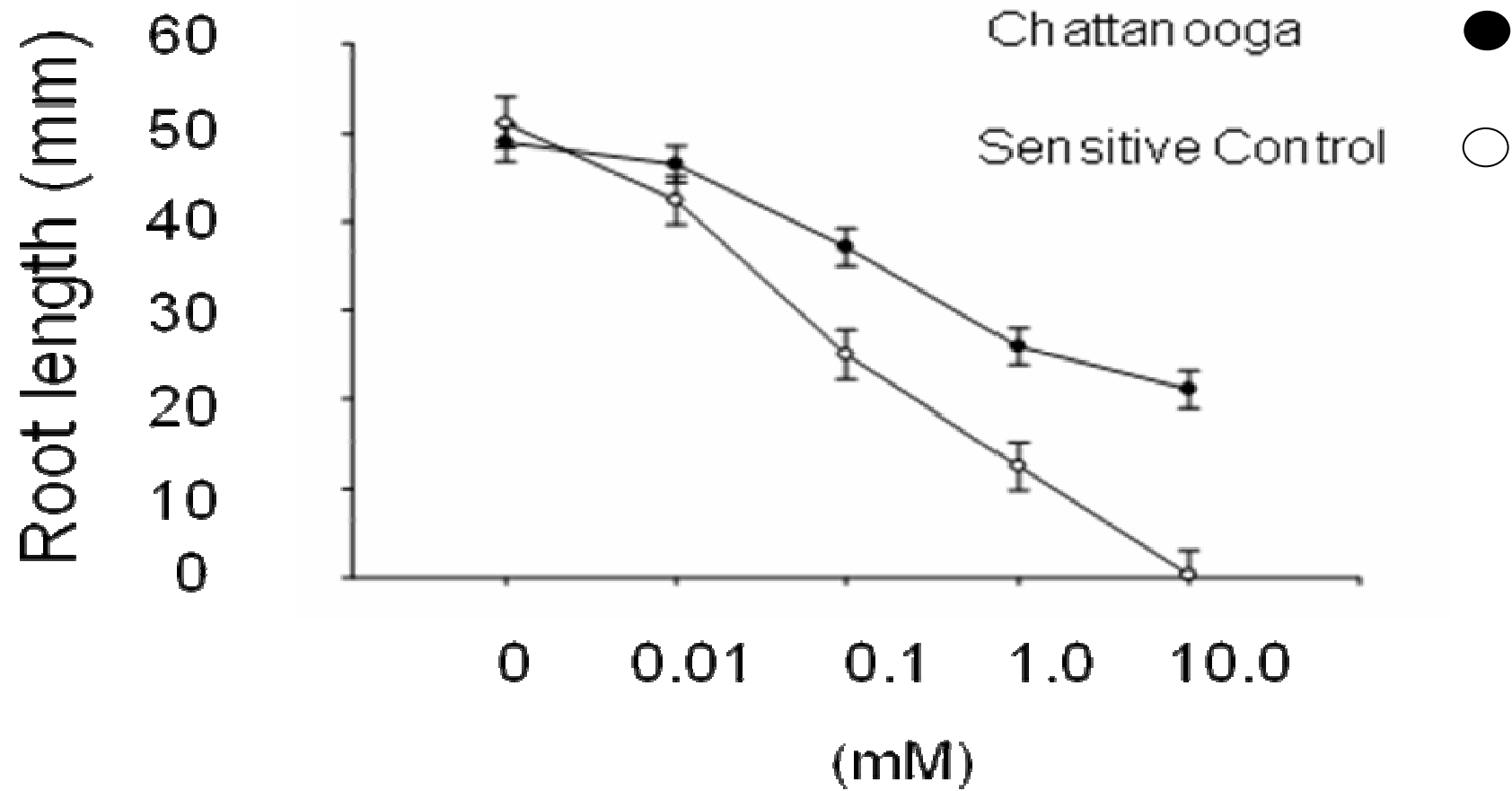


Fig 2.7 Effect of prodiamine concentration (mM) on root length (mm) of Chattanooga and Sensitive control ecotypes of *Poa annua* in hydroponics.

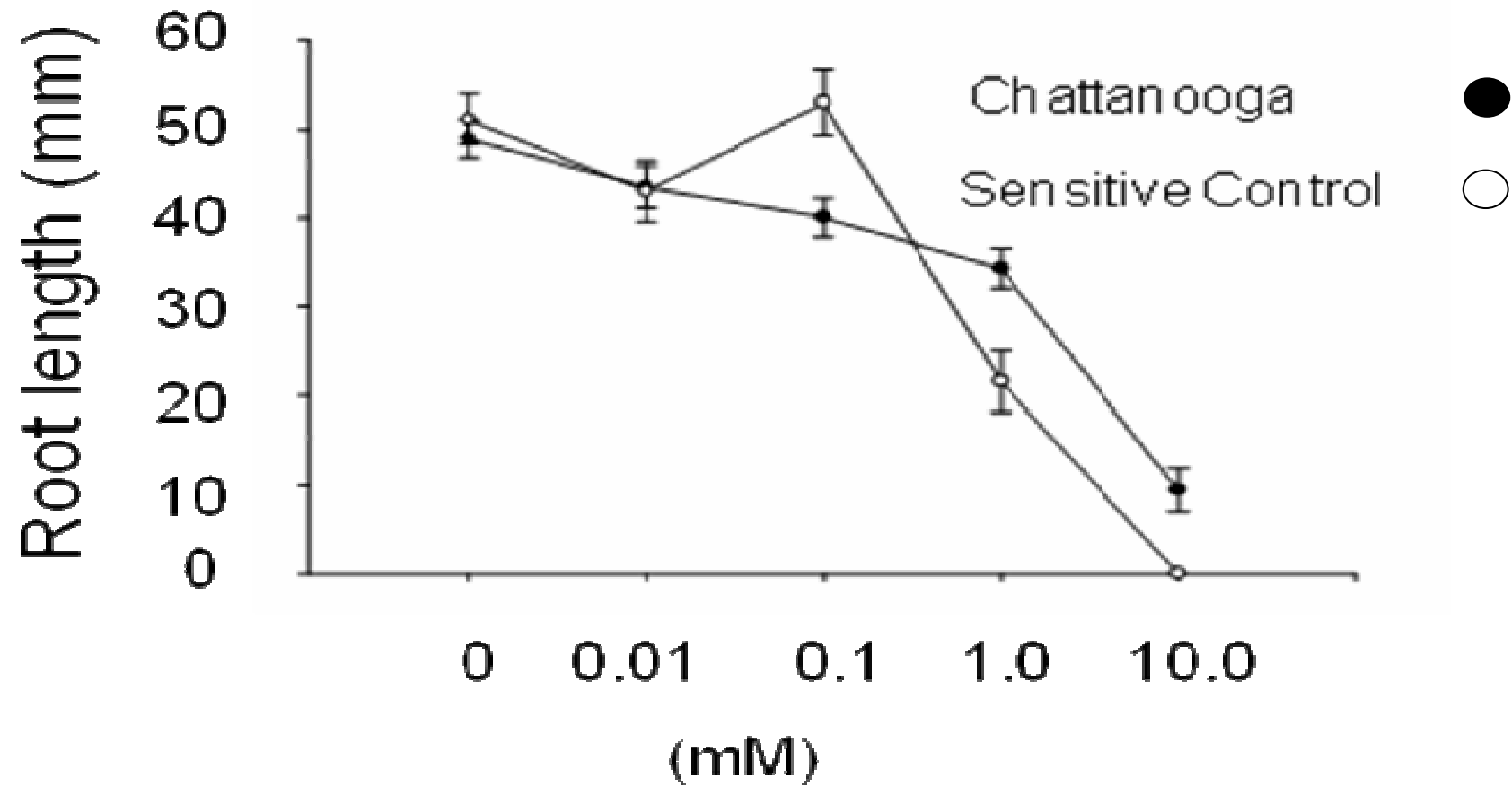


Fig 2.8 Effect of pendimethalin concentration (mM) on root length (mm) of Chattanooga and Sensitive control ecotypes of *Poa annua* in hydroponics.

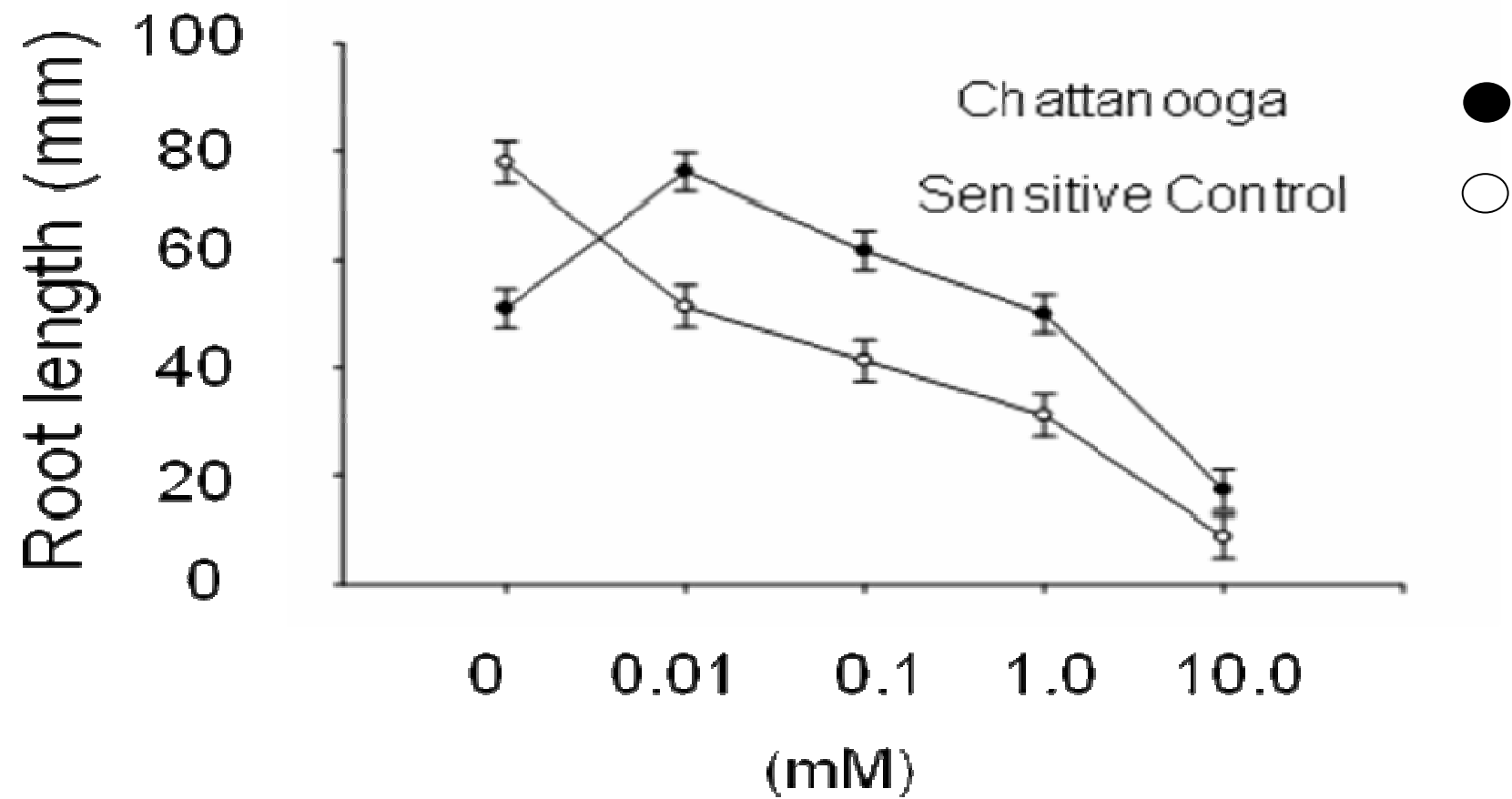


Fig 2.9 Effect of dithiopyr (mM) on root length for Chattanooga and Sensitive control ecotypes of *Poa annua* in hydroponics.

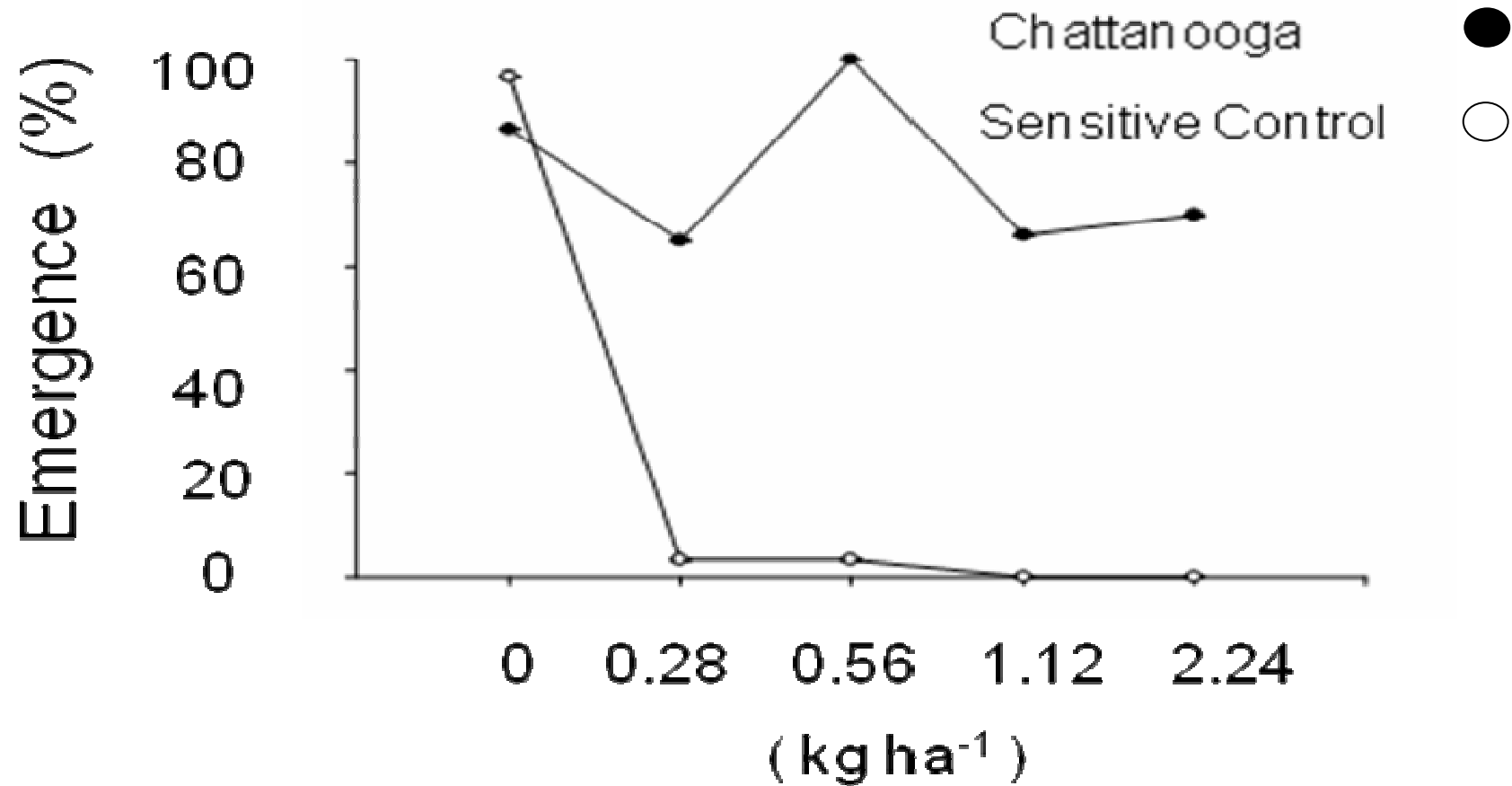


Fig 2.10 Effect of prodiamine (Kg ha^{-1}) on shoot emergence for Chattanooga and Sensitive control ecotypes of *Poa annua* in native soil.

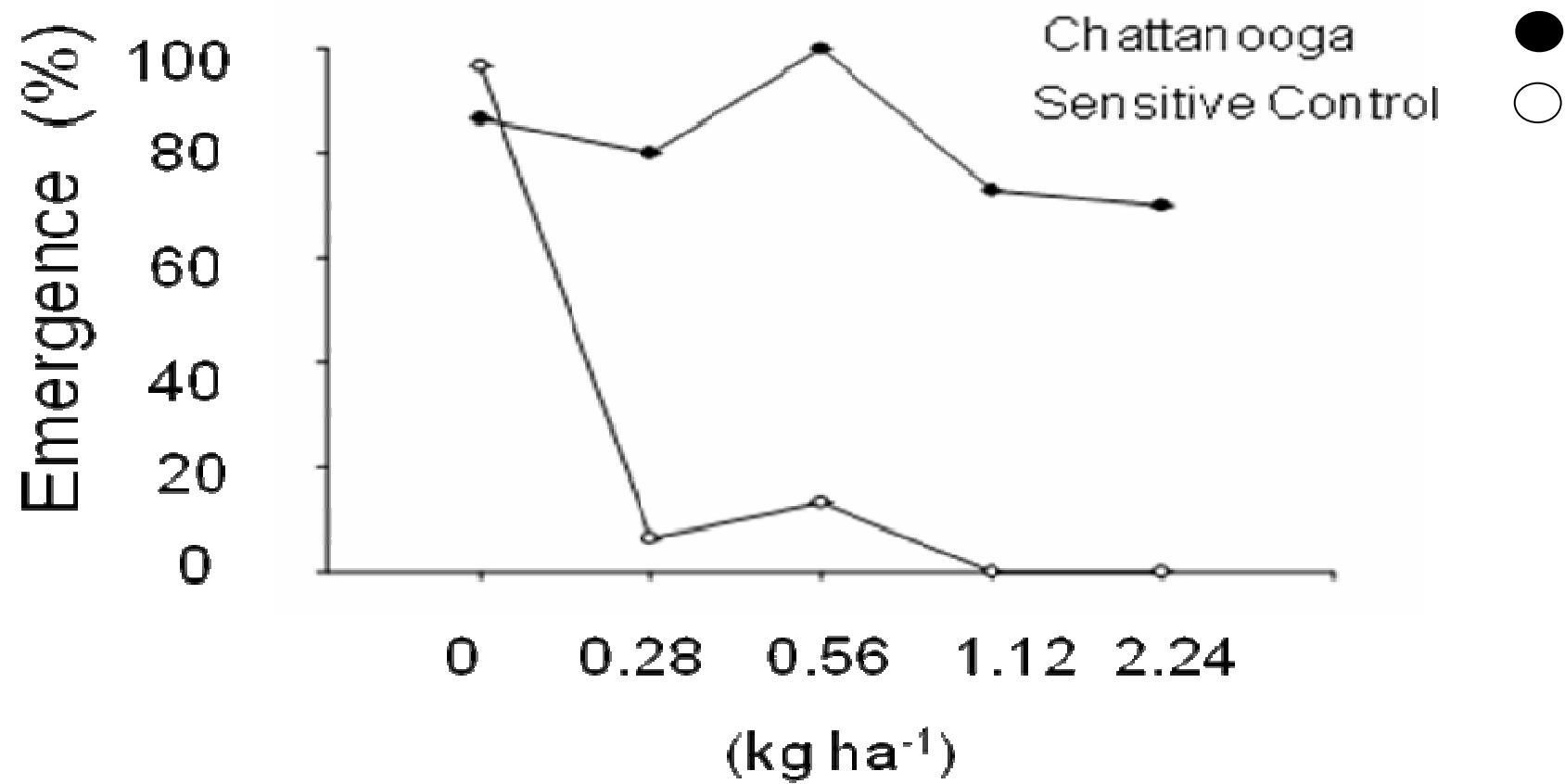


Fig 2.11. Effect of pendimethalin (Kg ha⁻¹) on shoot emergence for Chattanooga and Sensitive control ecotypes of *Poa annua* in native soil.

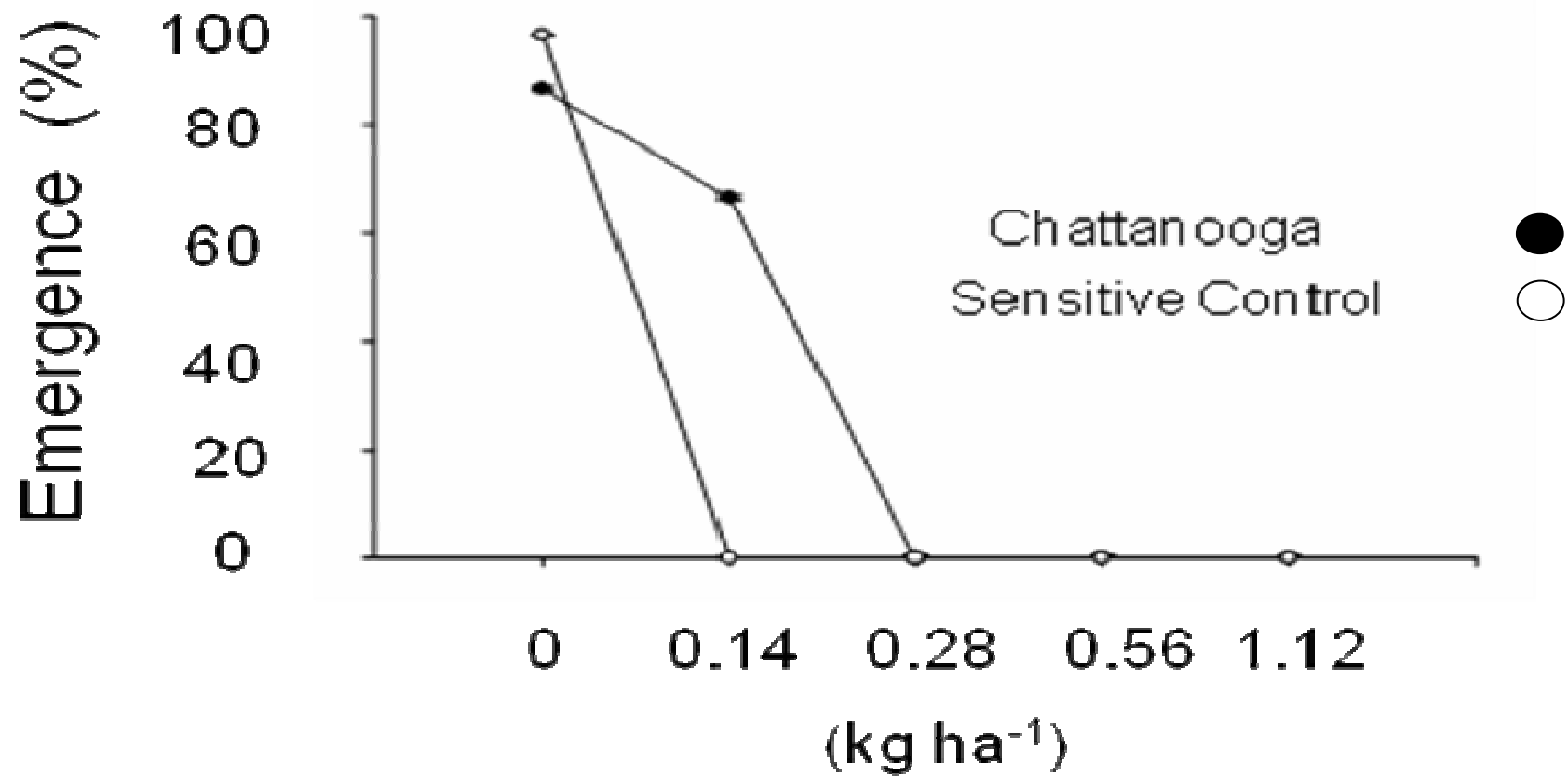


Fig 2.12. Effect of dithiopyr (Kg ha⁻¹) on shoot emergence for Chattanooga and Sensitive control ecotypes of *Poa annua* in native soil.

VITA

Matthew Anthony Cutulle, son of Lyn Alan Cutulle and Linda Gavitt Cutulle, was born on the 30th of April , 1982, in Alice Springs, Northern Territory, Australia. He has since lived in multiple places throughout the northern hemisphere. He graduated from Mt. Hebron High school in Ellicott City, Maryland in May, 2000. He entered Salisbury