EVALUATION OF THE BIOHERBICIDE *PHOMA MACROSTOMA* FOR USE IN SOUTHERN TURFGRASS SYSTEMS

A Thesis

by

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ABSTRACT

Phoma macrostoma Montagne is a fungus being developed as a natural herbicide for selective broadleaf weed control. The solid fermentation of *P. macrostoma* on grain produces a product that is applied as a dry granule. Herbicidal activity is characterized by foliar bleaching and necrosis of susceptible broadleaf weeds.

Previous research with this product was limited to cool-season climates, and information is limited regarding appropriate application rates or efficacy at higher temperatures and weeds associated with warm-season turf. Spectrum of weed control is still being explored, most recently in Texas.

Multiple years of field and greenhouse research were conducted in College Station, TX, to evaluate the efficacy of *P. macrostoma* for broadleaf weed control. Field studies showed that *P. macrostoma* was effective at controlling slender aster (*Aster subulatus* var**.** *ligulatus* Shinners*)*, with higher application rates providing 89 to 94% control. Field studies also showed that *P. macrostoma* was able to maintain efficacy at high temperatures, with maximum temperatures of 41°C. Greenhouse studies indicated a variable weed control spectrum, with *P. macrostoma* providing excellent control of dandelion (*Taraxacum officinale* Weber ex F.H. Wigg.), but little to no activity on other weeds including common mallow (*Malva neglecta* Wallr.) and common purslane (*Portulaca oleracea* L.). Growth chamber studies indicate that environmental factors such as temperature and moisture affect the efficacy by *P. macrostoma*, and cultural

practices such as nitrogen fertilization coupled with *P. macrostoma* application may enhance herbicidal activity.

Over the course of this research, no injury was observed on any species of warm-season turfgrass, making *P. macrostoma* a promising biorational option for natural weed control in lawns.

DEDICATION

This thesis is dedicated in memory of my brother Bill.

ACKNOWLEDGEMENTS

I would like to thank my committee chair, Dr. Wherley, my co-chair Dr. Senseman, and my committee members, Dr. Baumann and Dr. White, for their guidance and support throughout the course of this research.

Thanks also go to my friends and fellow graduate students and the department faculty and staff for making my time at Texas A&M University a great experience. I also want to extend my gratitude to the Scotts Miracle-Gro Company for affording me this amazing opportunity.

Finally, thanks to my family for their encouragement and to my wife and son for their love, support, and patience.

NOMENCLATURE

PM	*Phoma macrostoma*
WBG	Weed B Gon
CGM	Corn Gluten Meal
DAT	Days After Treatment
DIA	Digital Image Analysis
AMS	Ammonium sulfate

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CHAPTER I

INTRODUCTION AND

LITERATURE REVIEW

Over the past decade, synthetic herbicides have come under increased scrutiny around the world. Controversy surrounding pesticide use has led municipalities in Canada to restrict the use of pesticides for only cosmetic purposes, with concerns of cancer occurring due to pesticide exposure being the driving force behind the restrictions (Knopper and Lean, 2004). While some legislation has targeted herbicides for cosmetic use, others have banned the use of all weed and feed lawn products (herbicide + fertilizer combinations), only allowing the use of lawn herbicides for spot treatment applications (Anonymous, 2010). Concerns over water availability and the environmental consequences of inputs from turfgrass management have led some Florida communities to enact rules to reduce the area of landscaped turf, irrigation allowances, and applications of fertilizer (Cisar 2004). Herbicides are the most used pesticide in the U.S., with 498 and 531 million pounds of active ingredient used in 2006 and 2007, respectively (Environmental Protection Agency, 2011). Phenoxy herbicides, especially 2, 4-dichlorophenoxyacetic acid (2, 4-D), are among the herbicides being scrutinized. 2, 4-D is one of the most used herbicides in the U.S., for broadleaf weed control in many settings, ranging from agriculture to lawns and golf courses to industrial weed control. The Environmental Protection Agency (2011) estimated that 52 to 62 million pounds of 2, 4-D active ingredient were used in 2007. Frequent exposure to 2, 4-D has shown

increased instances of non-Hodgkin's lymphoma (NHL) in studies conducted in Sweden, Kansas, Nebraska, and Canada (Zahm and Blair, 1992). Atrazine is another herbicide that has come under scrutiny. The Environmental Protection Agency (2011) estimated 73 to 78 million pounds of active ingredient being used in 2007, in agriculture settings, primarily in corn. According to publicly available data from the U.S. Geological Survey (USGS) report in 2006, atrazine was the most frequently detected pesticide in U.S. stream and ground waters between 1992 and 2001. In October 2003, the European Union (EU) announced the ban of atrazine, while the U.S. EPA approved its continued use (Sass and Colangelo, 2006). This has become a heated debate, with opponents of these restrictions claiming there is a lack of scientific evidence to link cancer occurrences to pesticide use. With pressure to ban synthetic herbicides increasing, the need for finding effective alternative options for weed control has increased.

Few effective natural options for weed control in turfgrass systems are currently available. Among the alternative herbicides that are available for use are vinegar, essential oils (clove & cinnamon oil), citric acid, fatty acids (pelargonic acid), and combinations of these different products. These products are primarily used for nonselective weed control, with the effectiveness of these products dependent upon product concentrations. Vinegar, citric acid, and clove oils provide better control at higher use rates (Abouziena et al. 2009; Boyd and Brennan 2006; Evans et al. 2009). Use of higher product rates, however, results in greater potential for crop injury (Evans and Bellinder 2009).

Corn gluten meal (CGM) is a granular byproduct of commercial corn milling, containing approximately 10% nitrogen by weight. It is primarily used in turf as a crabgrass pre-emergence herbicide, but may inhibit other broadleaf and grassy weeds (Bingaman and Christians, 1995). One disadvantage to CGM is that it is effective only as a preemergence product, and has no postemergence activity on established weeds (Christians et al., 2010). Since CGM is applied as a granule, challenges associated with CGM include relatively high use rates (60 to 120 g m^{-2}) and inconsistent reports of weed control (Christians, 1993; Patton and Weisenberger, 2012; Stier, 1999). While natural herbicides do exist, finding products that are safe to turfgrass and provide consistent, effective weed control has been a challenge.

Phoma macrostoma Montagne (PM) is a naturally occurring fungus being developed as a natural herbicide, or bioherbicide, for weed control in turfgrass. *Phoma macrostoma* was discovered in Canada, when field isolates were collected from infected Canada thistle that were showing symptoms of bleaching and chlorosis (Graupner et al., 2003; Graupner et al.2006). These isolates were found to produce metabolites that were phytotoxic to susceptible broadleaf weeds, causing bleaching and chlorosis, and are part of a new family of herbicides, the macrocidins (Graupner et al., 2006). The isolate 94-44B was selected for developing the final product, which is produced from the solid fermentation of the fungus on grain, and leads to the production of mycelium that are applied to soil as a granule (Bailey et al., 2011b). It was registered in Canada in 2011 and the United States in 2012 (Bailey et al., 2013). The effects of PM were first reported in 2001, on the growth of dandelion (Bailey and Derby, 2001), as well as demonstrating

suppression of broadleaf weeds like Canada thistle, chickweed, and scentless chamomile Broadleaf weeds exhibit greater sensitivity than grasses to these metabolites (Bailey and Derby, 2001; Graupner et al., 2006). Plant symptoms observed with macrocidins are similar to those observed from hydroxyphenyl pyruvate dioxygenase (HPPD) inhibitors, however, inhibition of HPPD does not appear to be the mode of action, which at this time is unknown (Graupner et al., 2003). While the mode of action is still being studied, PM has exhibited two symptomologies; foliar photobleaching caused by phytotoxic macrodins and root inhibition (Bailey et al., 2011b). Bleaching and stunting appear the mostly in new growth of weeds that happen to be susceptible to PM, implying the macrocidin compounds are phloem mobile (Graupner et al., 2003).

Weed control with PM has been evaluated primarily in more northern climates on weeds ranging from pigweed to English daisy. In these studies, the bioherbicide has shown control of dandelion, Canada thistle, chickweed, and English daisy, with maximum efficacy reported at temperatures ranging from 15 to 25°C (Bailey et al., 2011a). Soil mobility of PM is limited, even at excessive application rates (Zhou et al. 2004), with retention and activity of the bioherbicide highest in clays rather than sand-based soils (Bailey et al. 2010).

To date, all cool-season turfgrass varieties tested exhibit high tolerance to PM (Bailey et al. 2011a), and there is no indication that PM negatively impacts the environment, even following very high application rates (Zhou et al., 2004). Therefore, use of PM for effective weed control in turfgrasses shows potential.

Further research is needed for determining whether PM could be effectively used at higher temperatures and with turfgrasses and weeds common to more southern climates. Major areas of needed research include 1) evaluation of spectrum of broadleaf weeds controlled and potential phytotoxicity to warm-season turfgrasses, 2) determining necessary field application rates, and 3) effects of moisture, temperature, and application placement on weed control efficacy.

CHAPTER II

EARLY SUMMER SLENDER ASTER CONTROL*

Introduction

In recent decades, synthetic herbicides have come under increased scrutiny around the world. While some legislation has targeted the removal of herbicides for cosmetic use, others have banned the use of all weed and feed products, only allowing the use of herbicides for spot treatment applications (Anonymous, 2010). With growing pressure to ban synthetic herbicides, the need for alternative weed control options has increased.

Currently, there are few effective natural options for weed control in turfgrass systems. Some alternative herbicides available for use include vinegar, essential oils (clove & cinnamon oil), citric acid, fatty acids (pelargonic acid), and combinations of these different products. These products are primarily used as nonselective herbicides, with effective weed control being dependent upon product concentrations with vinegar, citric acid and clove oils providing better control at higher use rates (Abouziena et al. 2009; Boyd and Brennan 2006; Evans et al. 2009). Use of higher product rates, however, results in greater potential for crop injury (Evans and Bellinder 2009).

Corn gluten meal (CGM) is a granular-applied herbicide that is a byproduct of commercial corn milling, containing approximately 10% nitrogen by weight. It is

*Reprinted with permission from "Early Summer Slender Aster Control in Bermudagrass Using Bioherbicide *Phoma macrostoma*" by J. Smith, B. Wherley, P. Baumann, S. Senseman, R. White, and S. Falk, 2013. *J Biofertil Biopestici* 4:139, Copyright 2013 by J. Smith, et al.

primarily used in turf as a crabgrass pre-emergence herbicide, but may inhibit other broadleaf and grassy weeds (Bingaman and Christians, 1995). One disadvantage to CGM is that it is effective only as a pre-emergence product, and has minimal post-emergent activity on established weeds (Christians et al., 2010). As a granular-applied product, challenges associated with CGM include relatively high use rates (60 to 120 g m^{-2}) and inconsistent reports of weed control (Christians, 1993; Patton and Weisenberger, 2012; Stier, 1999). While natural herbicides do exist, finding products that are safe to turfgrass and provide consistent, effective weed control has been a challenge.

The bioherbicide *Phoma macrostoma* Montagne is a natural herbicide being developed by the Scotts-Miracle Gro Company, Marysville, OH. It is produced from the solid fermentation of the fungus *Phoma macrostoma* on grain. *Phoma macrostoma* was discovered in Canada, when field isolates were collected from infected Canada thistle exhibiting symptoms of bleaching and chlorosis (Graupner et al. 2003). To date, this bioherbicide has been evaluated primarily in northern climates on weeds including dandelion (*Taraxacum officinale* Weber ex F. H. Wigg.), Canada thistle (*Cirsium arvense* (L.) Scop.), chickweed (*Stellaria media* (L.) Vill.), and English daisy (*Bellis perennis* L.) with maximal reported efficacy at temperatures ranging from 15 to 25°C (Bailey et al., 2011). Currently, limited research has been conducted to determine if this product could provide weed control at higher temperatures associated with southern climates in which warm-season turfgrass is managed.

Slender aster (*Aster subulatus* var**.** *ligulatus* Shinners) is a troublesome summer annual weed that thrives under high temperatures in many areas of the southern U.S. This plant's ability to grow in a prostrate growth pattern allows it to survive mowing, making it problematic in southern turfgrass. Slender aster can be difficult to control if not treated early on in its growth cycle, because it becomes woody as the season progresses, necessitating repeated herbicide applications. Early summer applications of synthetic herbicide formulations, 2, 4-D + MCPP + dicamba, provide good control of slender aster (Paul Baumann, personal communication), but the focus of this study was to evaluate the activity of the bioherbicide on slender aster.

The objectives of this study were to 1) evaluate efficacy of the bioherbicide *Phoma macrostoma* at elevated temperatures following early summer applications, 2) determine effective application rates for slender aster control, and 3) evaluate potential phytotoxicity on common bermudagrass (*Cynodon dactylon* L. Pers.*)*.

Materials and Methods

Field studies were conducted during spring and summer of 2011 and 2012 at the Texas A & M University Turfgrass Research Field Laboratory, College Station, TX. In 2011, trials were initiated on 1 June 2011, and carried out until 12 August 2011. The 2012 trials were initiated on 2 May 2012, and carried out until 26 June 2012. The studies were conducted on an established stand of common bermudagrass (*Cynodon dactylon* L. Pers.*),* intermixed with slender aster (*Aster subulatus* var**.** *ligulatus* Shinners*)* at the 3- to 4-leaf stage, and approximately 7 cm tall at treatment. Soil at the site was a Lufkin fine sandy loam with a pH of 9.8. Field plots were arranged as a randomized

complete block design (RCBD) with four replications. Individual plots measured 0.91 m x 0.91 m with a 0.3 m buffer between plots. Turf was mowed weekly to a height of 6.4 cm. Just prior to initiation, the study area was fertilized at a rate of 49 g N ha^{-1} using 46-0-0 (N: P: K) urea fertilizer.

Granular bioherbicide was produced on grain using solid state fermentation under contract for The Scotts Miracle-Gro Company, at a pilot scale manufacturing facility. The experimental bioherbicide product supplied for this experiment had half the potency that will be delivered in the commercial product, therefore higher application rates were used in this study. To initiate the studies, plots were irrigated to dampen weed and turf foliage and treatments were applied to the dampened foliage via shaker jar at application rates of 32, 64, or 128 g m^{-2}. In 2011, half of the herbicide treatment was applied at trial initiation and half applied 28 days after initial treatment (DAT). For 2012, the same overall rate of herbicide was applied, but treatments were applied once at trial initiation. Granules were left on weed foliage for 24 hours, and then granules were washed off of plant foliage and into the soil by irrigation.

Temperature and rainfall data were recorded by an onsite weather station during the studies. Mean daily air temperature for 2011 was 31°C, with an absolute maximum of 41°C and minimum of 20°C during the study period. For 2012, mean daily temperature was 27°C, with maximum of 41°C and minimum of 17°C. During the study period, plots were irrigated 4 to 5 times weekly receiving a total of 25 mm of water per week. Additionally, rainfall of 81 mm and 93 mm were received during the 2011 and 2012 study periods, respectively.

Slender aster plant counts were made using a 0.91 m^{-2} grid rating system consisting of thirty-six 12.7 cm x 12.7 cm squares. Squares which contained green slender aster plants were totaled (0-36) and used to calculate percent weed control based on the Henderson-Tilton Method, (1 – *Ta* x *Cb* / *Tb* x *Ca*) x 100, where *Tb* is the number of grids per plot before treatment, *Ta* the number of grids after treatment, *Cb* the number of grids in the untreated plot before treatment, and *Ca* the number of grids from the untreated plot after treatment (Henderson and Tilton, 1955). Weed counts were recorded prior to treatment applications and biweekly thereafter for the duration of the trials. Weed chlorosis/necrosis was also evaluated biweekly using a scale of 0 to 5, with 0 = no chlorosis injury, and 5 = complete necrosis. Phytotoxicity of common bermudagrass in plots was monitored using a scale of 0 = no injury to 5 = complete chlorosis.

Data were subjected to analysis of variance using the general linear model, univariate test procedure using SPSS ver. 21.0 (IBM Corp, Armonk, NY) to determine statistical significance of the results. Means separation procedures were performed using Tukey's test at the $P \leq 0.05$ level.

Results and Discussion

Initial slender aster populations in the selected test areas were very high. Grid counts were taken prior to study initiation and showed test plots contained slender aster in ~34 out of 36 grids in 2011, and ~33 out of 36 grids in 2012. By the conclusion of the study, significant reductions in weed populations were seen in both 2011 and 2012 with the higher two rates of the bioherbicide. The final grid counts for the 128 g m^{-2} rate were 3.5 and 1.8 out of 36 grids in 2011 and 2012, respectively. The 64 g m^{-2} ended the

study with slender aster in 5.8 and 9.8 out of 36 grids in 2011 and 2012, respectively. The 32 g m^{-2} rate did not significantly reduce final slender aster populations in 2011 (17 grids out of 36), however, weed populations were significantly reduced in 2012, ending the study with slender aster in 10.5 out of 36 grids. While the same total application amounts were applied in both years, the bioherbicide treatments were split-applied between day 0 and day 28 in 2011, and applied entirely at day 0 in 2012. No injury to bermudagrass was observed following application in either year. Ongoing research with this product using other warm-season species has shown no injury in other major warm-season grasses (Smith et al. 2013a, unpublished data).

Chlorosis and Bleaching

A potential concern of natural products may be slow or delayed efficacy, relative to synthetic products. Turfgrass managers and home owners prefer rapid control, indicating that the treatments are working. Therefore, rapid visual weed injury and decline following application is an important characteristic of an effective natural consumer product. During both years, initial foliar chlorosis and bleaching of slender aster became evident at all rates within 3 to 4 DAT. By 13 DAT in the 2012 study, moderate (3.25/5) foliar bleaching of weeds was observed in plots receiving both the 64 and 128 g m^{-2} application rates, with slightly less (2.5/5) chlorosis noted where the 32 g m^{-2} rate was applied (Figure 1). Photobleaching of susceptible weeds was followed by necrosis and gradual decomposition of weeds over the course of 2 to 4 weeks.

Weed Control

While equivalent total rates of the bioherbicide were applied in both studies, the split applied applications of 2011 resulted in considerably delayed control relative to the single application of 2012 (Figure 2). Herbicide-induced chlorosis was quickly evident in treated plots within the first two weeks of both years, but these did not result in significant differences in control until weeds had fully decomposed. In 2011, by 28 DAT, the 64 and 128 g m^{-2} bioherbicide rates exhibited significantly improved control (18 and 11% control, respectively) compared to untreated plots (Figure 2). By the end of the 2011 study (72 DAT), the highest rate of bioherbicide (128 g m^{-2}) provided significantly improved slender aster control (88%) when compared to the untreated. In addition, the lower two rates of bioherbicide (32 and 64 g m^{-2}) provided marginal control (41 and 54%, respectively) when compared to the untreated.

Onset of weed injury and subsequent control following application occurred much more rapidly in 2012 compared with 2011, likely due to bioherbicide treatments being applied as a single application. Another factor which could have contributed to the more rapid weed injury in the 2012 trial is that treatments were applied in May, on slightly younger weeds. Herbicide activity has been shown to occur much more rapidly when applied early in the weed life cycle to younger weeds when herbicide uptake and translocation are favored (McCarty and Murphy, 1994).

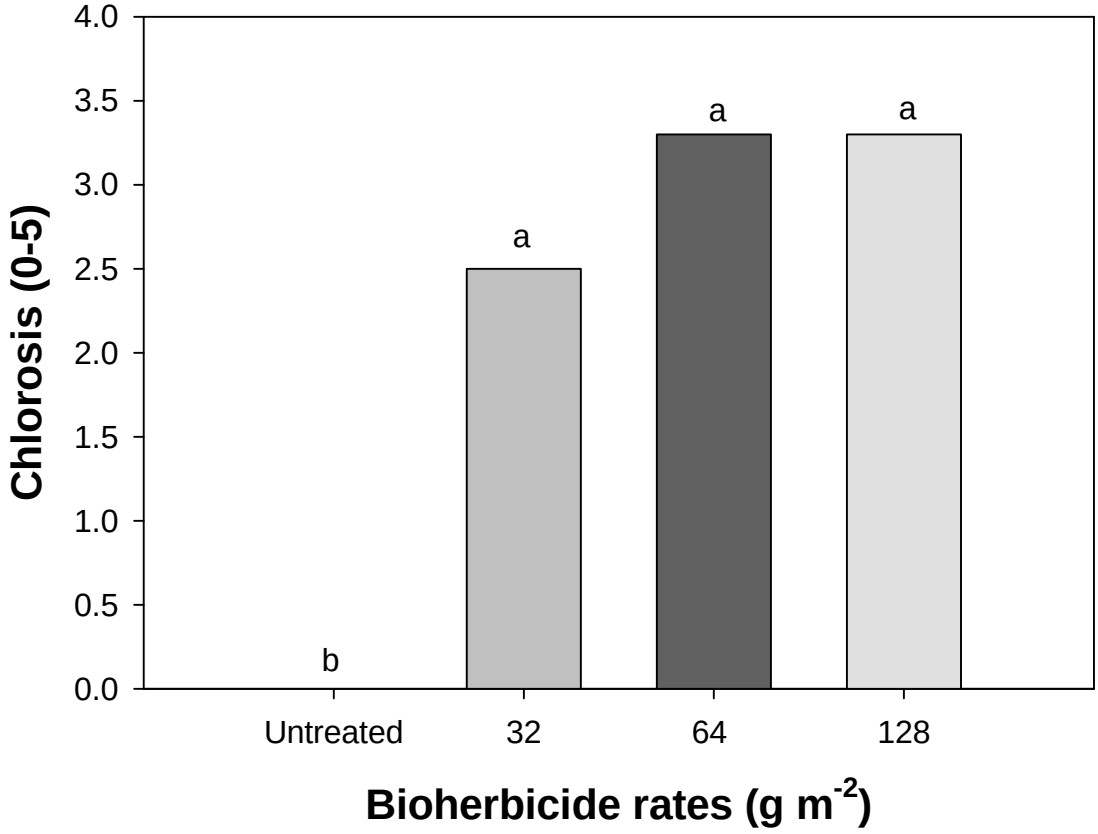

Figure 1. Bleaching and chlorosis of slender aster 13 days after treatment (DAT). (0 = no bleaching or chlorosis, 5 = complete necrosis). Trial was initiated on May 2, 2012, in College Station, TX, with single bioherbicide applications made at the beginning of the trial. Means followed by the same letter are not significantly different according to Tukey's Test ($P \leq 0.05$).

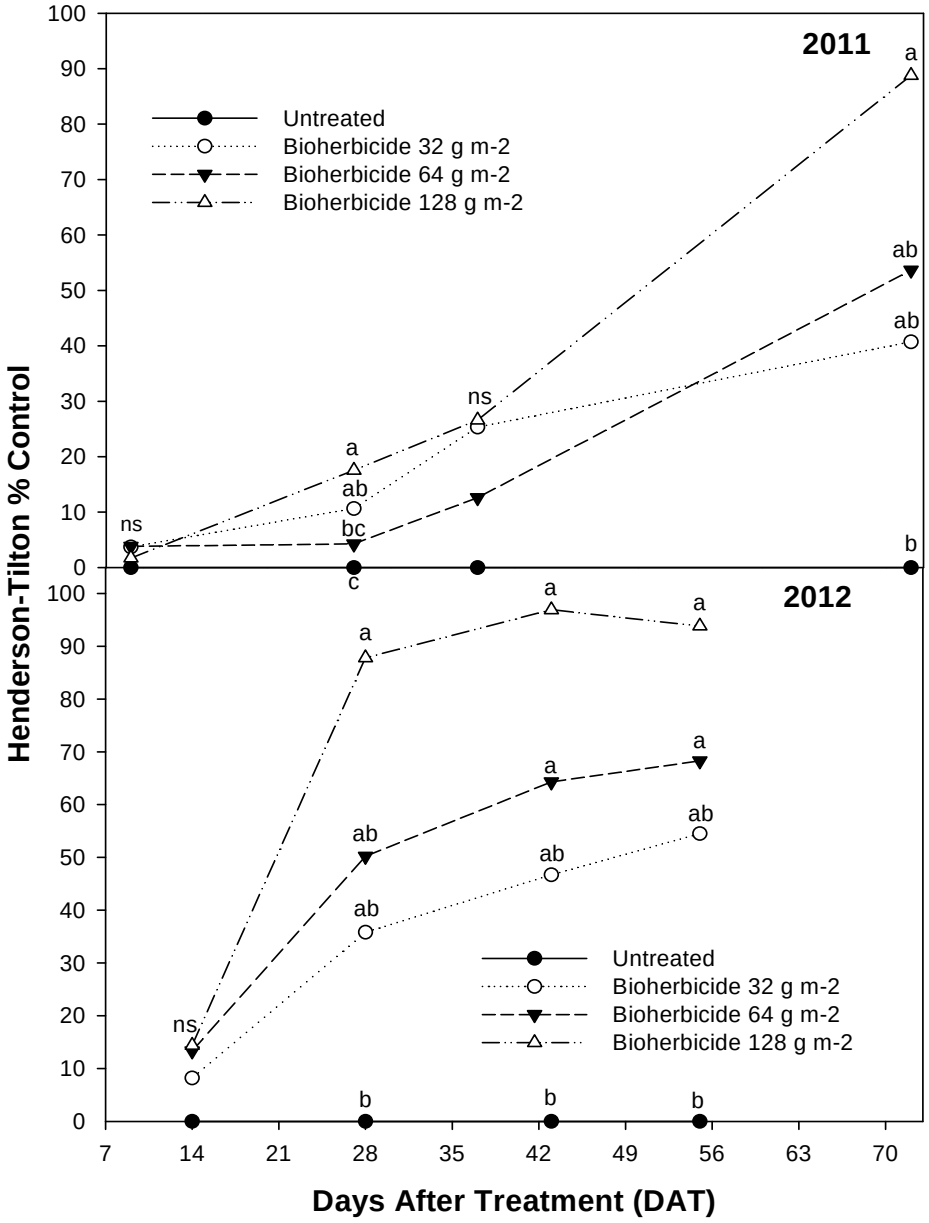

Figure 2. Henderson-Tilton % control of slender aster. Percentages were calculated using the Henderson-Tilton formula based on treated vs. untreated weed grid counts. Trials were initiated on June, 1 2011, and May 2, 2012, in College Station, TX. Bioherbicide was split applied in 2011, and single applications were made in 2012 at trial initiation. Means followed by the same letter are not significantly different according to Tukey's Test ($P \leq 0.05$).

As in 2011, no significant differences in weed control were observed in any treatment until 28 DAT, at which time the 128 g m^{-2} bioherbicide treatment provided 88% control. Rates of 32 g m^{-2} and 64 g m^{-2} provided marginal (~50%) control; however, these were not statistically different from untreated plots, due to a naturally occurring decline in weed population in untreated plots. By the end of the 2012 study (55 DAT), both the 64 and 128 g m^{-2} bioherbicide rates provided significantly improved control relative to untreated plots, (68 and 94%, respectively). Final levels of slender aster control were similar between both years, with the 128 g m^{-2} application rate providing 88 to 94% control, the 64 g m^{-2} application providing 55 to 65% control, and the 32 g m^{-2} application rate providing 40 to 50% control.

Temperature Effects

Previous research has shown this bioherbicide effectively controlling weeds under mild temperatures ranging from 15°C to 25°C (Bailey et al., 2011). However, prior to this research, it was not known what levels of control could be expected under higher temperatures. Despite the high temperatures around 41°C (with mean temperatures of 31°C in 2011 and 27°C in 2012) in both years, the bioherbicide provided effective control of slender aster in this study. Based on these results, the bioherbicide appears to retain good efficacy as a natural weed control product during summer months in areas receiving high temperatures following application. It should be noted, however, that irrigation was provided frequently (4 to 5 times weekly) during this study, and may have also contributed to success of the bioherbicide under these conditions. Daily irrigation may not be agronomically appropriate or feasible in some situations, especially

where municipal water restrictions limit the frequency of irrigation allowable on a landscape.

Potential Carryover

Another area of interest is the potential of this product to persist and carry over into subsequent seasons in the soil. Although no analysis of microbial fractions were attempted in this study, field observations indicate limited to no carryover into the following year, as successive weed seeds germinated in plots and produced green, healthy slender aster plants. However, research has shown that clay soils will retain the bioherbicide product longer than sandy loam soils (Bailey et al., 2010) similar to those used in this study. Our observations are consistent with the findings of Zhou et al. (2004) who found that residual activity of *Phoma macrostoma* begins to decline after 4 months, with no negative effects seen on susceptible plants in the year following application.

Implications on Use in Lawns

The results from this study demonstrate that the bioherbicide is effective at controlling slender aster, with a final level of control dependent on rate applied. Though significant control was not observed either year at the lowest application rate (32 g m^{-2}), limited activity was observed. Again, it should be noted that the material tested only contained half of the potency of the target commercial product intended for consumer use. Therefore, the amount of product used in these studies was twice that of the anticipated final commercial product. While a 128 g m^{-2} rate may be required for lawns with high weed pressure, lower rates (32 and 64 g m^{-2}) may be adequate for light weed

infestations. Furthermore, unlike CGM, which must be broadcast applied at high rates over the entire lawn for preemergence control, the bioherbicide could be used as a remedial postemergence weed spot-treatment product, thereby reducing the total application amount substantially.

Conclusions

Effective weed control using the bioherbicide has been observed under a range of temperatures and appears to be limited to the season of application and site of placement, with no apparent phytotoxicity to desirable warm-season turfgrass. While future research is needed to more clearly define the spectrum of weed species controlled by this bioherbicide, it appears to be a suitable candidate for use as a natural broadleaf weed control option for lawns.

CHAPTER III

WEED CONTROL SPECTRUM AND TURFGRASS TOLERANCE

Introduction

Over the past decade, synthetic herbicides have come under increased scrutiny around the world. Controversy surrounding pesticide use has led municipalities in Canada to restrict the use of pesticides for only cosmetic purposes. A concern of cancer occurring due to pesticide exposure is one of the driving forces behind these restrictions (Knopper and Lean, 2004). While some legislation has targeted herbicide use only for cosmetic purposes, others have banned the use of all weed and feed lawn products, allowing the use of herbicides only for lawn spot treatment applications (Anonymous, 2010).

Few effective natural options for weed control in turfgrass systems are currently available. Among the alternative herbicides that are available for use are vinegar, essential oils (clove & cinnamon oil), citric acid, fatty acids (pelargonic acid), and combinations of these different products. These products are primarily used for nonselective weed control, with the effectiveness of these products dependent upon product concentrations, with vinegar, citric acid, and clove oils providing better control at higher use rates (Abouziena et al. 2009; Boyd and Brennan 2006; Evans et al. 2009). Use of higher product rates, however, results in greater potential for crop injury (Evans and Bellinder 2009).

Some currently available bioherbicides derived from fungi include DeVine®, (*Phytophthora palmivora* (Butler) Butler sensu lato), which is used for the control of *Morrenia odorata* (Hook. & Arn.) Lindl. (stranglervine or milkweed vine) in citrus, Collego® (*Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. f.sp. *aeschynomene*), which is used to control *Aeschynomene virginica* (L.) BSP (northern jointvetch) in rice and soybean crops, BioMal® (*Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. f.sp.*malvae*), which is used for the control of *Malva pusilla* Sm. (round-leaved mallow) in Canada, and Dr. BioSedge®, (*Puccinia canaliculata* (Schw.) Lagh.), used for the control of *Cyperus esculentus* L. (yellow nutsedge) (Charudattan and Dinoor, 2000). None of these products are currently targeted for use in turfgrass, and the latter two are unavailable for commercial use. *Sclerotinia minor* Jagger has shown good control of dandelion in turfgrass settings, but is thought to be reliant on competition from turf to help reduce weed populations (Abu-Dieyeh and Watson, 2007).

Phoma macrostoma Montagne (PM) is a naturally occurring fungus being developed as a natural herbicide, or bioherbicide, for lawn weed control, produced from the solid fermentation of the fungus on grain. The effects of PM were first reported in 2001, on the growth of dandelion (Bailey and Derby, 2001), as well as, demonstrating suppression of broadleaf weeds like Canada thistle, chickweed, and scentless chamomile. *Phoma macrostoma* was discovered in Canada, when field isolates were collected from infected Canada thistle that were showing symptoms of bleaching and chlorosis (Graupner et al., 2003; Graupner et al.2006). These isolates were found to produce metabolites that were phytotoxic to susceptible broadleaf weeds, causing

bleaching and chlorosis (Graupner et al., 2006). Broadleaf weeds exhibit greater sensitivity than grasses to these metabolites (Bailey and Derby, 2001; Graupner et al., 2006).

The objectives of this study were to evaluate and confirm 1) the spectrum of broadleaf weeds controlled, 2) appropriate application rates of PM necessary to provide weed control under controlled greenhouse conditions, and 3) compare levels of control to a commercially available selective herbicide.

Materials and Methods

Greenhouse studies were conducted during 2012 and 2013 at the Texas A&M University Borlaug Center for Southern Crop Improvement, College Station, TX. In 2012, trials were initiated on 13 June 2012, and carried out until 1 August 2012. The 2013 trials were initiated on 20 June 2013, and carried out until 8 August 2013. The studies were conducted on the following broadleaf lawn weeds: common dandelion (*Taraxacum officinale* Weber ex F.H. Wigg.), annual sowthistle (*Sonchus oleraceus* L.), California burclover (*Medicago polymorpha* L.), slender aster (*Aster subulatus* var**.** *ligulatus* Shinners), common purlsane (*Portulaca oleracea* L.), common mallow (*Malva neglecta* Wallr.), yellow woodsorrel (*Oxalis stricta* L.), henbit (*Lamium amplexicaule* L.*)*, common chickweed (*Stellaria media* (L.) Vill.), shepherd's purse (*Capsella bursa-pastoris* (L.) Medikus), and spotted spurge (*Euphorbia maculata* L.). Poor germination led to only one year of data for yellow woodsorrel, henbit, common chickweed, shepherd's purse, and spotted spurge. Therefore, data for these weeds have not been presented herein.

Weed species were seeded and given 4 to 6 weeks to become established prior to treatment, with weeds being thinned to a desired final density of 5 plants pot^{-1}. For better consistency with field experiments, native field topsoil was used as a growth medium. This soil was a Lufkin fine sandy loam (fine, montmorillonitic, thermic, Vertic Albaqualf) with a pH of 5.3.

Potted weed treatments were arranged as a randomized complete block (RCBD) with four replications. Individual treatments consisted of a single 10 x 10 cm^{-1} pot. A complete slow-release fertilizer (12-4-8) was applied at seeding to supply 3.7 g N, 0.5 g P, and 2.0 g K m^{-2}. During the course of the studies pots were watered daily to field capacity so that weeds did not experience moisture stress. For both studies the greenhouse was maintained at 27°C/24°C (day/night) temperatures. Based on light measurements obtained during cloudless days at solar noon during both studies, photosynthetic photon flux within the study area was 890 (±5) μmol m^{-2} s^{-1}, and did not differ between studies.

To initiate the studies, granular bioherbicide treatments were applied to damp weed foliage at application rates of 32, 64, or 128 g m^{-2} in 2012, and 30, 60, and 120 g m^{-2} in 2013 (1/2x, 1x, and 2x, respectively). Application rates differed between years due to slight differences in the PM concentration of the formulated products for 2012 and 2013 studies. Based on preliminary research trials, target rates were designed to supply 6,600 MU/m^{-2} (preemergence herbicide rate), 12,667 MU/m^{-2} (postemergence herbicide rate), or 25,000 MU/m^{-2} (spot treatment rate) (Stuart Falk, personal communication). Bioherbicide treatments were compared to a synthetically produced

industry standard herbicide Weed B Gon Southern Concentrate (WBG) (2, 4-D + MCPP + dicamba + carfentrazone), applied at 0.95 ml m^{-2}, and an untreated control. Granules were left on weed foliage for 24 hours before being watered into the soil by irrigation.

Weed evaluations included weed injury (chlorosis + necrosis) ratings taken 14 days after application to assess the short-term injury symptoms and the severity of the injury. A scale of 0 to 5 was used with 0 = no injury, 1 = slight chlorosis/minimal injury, 2 = many plants chlorotic/injured, 3 = many plants photobleached/ necrotic, 4 = most plants necrotic/photobleached, 5 = plant death.

Weed necrosis was also evaluated over the entire length of the study, based on visual estimation of the percentage of entire weed leaf canopy exhibiting brown, necrotic symptoms. For example, if 70% of the remaining leaf tissue in the canopy was brown and 30% was green, then it was evaluated as a necrosis level of 70%.

Other weed evaluations included percent digital image analysis (DIA) of weed cover, which involved light box photos for digital imaging analysis of percent (%) green cover using Sigma Scan software (Richardson et al., 2001). Photographs were taken with the aid of a light box to ensure consistency in light quality. The light box was constructed of a 15.2-cm PVC pipe cut to 20.3-cm in length. A cap was placed on one end, with an aperture cut to fit a camera lense. Lights from four LED flashlights were used as the light source, and spaced evenly around the cap to give even light distribution. A piece of felt was placed over the light bulbs to dissipate the light, and prevent glare on the weed leaves. Pots contained the same number of weeds at trial initiation and final counts were taken at trial completion. Weed counts were used to calculate percent

control based on the Henderson-Tilton Method, (1 – *Ta* x *Cb* / *Tb* x *Ca*) x 100, where *Tb* is the number of grids per plot before treatment, *Ta* the number of grids after treatment, *Cb* the number of grids in the untreated plot before treatment, and *Ca* the number of grids from the untreated plot after treatment (Henderson and Tilton, 1955). Visual percent cover (0-100), visual percent necrosis (0-100), and visual chlorosis (0 = no bleaching, 5 complete necrosis) were evaluated at 7, 14, 21, 29, and 49 DAT. At the completion of the study, total weed biomass (above and below ground) was determined by oven drying plants that been washed free of soil at 65℃ for 72 hours.

Turfgrass tolerance experiments were conducted alongside the broadleaf weed control experiments to test whether PM applied at 1/2x, 2x, and 4x rates would cause injury to commonly used warm-season turfgrasses. Species evaluated included bermudagrass (cultivars 'Celebration' and 'Tifway'), zoysiagrass (cultivars 'Palisades' and 'Cavalier'), St. Augustinegrass (cultivars 'Raleigh' and 'Floratam'), common centipedegrass, and common buffalograss. Turf plugs were harvested from the Texas A&M Turfgrass Field Lab using a 10.8-cm golf hole cup cutter. Excess soil was trimmed off creating a 2.5-cm soil depth. Plugs were planted into 15.3-cm round pots filled with Sunshine® MVP Professional Growing Mix (Sun Gro Horticulture Canada Ltd., Seba Beach, AB, Canada) and given 4 weeks to become established. Potted turf plug treatments were arranged as a randomized complete block (RCBD) with four replications. During the course of theses studies, pots were watered daily to field capacity to prevent moisture stress. Granular bioherbicide treatments were applied to damp turf foliage. Granules were left on turf foliage for 24 hours before being watered

into the soil by irrigation. Weekly turf injury ratings were taken using a scale of 0 to 5 with 0 = no injury, 5 = turf death.

At the conclusion of the study, data were subjected to analysis of variance using PROC GLM (SAS, Cary, NC) to determine statistical significance. Mean separation procedures were performed using Fisher's Protected LSD at the $P = 0.05$ level.

Results

A rate-dependent response effect was observed during both studies, with PM showing increasing efficacy as the application rates increased. This response was seen in terms of percent control, weed necrosis, weed chlorosis, DIA, and dry biomass. A significant replication effect was also observed with shoot dry weights and DIA (Tables 1 and 2, respectively). This can be attributed to variation in weed growth rates prior to the experiment, and was accounted for by blocking similar sized weeds within replications prior to initiating the experiment. Thus, the last block (replication) contained weeds that were slightly smaller and less developed than the first block.

Table 1. ANOVA for percent control, shoot, root, and total biomass dry weights of greenhouse studies.

	P values			
	Final Weed Control	Final Dry Weights		
	%	Shoots	Roots	Total
Study	NS	NS	***	***
Species	***	***	**	***
Treatment	***	***	***	***
Study x Treatment	NS	NS	*	*
Replication	NS	*	NS	NS
Species x Treatment	***	***	***	***
Study x Species	*	*	NS	*

* Significant at 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

NS, not significant at $P \leq 0.05$.

Table 2. ANOVA for chlorosis, necrosis, and DIA of greenhouse studies.

	P values		
	Injury	Necrosis	DIA Cover
Study	***	**	***
Species	***	***	***
Treatment	***	***	***
Replication	NS	NS	***
Timing	***	***	***
Study x Species	**	***	***
Study x Treatment	***	NS	NS

* Significant at 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

NS, not significant at $P \leq 0.05$.

Final Biomass Dry Weights

At the conclusion of the studies weeds were harvested, with shoots and the roots dried separately and weighed to determine the influence of PM on final weed biomass. ANOVA revealed a significant study main effect for root and total biomass, and a significant study x species interaction in terms of final shoot biomass, therefore data have been presented separately for study 1 (2012) and study 2 (2013) (Table 1).

A visible rate dependent response effect was seen with the shoot and root dry weights decreasing as the rate of PM increased both years; however differences were of greater statistical significance in study 1 (Table 3). In study 1, when pooling across all weed species, the highest rate (2x) of PM significantly reduced the shoot and root dry weights of weeds (1.24 and 0.42 g for shoots and roots, respectively) compared to the lowest rate of PM (1.72 and 0.88 g for shoots and roots, respectively). In fact, the reduction in shoot and root biomass was similar to that caused by WBG treatment (0.92 and 0.50 g for shoots and roots, respectively). The middle rate (1x) of PM did not significantly differ from low or high rates of PM in terms of shoot, root, or total biomass.

In study 2, fewer differences were seen between the PM rates, with no significant differences in total biomass dry weights (Table 3). However, the highest rate applied resulted in significantly less shoot dry weights than the middle PM rate (1.3 vs. 1.9 g, respectively), but was not significantly different than the low PM rate (1.6 g). As in study 1, WBG caused the greatest reduction in weed biomass dry weights, significantly less than all other treatments (0.58 and 0.60 g for shoots and roots, respectively).

Final Weed Control

ANOVA for final percent control revealed a significant study x species interaction, so data have been presented separately by study and weed species (Table 1). Final percent control increased as the application rates of PM increased, as seen in the dry weight data, with a visible rate response effect (Table 3). When pooling across all weed species, the lowest (1/2x) rate provided 11 to 18% control, the middle (1x) rate gave 19 to 23% control, and the highest (2x) rate gave 39 to 34% control, for studies 1 and 2, respectively. The slight decrease in control may have been associated with the slightly lower application rate used in study 2.

Based on orthogonal contrasts, which were pooled across weed species and rates, PM offered significantly better overall final control relative to untreated controls in both studies. PM also significantly reduced total weed biomass compared to untreated controls in study 1, but not in the study 2. In terms of both percent control and biomass, PM was generally less effective than WBG in both studies (Table 3).

Table 3. Data for final control, shoot, root, and total biomass dry weight across species for the two years of greenhouse studies.

	Study 1				Study 2			
	Final Control	Dry Weights (grams)			Control	Dry Weights (grams)		
Treatment	%	Shoots	Roots	Total	%	Shoots	Roots	Total
Untreated	0.00 c	1.57 ab	1.41 a	2.99 a	0.00 c	1.43 b	2.65 a	4.10 a
PM 1/2x	17.71 b	1.71 a	0.88 b	2.60 ab	11.04 c	1.58 ab	2.28 a	3.80 a
PM 1x	23.33 b	1.54 ab	0.64 bc	2.20 bc	18.67 c	1.87 a	2.30 a	3.86 a
PM 2x	39.21 a	1.24 bc	0.42 c	1.66 cd	34.30 b	1.26 b	1.98 a	3.56 a
Weed B Gon	51.80 a	0.92 c	0.50 c	1.42 d	64.50 a	0.58 c	0.60 b	1.20 b
P-value	***	***	***	***	***	***	**	***
				Contrast statements				
Untreated vs. treated	***	NS	***	***	***	NS	NS	*
Untreated vs. Bioherbicide	***	NS	***	**	***	NS	NS	NS
Bioherbicide vs. Weed B Gon	***	***	NS	**	***	***	***	***

Means within the same column followed by the same letter do not differ significantly according to Fisher's protected LSD ($P = 0.05$).
Percent control calculated using Henderson-Tilton Method with beginning and final weed counts.
* Significant at 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.
NS, not significant at $P \leq 0.05$.

Weed Species Control

Weed control provided by PM varied between species and studies, with rates of control ranging from 0 to nearly 100%, depending on the species. It should be noted that the weed density (5 per pot) used in these experiments was higher than what would generally be encountered in a typical lawn, and thus, represented a worst-case scenario in some respects. As such, even Weed B Gon Southern Concentrate (WBG) offered only low to moderate control in many cases.

Dandelion was the most susceptible of the six weeds evaluated, with the highest PM rate giving 100% dandelion control both years (Figure 3). The middle PM rate gave 95% dandelion control in study 1, but control decreased to 65% in study 2, possibly due to the slightly lower application rates in study 2. The lowest PM rate gave only 50 and 30% control of dandelion in studies 1 and 2, respectively, significantly lower than the higher two application rates. Dandelion control from PM at both the 1x and 2x rates was comparable to WBG application in both years.

Annual sowthistle also showed susceptibility to PM in both studies. The highest PM rate provided 75% control of annual sowthistle in study 1, but control dropped to only 15% in study 2 (Figure 3). The lower two rates gave 0 to 20% annual sowthistle control both years. Annual sowthistle control from WBG approached 45% both years, which was less than that offered by the 2x rate of PM in study 1, but nearly double that of the 2x PM rate in study 2.

The highest PM rate gave 55% control of slender aster in study 2, and 25% in study 1, while the lower two rates gave only 10 to 20% slender aster control in both

studies (Figure 3). However, the levels of control offered by PM were comparable to or superior to that of WBG in both years, which was 20% or less.

Phoma macrostoma rates provided consistently low (0 to 30%) control of California burclover in both studies (Figure 3), which was substantially less than that offered by WBG, which provided 80 to 90% control of California burclover.

Whereas limited (0 to 30%) control was observed from PM on common mallow in study 1, no activity was seen in study 2. Conversely, PM provided no control of common purslane in study 1, and only 0 to 5% control in study 2. In comparison, WBG provided between 50 and 100% control of both species (study 1 and 2, respectively).

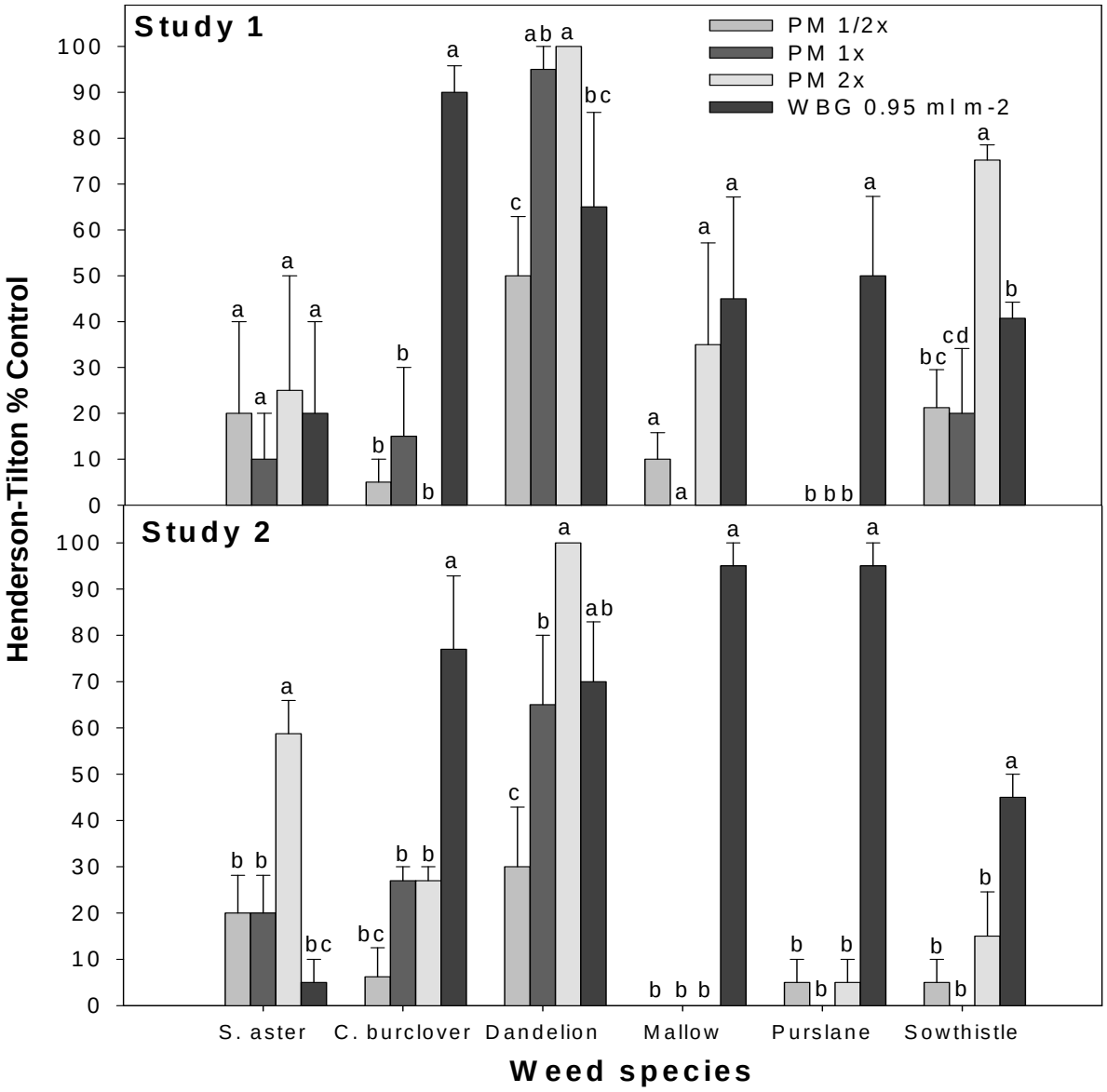

Figure 3. Henderson-Tilton % control of broadleaf weeds in greenhouse studies. Percent control was calculated using Henderson-Tilton Formula based on initial and final weed counts in each pot. Means followed by the same letter are not significantly different according to Fisher's Protected LSD ($P = 0.05$). Bars represent standard error.

Weed Injury

While total weed elimination following PM application may require a period of weeks or months, onset of initial injury is an important consideration, and is of practical importance to the home consumer, who may wish to determine whether the product is 'working' or not. Weed injury ratings were therefore taken at 14 days after application to assess the short-term injury symptoms and the severity of the injury. A scale of 0 to 5 was used with 0 = no injury, 1 = slight chlorosis/minimal injury, 2 = many plants chlorotic/injured, 3 = many plants photobleached/ necrotic, 4 = most plants necrotic/photobleached, 5 = plant death.

ANOVA revealed a significant study x treatment interaction for injury; thus, data have been presented separately by study (Table 2, Figure 4). As with the other ratings, injury in response to PM application was rate-dependent. In study 1, all PM treatments caused significant injury (1 to 2 out of 5) compared to the untreated (Figure 4), with injury (primarily chlorosis) increasing as PM rates increased, yet, PM caused less weed injury compared to WBG (3.5 of 5).

A similar response from PM was seen in study 2, but somewhat lower overall weed injury occurred for all treatments, relative to study 1. PM rates in study 2 provided injury ratings of 0.75 to 1.75 out of 5, and 1x and 2x PM rates provided similar levels of injury to WBG (Figure 4).

Percent Weed Necrosis

Percent necrosis ratings were taken weekly during the studies as a measure of the percentage of necrotic weed tissue in each pot. ANOVA revealed a significant study x

species interaction, as well as timing, treatment, and species main effects for necrosis. For the purposes of understanding overall effects and comparison of herbicide treatments, necrosis data were pooled across all weed species and presented by week (Table 2, Figure 5).

Again, a rate dependent response effect of PM on necrosis was observed, with the percentage of necrosis increasing with the application rates (Figure 5). The WBG treatment led to the greatest necrosis across all weeds evaluated throughout the studies. Weeds treated with WBG had 50 to 57% necrosis at 7 DAT in study 1 and study 2, respectively, showing significantly greater speed of activity of the product. The WBG finished the studies with 70 and 58% necrosis 49 DAT in study 1 and study 2, respectively (Figure 5), significantly greater than all of the PM rates. The PM was much slower acting, with little necrosis seen after 7 DAT, and only 15 to 20% necrosis 14 DAT at the highest PM rate. The highest PM rate had significantly greater necrosis than the lower two rates, exhibiting 48% in study 1, and 30% in study 2 at 49 DAT. The lower two PM rates were not significantly different than the untreated by study completion in either year, with each exhibiting 10 to 25% necrosis both years.

Turfgrass Injury

Parallel turf experiments were also conducted alongside the broadleaf weed experiments to test whether PM applied at 1/2x, 2x, and 4x rates would cause injury of commonly used warm-season turfgrasses, including bermudagrass (cultivars ‘Celebration’ and ‘Tifway’), zoysiagrass (cultivars ‘Palisades’ and ‘Cavalier’), St.

Augustinegrass (cultivars ‘Raleigh’ and ‘Floratam’), common centipedegrass, and common buffalograss.

Based on these experiments, no injury was observed at any time during the 49 day experiment due to PM application (data not shown). This is also consistent with our observations from field studies, where injury has never been observed due to PM application to common bermudagrass (*Cynodon dactylon* [L.] Pers.), ‘Raleigh’ St. Augustinegrass (*Stenotaphrum secundatum* [Walt.] Kuntze), or ‘Tifway’ bermudagrass (*Cynodon dactylon x C. transvaalensis* Burtt-Davy).

Percent Digital Image Analysis Weed Cover

ANOVA revealed a significant study x species interaction, as well as timing, treatment, and species main effects for digital image analysis (DIA) weed cover. As with necrosis data, herbicide x timing data have been presented with data pooled across weed species to better ascertain the effects of herbicide treatments over time as they relate to untreated and WBG applications (Figure 6).

As with percent control and necrosis, a rate-dependent response of PM on weed cover was observed, with weed cover decreasing as the PM application rates increased. In study 1, green vegetative cover by weeds was initially between 75 and 80% weed green cover. Over the course of the study, a PM rate response was seen, with green cover decreasing as PM rates increased (Figure 6). In study 1, at around 21 DAT, there was a noticeable decline in green cover across all treatments. This is attributed to monthly greenhouse pest maintenance with the greenhouse being unavailable for 24 hours, which

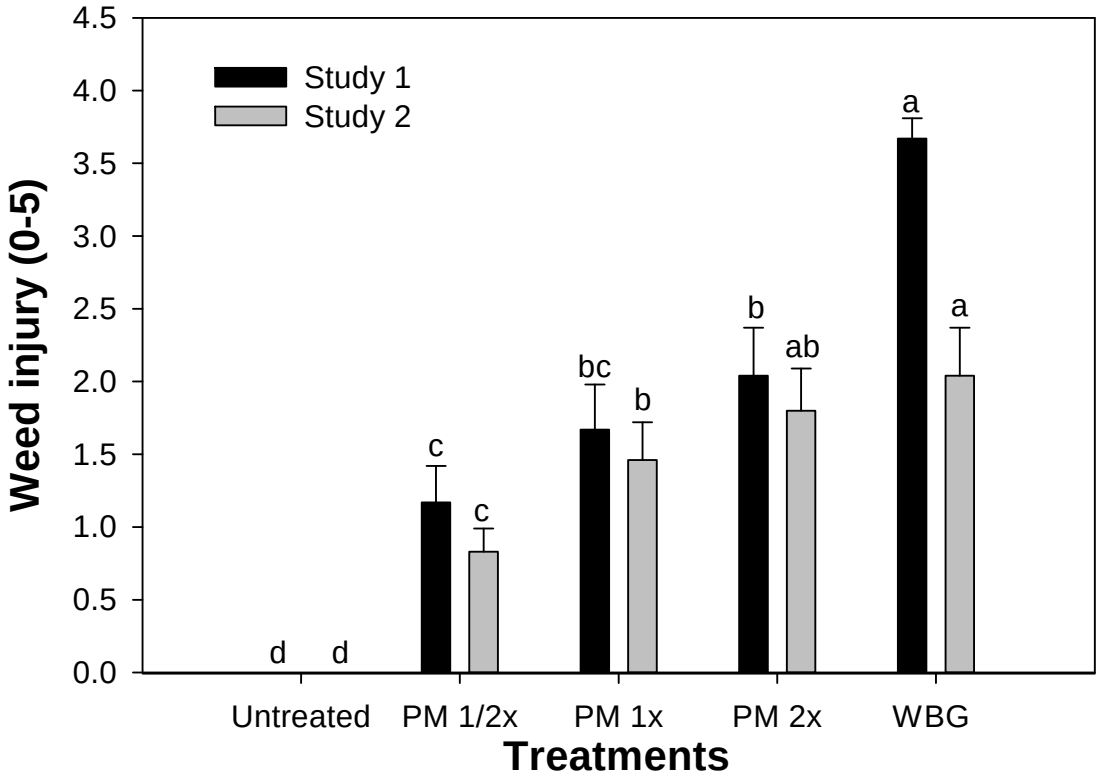

Figure 4. Weed injury of broadleaf weeds in greenhouse studies 14 DAT. 0 = no injury, 3 = majority of plants photobleached/injured, 5 = plant death. Means were pooled across all weed species. Means followed by the same letter are not significantly different according to Fisher's Protected LSD ($P = 0.05$). Bars represent standard error.

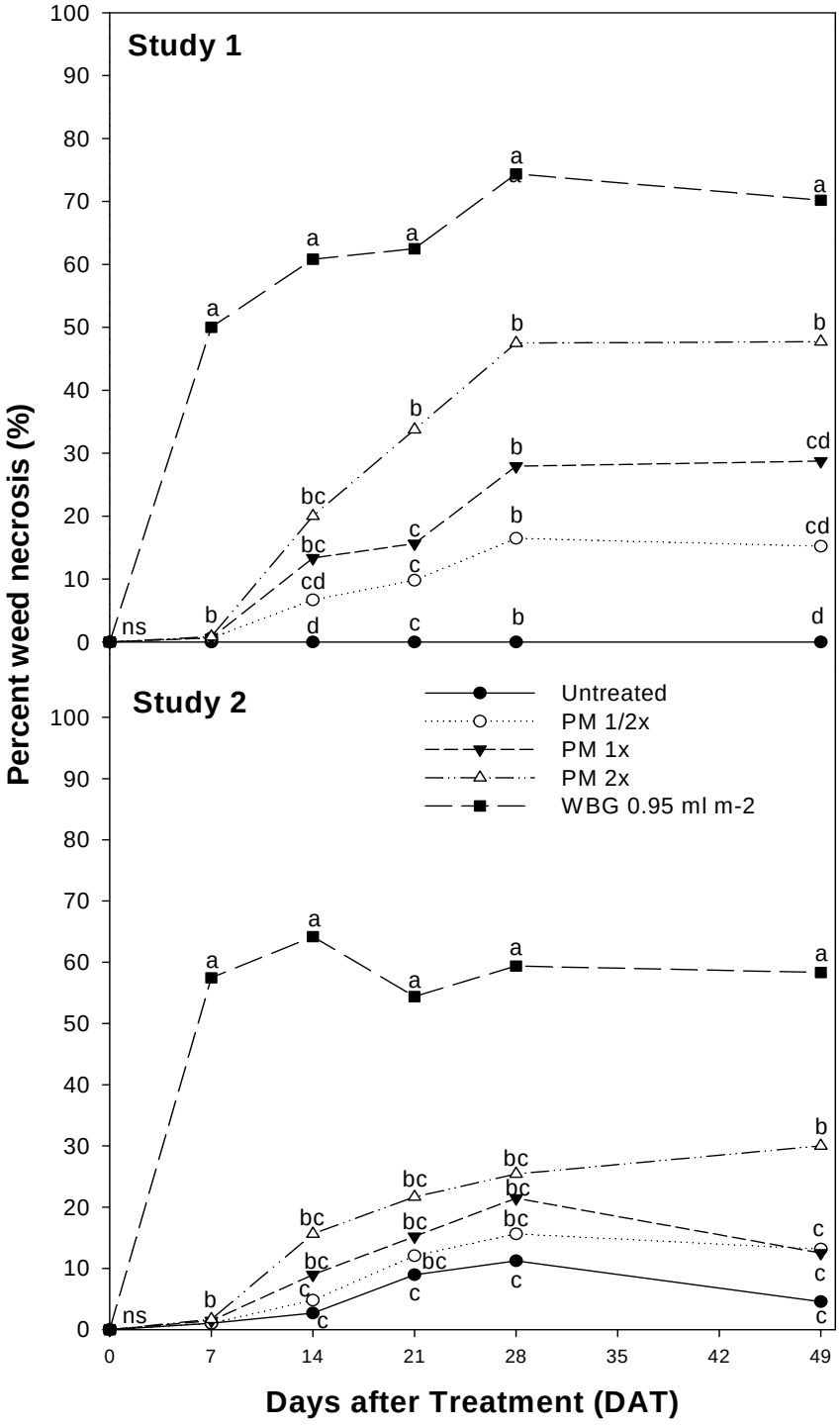

Figure 5. Percent necrosis (%) of broadleaf weeds in greenhouse studies. Means were pooled across all weed species evaluated. Means followed by the same letter are not significantly different according to Fisher's Protected LSD ($P = 0.05$).

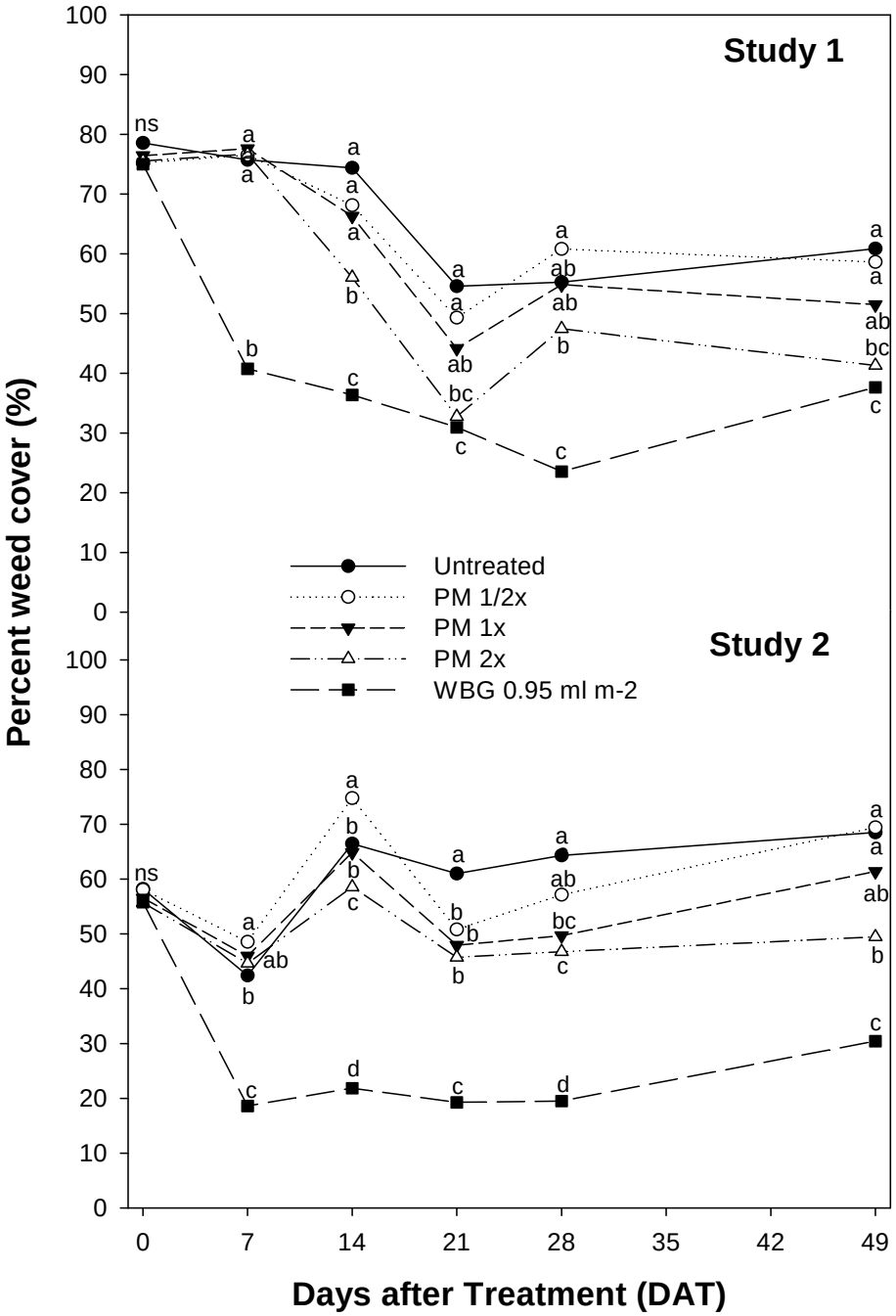

Figure 6. Percent cover (%) of broadleaf weeds in greenhouse studies. Cover was calculated using light box photographs analyzed in Sigma Scan software (Richardson et al., 2001) for % green cover. Means were pooled across all weed species evaluated. Means followed by the same letter are not significantly different according to Fisher's Protected LSD ($P = 0.05$).

resulted in plants missing a day of irrigation, causing wilting of the weeds. However, once irrigation was resumed, wilting decreased quickly.

Little reduction in green cover was seen by the PM treatments until 14 DAT in study 1, when the highest rate caused significant reduction in green cover compared to the lower two PM rates. By comparison, WBG provided significantly more rapid and substantial (nearly double) reductions in green cover compared to PM, causing significant reductions as early as 7 DAT compared to all other treatments, with differences sustained for most of the trial.

By the end of study 1, the highest PM rate reduced weed cover compared to the untreated and lowest PM rate, but was not different than the middle PM rate or the WBG treatment. The middle PM rate was also not different from either the untreated or lowest PM rate. By the conclusion of the study, WBG caused the greatest reduction in green cover with 40% green cover remaining 49 DAT.

In study 2, experiments were initiated with slightly less (55 to 60%) weed green cover than in study 1. A slight reduction in green cover by all PM treatments and the untreated was observed 7 DAT, and a substantial decline in cover occurred 7 DAT in response to WBG application. This was followed by a slight rebound in green cover at 14 DAT by the PM and untreated treatments, thereafter increasing slightly for the ½x and 1x treatments, but leveling off for the 2x and untreated controls for the duration of the study.

WBG application again led to the greatest reduction in green cover finishing with 30% green cover, significantly less cover than that remaining in all other treatments.

Application of the highest PM rate resulted in a slight decline to 45% green cover while the lower two PM rates and the untreated finished with statistically similar green cover than when the study was initiated, 55 to 60%, suggesting poor efficacy at these lower two rates.

Discussion

When evaluating these results in their entirety, it is apparent that PM is effective for controlling some, but not all weed species. While PM worked quite effectively on dandelion, it offered little control of other weeds including common mallow and purslane. Also, while injury effects (chlorosis/necrosis) were observed in most species, many were cosmetic in nature, with complete necrosis never taking place. As such, it seems that follow-up or repeat applications may be necessary to achieve better control of many of the weeds tested. While future research is needed to more clearly define the spectrum and benefits of repeat applications on control of various weed species controlled by this bioherbicide, PM offers potential for use as a natural broadleaf weed control option for specific broadleaf weeds.

In the current study, dandelion was clearly the most susceptible of the weeds evaluated, with the highest PM rate providing 100% control. The middle rate gave 65 to 95% while the lowest PM rate gave 30 to 50% control of dandelion. The results reported here are consistent with those of Bailey et al. (2011a) that reported PM caused up to 92% mortality of dandelion under greenhouse conditions, and 70 to 90% control of dandelion under field conditions. Similarly, Zhou et al. (2004) reported 77% control of dandelion using PM application rates of 63 g m^{-2} and 90% control at 125 g m^{-2} under field settings.

The results reported by Zhou et al. (2004) are within the range of rates and control in the current greenhouse study.

Our results also indicated that the highest PM rate provided 75% control of annual sowthistle in 2012, but only 15% in 2013, with the lower two PM rates providing 0 to 20% control. While there are no published data regarding PM control of annual sowthistle, Bailey et al. (2011a) reported 33% mortality of perennial sowthistle, a weed from the same family and similar in appearance, but with different root and reproductive characteristics.

The highest PM rate in our study provided 25 to 55% control of slender aster, while the lower two rates gave 20% control or less in both years. This is much lower than observed under field conditions with similar application rates where PM provided 50 to 94% control of slender aster (Smith et al., 2013b). This is likely attributed to the slender aster plants in the greenhouse being more mature and established than plants in the field, as well as from possible competition with the turf.

The PM also provided between 0 to 30% control of California burclover across all application rates. This level of California burclover control is much lower than observed by Bailey et al. (2011a) who reported 78% California burclover mortality. However, it should be noted that the authors achieved this level of control using a 4x rate, essentially double that of the highest rate tested in this study.

In this study, we observed little to no activity from PM on common mallow and common purslane. However, Bailey et al. (2011a) reported 52% mortality of purslane,

and similar to the findings of Zhou et al. (2004), found the greatest activity occurred within the first 30 days following PM applications.

On dandelion, PM offered similar or even superior control to that of WBG. Despite this, WBG provided significantly better control of the majority of the weeds PM struggled to control, including common purslane, California burclover, and common mallow. Thus, while PM clearly does not offer the broad spectrum of weed control of WBG, it showed good efficacy on some broadleaf weeds.

Unlike other bioherbicides which commonly offer control of only a specific weed, PM has shown activity on many broadleaf weeds. However, based on the mild to moderate injury and low to inadequate levels of control offered for many species tested, it seems likely that increased rates, more concentrated formulations, or repeat applications would be necessary for controlling these weeds .

Conclusions

This study evaluated weed control following application of various rates of PM on broadleaf weeds including slender aster, California burclover, common dandelion, common mallow, common purslane, and annual sowthistle, as well as the tolerance of warm-season turfgrass cultivars. While no injury was observed from PM on any turfgrasses, activity on the broadleaf weeds was variable. Although all weeds showed susceptibility to PM, as evidenced by some extent of chlorosis following application, levels of control differed by weed species and study. The PM had the greatest efficacy on dandelion, which was equal to or superior to WBG in both studies, but showed little to no efficacy on common purslane and to some extent, common mallow. These results

suggest that PM may not offer effective broad spectrum broadleaf weed control similar to that of common synthetic herbicides, (i.e. WBG) at the rates used in this study.

Weed size and application timing may have also impacted the variable efficacy of PM, with weed control decreasing as weeds became larger and more mature. This is evident by the difference in control of slender aster from the field to the greenhouse. Plants in the field studies were younger and much less mature, with applications being made early in the growing season.

Additionally, it should be emphasized that chlorosis and bleaching do not necessarily lead to necrosis and death of weeds. The different weeds tested showed varying levels of susceptibility to PM, with some exhibiting greater chlorosis and plant death than others. California burclover showed signs of chlorosis, but not all affected plants died. Application rates may have played a role in this with PM having better activity at higher application rates on less sensitive weeds as also reported by Bailey et al. (2011a) when a 4x application rate saw 78% mortality of California burclover.

While higher concentrated formulations, elevated application rates, or repeat applications may overcome the limited and variable control seen in some of the weed species, this may not be feasible, practical, or acceptable in the context of turfgrass management, and may impact the potential commercial success of such a product in the market place.

CHAPTER IV

THE EFFECTS OF IRRIGATION FREQUENCY, TEMPERATURE, AND *PHOMA MACROSTOMA* APPLICATION TECHNIQUE ON DANDELION CONTROL

Introduction

Phoma macrostoma (PM) is a naturally occurring fungus being developed as a natural herbicide for lawn weed control, produced from the solid fermentation of the fungus on grain. The effects of PM were first reported in 2001, on the growth of dandelion as well as demonstrating suppression of broadleaf weeds like Canada thistle, chickweed, and scentless chamomile (Bailey and Derby, 2001). PM was discovered in Canada, where field isolates were collected from infected Canada thistle that were showing symptoms of bleaching and chlorosis (Graupner et al., 2003; Graupner et al., 2006). These isolates were found to produce metabolites that were phytotoxic to susceptible broadleaf weeds, causing bleaching and chlorosis (Graupner et al., 2006). Broadleaf weeds exhibit greater sensitivity than grasses to these metabolites (Bailey and Derby, 2001; Graupner et al., 2006). Although plant symptoms are similar to those observed from hydroxyphenyl pyruvate dioxygenase (HPPD) inhibitors, the mode of action does not inhibit this pathway and is still unknown (Graupner et al., 2003). While the mode of action is still being studied, PM has exhibited two modes of action; foliar photobleaching caused by phytotoxic macrodins and root inhibiton (Bailey et al., 2011b).

Weed control with PM has been evaluated primarily in more northern climates on weeds ranging from pigweed to English daisy. In these studies, the bioherbicide has shown control of dandelion, Canada thistle, chickweed, and English daisy, with maximal efficacy reported at temperatures ranging from 15 to 25°C (Bailey et al., 2011a). Soil mobility of PM is limited, even at excessive application rates (Zhou et al. 2004), with retention and activity of the bioherbicide highest in clays rather than sand-based soils (Bailey et al. 2010).

The objective of this study was to evaluate the effects of irrigation frequency, temperature, and application technique on dandelion control efficacy in a controlled environment chamber.

Materials and Methods

Growth chamber studies were conducted during 2013 at Texas A&M University, College Station, TX, in the Herman F. Heep Center for Soil & Crop Sciences. Trials were initiated on 17 May 2013, and 4 September 2013, and carried out for 35 days. The studies were conducted on common dandelion (*Taraxacum officinale* Weber ex F.H. Wigg.).

Dandelions were seeded in a greenhouse and given 4 to 6 weeks to become established. Weeds were placed in growth chambers 7 days prior to treatment, with weeds being thinned to a desired final density of 5 plants pot^{-1}, and allowed to acclimate to growth chamber test conditions (Table 4.). Two Conviron CMP4030 (Controlled Environments Limited, Winnipeg, Manitoba, Canada) growth chambers were used, with one set at 20°C and one set at 30°C constant temperatures for the duration of the

Table 4. Growth chamber light intensity and temperature readings.

Target Temperature	Light intensity (μmol m^{-2} s^{-1})		Temperature °C					
			Study 1			Study 2		
(°C)	Study 1	Study 2	Average	Low	Max	Average	Low	Max
20°	569 ±12	442 ±30	20.35 ±0.64	19.80	22.40	20.20 ±0.50	19.40	22.10
30°	553 ±12	524 ±31	30.00 ±0.60	29.10	32.30	30.30 ±1.25	28.70	33.10

studies. Each growth chamber was equipped with five 400-watt metal halide and five 400-watt high pressure sodium bulbs (Philips Lighting Company, Somerset, N.J.). Lighting was programmed to provide a 14/10 hour (day/night) photoperiod, with light intensity ramping up in the morning and ramping down in the evening, to simulate sunrise and sunset. HOBO® temperature loggers (Onset Computer Corporation) were used to monitor growth chamber temperatures throughout these studies. For better consistency with field experiments, native field topsoil was used. This soil was a Lufkin fine sandy loam (fine, montmorillonitic, thermic, Vertic Albaqualf) with a pH of 5.3.

Potted weed treatments were arranged as a completely randomized design (CRD) with 5 replications. Experimental units consisted of 5 dandelions in a single 10 x 10 cm^{-1} pot. A complete slow-release fertilizer (12-4-8) was applied at seeding to supply 3.7 g N, 0.5 g P, and 2.0 g K m^{-2}.

Both chambers contained the same treatments. Treatments were 1) untreated control watered every other day (EOD), 2) soil applied PM watered EOD, 3) soil applied PM + ammonium sulfate (AMS) watered EOD, 4) untreated control watered daily, 5) soil applied PM watered daily, 6) soil applied PM + AMS watered daily, and 7) foliar

applied PM to wet weed foliage watered daily (applied by misting the tissue with distilled water prior to PM granule application). All PM treatments received the same application rate of (60 g m^{-2}) of product. Treatments with AMS added received a nitrogen application rate of 2.5 g N m^{-2}. All treatments received irrigation amounts of 100% x actual evapotranspiration (ET_a) during the study (determined by averaging the daily gravimetric change in weight of 5 reference pots). Mean ET_a was 40 (+/- 3.8) ml per day for the 20°C chamber and 80 (+/- 3.4) ml per day for the 30°C chamber for both studies. At the conclusion of the first study, the chamber temperatures were swapped (20°C chamber set at 30°C, 30°C chamber set at 20°C) and the study was repeated.

Weed evaluations included digital image analysis (DIA) of percent weed green cover using Sigma Scan (Richardson et al., 2001). Photographs were taken with the aid of a light box to ensure consistency in light quality. Plots contained the same number of weeds at trial initiation and final counts were taken at trial completion. Weed counts were used to calculate percent control using the Henderson-Tilton formula, (1 – *Ta* x *Cb* / *Tb* x *Ca*) x 100, where *Tb* is the number of grids per plot before treatment, *Ta* the number of grids after treatment, *Cb* the number of grids in the untreated plot before treatment, and *Ca* the number of grids from the untreated plot after treatment (Henderson and Tilton, 1955). Visual percent cover (0-100), visual percent necrosis (0-100), and visual chlorosis (0 = no bleaching, 5 complete necrosis) were also taken at 7, 14, 21, 28, and 35 DAT.

At the completion of the study, total dandelion biomass (above and below ground) was determined by oven drying plants that had been washed free of soil at 65°C

for 72 hours. Data were subjected to analysis of variance using the general linear model, univariate test procedure using SPSS ver. 21.0 (IBM Corp, Armonk, NY) to determine statistical significance of the results. Mean separation procedures were performed using Tukey's HSD at the $P \leq 0.05$ level.

Results

Percent Control

Percent control was calculated using the Henderson-Tilton Method, using initial and final weed counts. Study main effects were not significant for final percent control, so data were pooled across experiments. In terms of final percent control, there was a highly significant temperature x treatment interaction, as well as significant temperature and treatment main effects (Table 5). Surprisingly, little to no control was observed in

Table 5. Growth chamber ANOVA for weed biomass dry weights and percent control.

	P values						
	Study 1			Study 2			
	Dry Weights			Dry Weights			Control
	Shoot	Root	Total	Shoot	Root	Total	%
Temperature	NS	NS	NS	**	NS	NS	***
Treatment	**	**	**	***	***	***	**
Temperature x Treatment	*	NS	*	NS	NS	NS	***

Means for percent control pooled for both studies.

* Significant at 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

NS, not significant at $P \leq 0.05$.

the 20°C chamber, regardless of treatment in either study. Within the 20°C treatment, addition of AMS showed slight (but not significant) benefit compared to PM alone, regardless of irrigation treatment, providing 2% control of dandelions (Table 6.).

Better weed control occurred in the 30°C chamber in both studies, with PM treatments watered EOD providing generally superior control to those watered daily. Within the EOD treatments, PM + AMS EOD provided the highest levels of control (34%), but was statistically similar to PM EOD (20% control). Both PM treatments caused significantly greater levels of control relative to untreated EOD treatments. Within the daily irrigated plants, PM + AMS provided higher, but not statistically different control to PM daily or untreated controls. Within the daily irrigated treatments, foliar applications provided the greatest levels of control (16%), but these were not statistically different from other treatments.

Table 6. Growth Chamber Henderson-Tilton % control as influenced by the treatments. Means followed by the same letter are not significantly different, according to Tukey's HSD (P =0.05).

	Henderson-Tilton % Control Temperature	
Treatment	20°C	30°C
Untreated EOD	0.00 a	0.00 b
PM EOD	0.00 a	20.00 ab
PM + AMS EOD	2.00 a	34.00 a
Untreated Daily	0.00 a	0.00 b
PM Daily	0.00 a	8.00 b
PM + AMS Daily	2.00 a	12.00 b
PM Foliar Daily	0.00 a	16.00 ab

Biomass

Study main effects were significant for dry tissue biomass, and therefore data are presented separately by study for these parameters. In study 1, there were significant temperature x treatment interactions for shoot and total dry weights, but not for roots (Table 5). Treatment main effects were also significant for shoot and total dry weights. For study 2, a highly significant treatment main effect was observed on shoot, root, and total dry weights, while only shoot dry weights were affected by temperature (Table 5). PM treatments had little effect on shoot biomass during both studies, regardless of temperature (Table 5). While there were some treatment differences at 20°C in study 1 and both temperatures in study 2, these were of little practical significance, as untreated plants never statistically differed from their treated counterparts in either irrigation regime. PM appeared to have the greatest effect on root biomass in the studies, which influenced total biomass as well (Table 6).
For example, in experiment 1, both 'PM + AMS Daily' and 'PM Foliar Daily' treatments had significantly lower root and total biomass relative to the 'untreated daily' control treatments at the 30°C temperature. Also, in study 2, 'PM + AMS EOD' and 'PM EOD' each had significantly lower root and total biomass, relative to their untreated EOD counterparts.

Foliar application of the product to wet vs. dry foliage also showed significantly better activity on roots in study 1 at the 30 C temperature; however, differences in root biomass were not significant between these two treatments at either temperature in study 2.

Clearly, PM exhibited strong activity on roots, although effects differed by irrigation level between studies. Although rapid chlorosis was observed in treated plants during the study, this did not translate into decreased shoot biomass. This was likely because under the conditions of the growth chamber study, chlorotic plants were extremely slow to progress to a necrotic stage. Also, resurgence, or rebound of lower leaf tissue in treated plants sometimes was observed following treatment injury.

Chlorosis

Visual ratings were taken to assess the amount of chlorosis and photobleaching present on dandelions. Significant study main effects also occurred for chlorosis, so data are presented separately by study. A significant 3-way interaction between temperature x treatment x timing occurred for chlorosis in study 1. In study 2, a significant treatment x timing interaction also occurred for chlorosis (Table 7).

Study 1

In study 1, the 30°C temperature led to generally increased chlorosis relative to the 20°C chamber (Figure 7). Chlorosis became apparent within 7 DAT in both 20°C and 30°C growth chambers, though differences were not significant until 14 DAT (Figure 7). No significant differences were observed between the PM rates when watered EOD in the 20°C chamber for the length of the study. After 14 DAT, chlorosis from PM + AMS watered daily (1.2 out of 5) and PM foliar watered daily (0.8 out of 5) exhibited significantly greater chlorosis than the untreated watered daily. This trend continued throughout the study with all PM treatments watered daily having

Table 7. Growth chamber ANOVA for chlorosis, visual cover, DIA, and necrosis.

	P values						
	Study 1			Study 2			
	Chlorosis	% Cover	DIA	Chlorosis	% Cover	DIA	Necrosis
Temperature	***	***	***	NS	***	***	***
Treatment	***	NS	***	***	***	**	***
Timing	***	***	***	***	***	***	***
Temperature x Treatment	***	**	***	NS	***	**	***
Temperature x Timing	***	***	***	*	***	***	***
Treatment x Timing	***	*	***	***	***	***	***
Temperature x Treatment x Timing	**	NS	**	NS	***	NS	***

Means for necrosis were pooled for both studies.

* Significant at 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

NS, not significant at $P \leq 0.05$.

Table 8. Growth chamber data for shoot, root, and total biomass dry weights. Study 1 (upper) and 2 (lower). Means within the same column followed by the same letter do not differ significantly according to Tukey's HSD ($P = 0.05$).

Study 1

	Dry Weights (grams)											
	20°C						30°C					
Treatment	Shoots		Roots		Total		Shoots		Roots		Total	
Untreated EOD	1.22	ab	6.80	a	8.02	a	1.26	a	6.97	abc	8.24	abc
PM EOD	1.21	ab	8.20	a	9.37	a	1.04	a	10.04	ab	11.10	ab
PM + AMS EOD	1.74	a	8.24	a	9.98	a	1.01	a	2.32	c	3.33	c
Untreated Daily	1.28	ab	9.31	a	10.60	a	1.70	a	12.92	a	14.60	a
PM Daily	1.42	ab	7.13	a	8.55	a	1.34	a	7.90	abc	9.25	abc
PM + AMS Daily	1.60	a	4.31	a	5.91	a	1.56	a	4.60	bc	6.13	bc
PM Foliar Daily	0.98	b	6.92	a	7.90	a	1.20	a	4.30	bc	5.44	bc

Study 2

	Dry Weights (grams)											
	20°C						30°C					
Treatment	Shoots		Roots		Total		Shoots		Roots		Total	
Untreated EOD	0.67	ab	6.91	a	7.58	a	0.88	ab	5.78	a	6.66	a
PM EOD	0.67	ab	3.06	b	3.73	b	0.74	b	2.96	b	3.70	b
PM + AMS EOD	1.04	a	3.78	b	4.82	ab	0.98	ab	3.14	b	4.11	ab
Untreated Daily	0.59	b	5.11	ab	5.71	ab	0.87	ab	5.27	ab	6.14	ab
PM Daily	0.68	ab	2.70	b	3.39	b	0.77	b	2.75	b	3.52	b
PM + AMS Daily	0.86	ab	4.65	ab	5.51	ab	1.20	a	4.03	ab	5.23	ab
PM Foliar Daily	0.70	ab	2.78	b	3.48	b	0.64	b	3.01	b	3.65	b

significantly greater chlorosis compared to their respective untreated pots 21 and 28 DAT, with PM + AMS significantly greater than PM alone. After 35 DAT, only the PM foliar treatment (2.0 out of 5) was significantly different than its respective untreated counterpart. Differences in chlorosis were seen between the PM treatments throughout the study, but they were never significantly different from the untreated controls. Within the 20°C chamber, the addition of AMS increased the degree of chlorosis compared to PM alone, although differences were not significant until 28 DAT. Under similar watering conditions, PM applied to weed foliage had greater chlorosis (2.6 out of 5) than PM applied to soil (1.6 out of 5), although these too were not significantly different.

By 21 DAT within the 30°C chamber, all PM treatments, regardless of watering regimen, had significant chlorosis compared to their respective untreated treatments, and this was sustained for the duration of the study (Figure 7). Slight differences were seen between the PM treatments throughout the study, but were not significant. The addition of AMS did not exhibit the effect of added chlorosis seen in the 20°C chamber.

Study 2

In study 2, levels of overall chlorosis were relatively similar between the two temperature treatments. Within the 20°C chamber PM + AMS EOD and PM + AMS daily exhibited significant chlorosis at 7 DAT (Figure 8). Thereafter all PM treatments, except PM daily had significant chlorosis when compared to their respective untreated treatments, and were not significantly different from each other. Though not significantly different, foliar applied PM (3 out of 5) had slightly higher chlorosis than

PM applied to the soil (2.6 out of 5). The addition of AMS did not result in increased chlorosis as had occurred in study 1, with both PM and PM + AMS EOD having the similar levels of chlorosis (2.8 out of 5), and PM daily (2.8 out of 5) having slightly greater chlorosis than PM + AMS daily (2.6 out of 5) 35 DAT.

At 30°C, all PM treatments, regardless of irrigation regime, exhibited significant chlorosis 7 DAT, and for the duration of the study (Figure 8). Though not significant, PM + AMS had enhanced chlorosis compared to PM alone under both watering schedules.

As had occurred in study 1, wet foliar applications of PM caused a trend towards increased chlorosis compared to PM applied to soil, however, differences were not significant..

Also similar to study 1, the addition of AMS tended to result in more rapid and severe chlorosis than PM alone, regardless of irrigation. The exception was study 2 in the 20°C chamber, when the addition of AMS had similar chlorosis to PM EOD, and slightly less than PM daily.

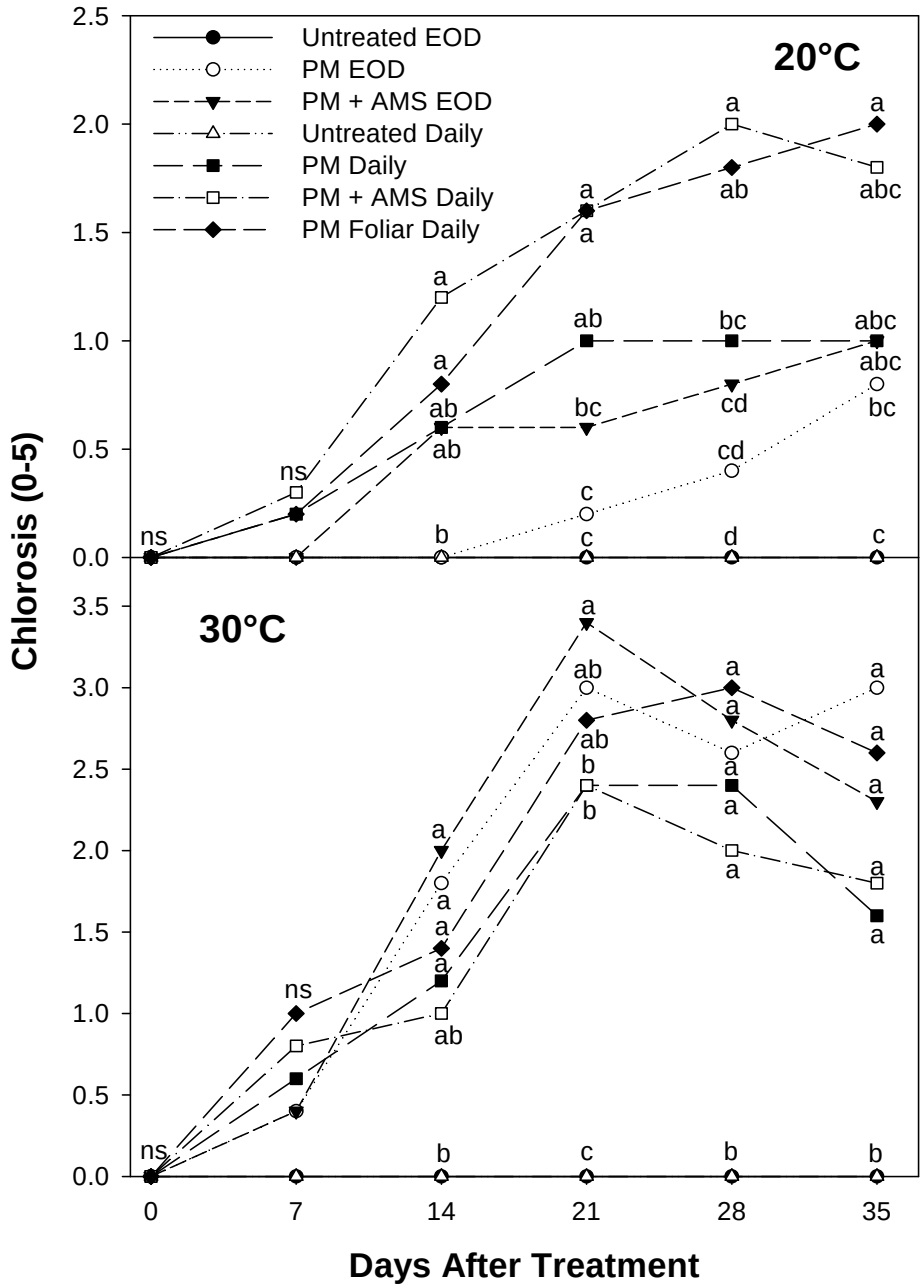

Figure 7. Growth chamber study 1 chlorosis. (0 = no chlorosis, 3 = many plants photobleached, 5 = plant death). Means followed by the same letter are not significantly different according to Tukey's HSD ($P = 0.05$).

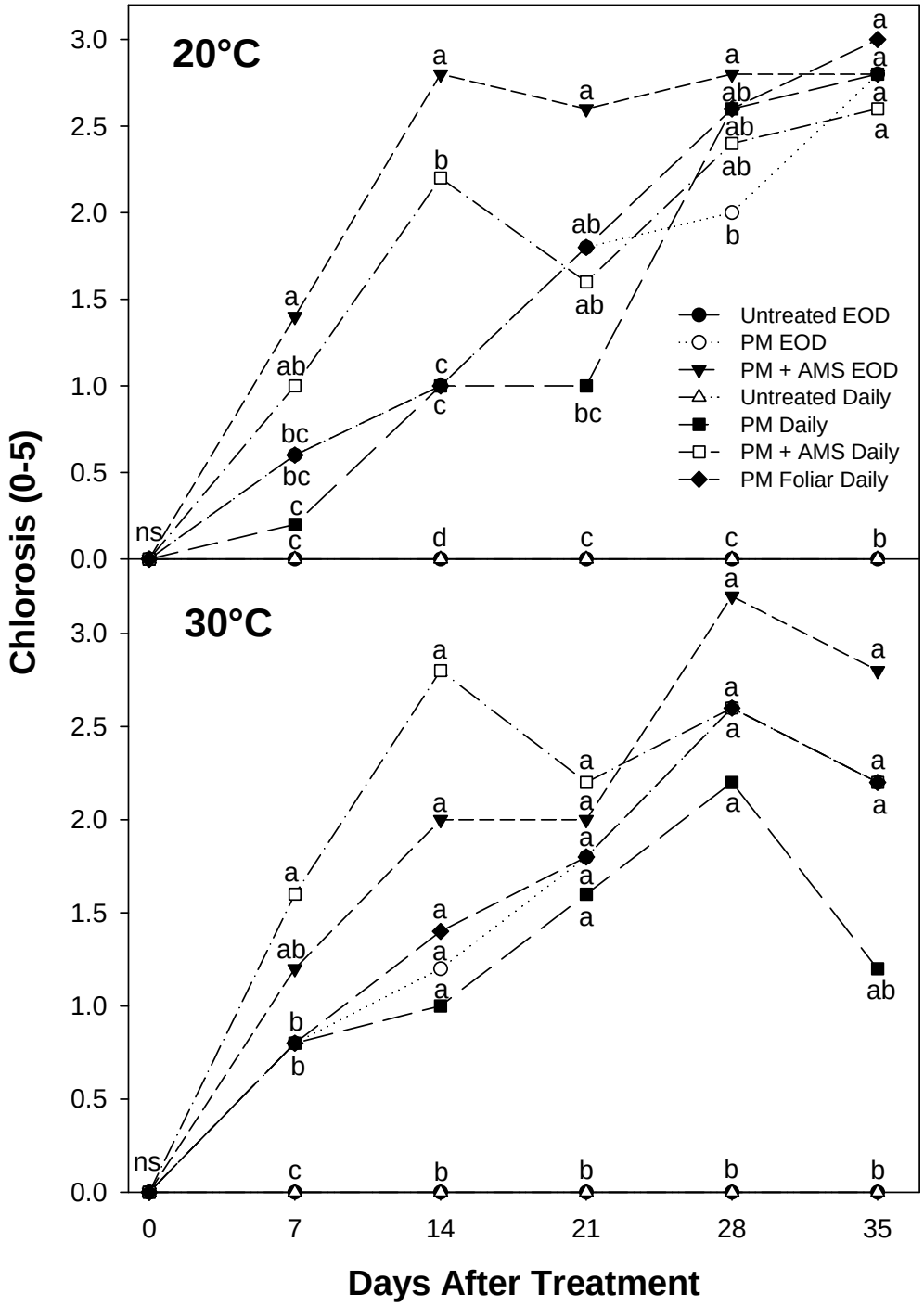

Figure 8. Growth chamber study 2 chlorosis. (0 = no chlorosis, 3 = many plants photobleached, 5 = plant death). Means followed by the same letter are not significantly different according to Tukey's HSD ($P = 0.05$).

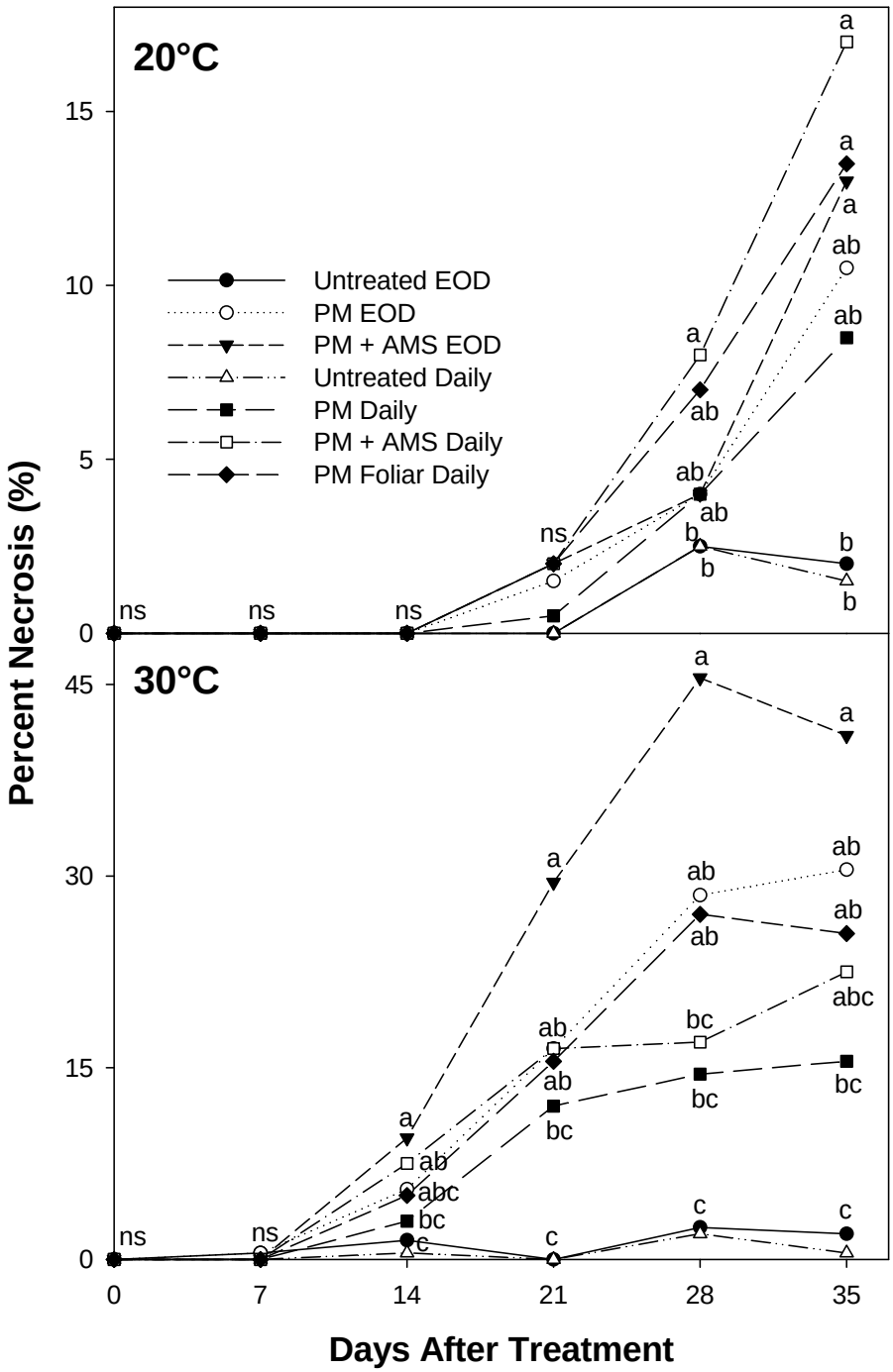

Figure 9. Growth chamber percent necrosis (%). Means were pooled for both studies. Means followed by the same letter are not significantly different according to Tukey's HSD ($P = 0.05$).

Necrosis

Although significant chlorosis occurred at both temperatures, this did not always progress into greater necrosis or dandelion control, especially in the 20°C chamber. As such, plants would express severe chlorosis and bleaching, but would not advance beyond that state, to reach necrosis and plant death.

Study main effect was not significant for necrosis, so data are pooled across studies. Also, a significant 3-way interaction between temperature x treatment x timing on necrosis was detected (Table 7).

In the 20°C chamber no significant differences were seen until 28 DAT, when PM + AMS daily (8%) had significant necrosis compared to the untreated. By 35 DAT PM + AMS watered EOD (13%), PM + AMS daily (17%), and PM foliar daily (13.5%) were significantly different than their respective untreated counterparts (Figure 9).

In the 30°C chamber, significant necrosis began to occur 14 DAT in PM + AMS EOD (9.5%) and PM+AMS daily (7.5%). After 21 DAT, PM and PM + AMS EOD had significant necrosis for the duration of the study, ending the study with necrosis levels of 30.5% and 41%, respectively. At 35 DAT PM foliar daily had the most necrosis (25.5%) of the treatments watered daily, significantly different than the untreated watered daily (Figure 9.).

The addition of AMS led to greater necrosis at both temperatures, though more of an impact was seen in the 30°C chamber. The higher temperature also led to more rapid necrosis of chlorotic and bleached leaf tissue.

Watering schedule had less of an effect in the 20°C chamber with similar necrosis seen between the different regimes. Treatments watered EOD in the 30°C chamber had close to double the necrosis of the treatments watered daily.

Visual Cover

Significant study main effects occurred for visual percent cover, so data are presented separately by study. A significant treatment x timing interaction occurred on percent cover in study 1. In study 2, a significant 3-way temperature x treatment x timing interaction occurred for percent cover (Table 7).

Study 1

During Study 1, there were no significant differences between PM treatments in the 20°C chamber, with all pots maintaining between 90 and 96% weed cover for the duration of the study. Despite weeds showing chlorotic leaves and photobleaching, leaf tissue never progressed beyond that to necrosis.

In the 30°C chamber, no significant differences were seen between the PM treatments until 21 DAT, when PM + AMS watered EOD (72%) had significantly lower cover than the untreated, as well as many of the other PM treatments (Figure 10). The same differences were seen at 28 DAT with PM + AMS watered EOD having 55% cover. At 35 DAT, there were no significant differences seen between any of the treatments.

Study 2

Study 2 saw a similar trend in the 20°C chamber, with no significant differences seen between the PM treatments throughout the study. There was a gradual increase in

percent cover until 21 DAT seen across all treatments, thereafter saw a slight decline through 35 DAT (Figure 11.).

The 30°C chamber had no significant differences between the treatments throughout the study, and saw the same gradual increase in percent cover until 21 DAT, and then all PM treatments showed a sharp decline in weed cover.

The 30°C chamber had slightly less percent weed cover than the 20°C chamber during both studies.

The addition of AMS had the biggest effect in both studies in the 30°C chamber. Though no significant differences were seen, there was a trend seen with the PM + AMS treatments having less weed cover than the other treatments.

Foliar applications of PM had the biggest effect in study 2 in the 30°C chamber. Though not significantly different than the other treatments, it had the lowest percent weed cover.

Watering schedule had little effect in study 2, but saw some significant differences by the PM treatments watered EOD in study 1. Both treatments were not significantly different than their respective untreated, but PM watered EOD had significantly less weed cover than some PM watered daily treatments.

Digital Image Analysis (DIA)

Significant study main effects occurred for DIA cover, so data are presented separately by study. A significant 3-way interaction between temperature x treatment x timing occurred for DIA in study 1. In study 2, a significant treatment x timing interaction occurred for DIA (Table 7).

Study 1

In Study 1, no significant differences in DIA were seen in the 20°C chamber until 21 DAT, when PM foliar watered daily had significantly less weed cover than the untreated and PM watered daily (Figure 12). Thereafter, no significant differences were seen for the duration of the study.

In the 30°C chamber, significant differences were seen 14 DAT, with PM + AMS EOD, PM + AMS daily, and PM foliar watered daily having significantly less weed cover than their respective untreated pots (Figure 12.). No significant differences were seen for the duration of the study.

Study 2

In Study 2, though significant differences were seen between PM treatments, they were not significantly different than their respective untreated treatments (Figure 13). On some rating dates, untreated treatments had significantly less weed cover than the PM treatments.

No benefit was seen by the addition of AMS in either study. Foliar applications had little benefit as well. Temperature had an effect in study 2, with weeds having slightly less cover in the 30°C chamber compared to the 20°C chamber. Watering regime also had no impact on weed cover either.

Discussion

Nitrogen, especially ammonium sulfate (AMS) has long been used in mixtures with herbicides and was reported to improve efficacy of herbicides (Wang and Liu, 2007), with increasing nitrogen levels enhancing herbicide performance (Kim et al.,

2007). It has been reported applications of nitrogen also increase populations of soil fungi (Reeleeder et al., 2006), which could lead to greater herbicidal activity from PM. For this study, the addition of AMS enhanced the activity of PM on dandelion. Adding AMS increased the percent of dandelions controlled by PM by 2% in the 20°C chamber, and by as much as 14% in the 30°C chamber compared to PM alone, depending on irrigation regime. This is similar to the findings of Bailey et al. (2013) who reported a 16 to 27% increase in dandelion reductions under greenhouse conditions, and 10 to 20% increases in dandelion reductions under field conditions when nitrogen fertilizer was applied with PM, though dandelion control in this study was much lower than that seen by Bailey et al. (2013). In some instances adding AMS also led to increases in shoot, root, and total biomass dry weights. Chlorosis was observed sooner with the addition of AMS, as well as an increase in the severity of chlorosis, but this did not necessarily lead to increased dandelion control. Plants in the 20°C chamber in this study became chlorotic and bleached and remained that way for most of the study. The addition of AMS also led to greater final necrosis with increases of 2 to 11%, depending on temperature and irrigation. The addition of AMS could have led to an increase, or stimulation of dandelion growth, with the growing roots taking up greater amounts of macrocidins from the PM granules in the soil, as noted in Bailey et al., (2013).

Temperature Effects

Temperature is an important factor for regulating microbial activity in soil (Pietikäinen et al., 2005), with fungal populations and activity increasing with increasing temperatures (Castro et al., 2010). Herbicidal activity of PM increased with higher

temperatures, with greater activity seen in the 30°C when compared to the 20°C growth chamber. The 20°C chamber only saw 2% control of dandelion, while the 30°C chamber saw up to 34% control. The 30°C chamber saw increased weed necrosis (15.5 to 41%) compared to the 20°C chamber (8.5 to 17%). Study 1 saw greater chlorosis in the 30°C chamber than the 20°C chamber, but in Study 2 the two chambers saw similar levels of chlorosis.

Irrigation Impacts

Soil fungi are aerobic (Griffin 1963), requiring oxygen to survive, and most fungi have poor survival at soil depths greater than 8 cm due to anaerobic conditions (Zhou et al., 2004). The PM treatments watered every other day had greater percent control, up to 34%, than PM treatments watered daily, up to 16%. Plants were watered at 100% ET, based on water lost from the pots in the growth chambers. Dandelion control may have been reduced by the treatments watered every day due to too much water being replaced. This possibly could have created an anaerobic environment, decreasing the activity of PM. The wetter soils may have also altered the dandelion's ability to take up the macrocidins produced by PM, leading to a decline in herbicidal activity. In saturated soils, macrocidins may be released into the water and carried away in run-off (Zhou et al., 2004). Dandelions were grown in pots, so soil shrinkage could have led to small cracks between the soil and pot sides possibly allowing macrocidins to be leached via preferential flow and thus reducing the concentrations available for weed uptake, leading to the reduced control seen in this study.

Conclusions

The PM has proven to be effective at controlling many broadleaf weeds, especially dandelion. Dandelion control increased with increase in temperature, but increased irrigation caused a decrease in control. Adding nitrogen in the form of ammonium sulfate increased the activity of PM and provided greater control of dandelion. The amount of control seen in this study was much lower than previously reported by others, where 100% control was observed. Future research is needed to more clearly understand the effects that moisture, temperature, and nutrient interactions have on PM.

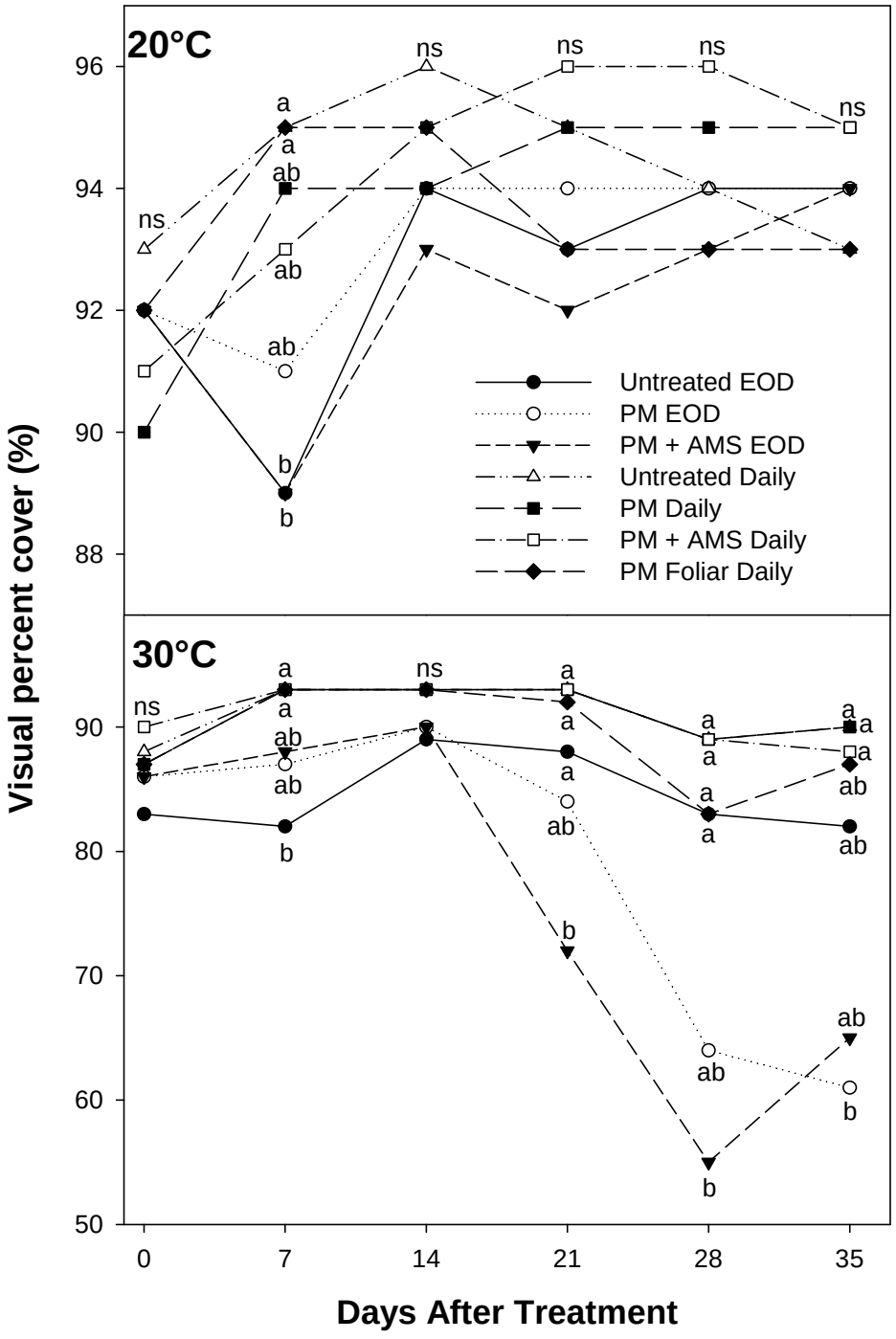

Figure 10. Growth chamber study 1 visual percent cover (%). Means followed by the same letter are not significantly different according to Tukey's HSD ($P = 0.05$).

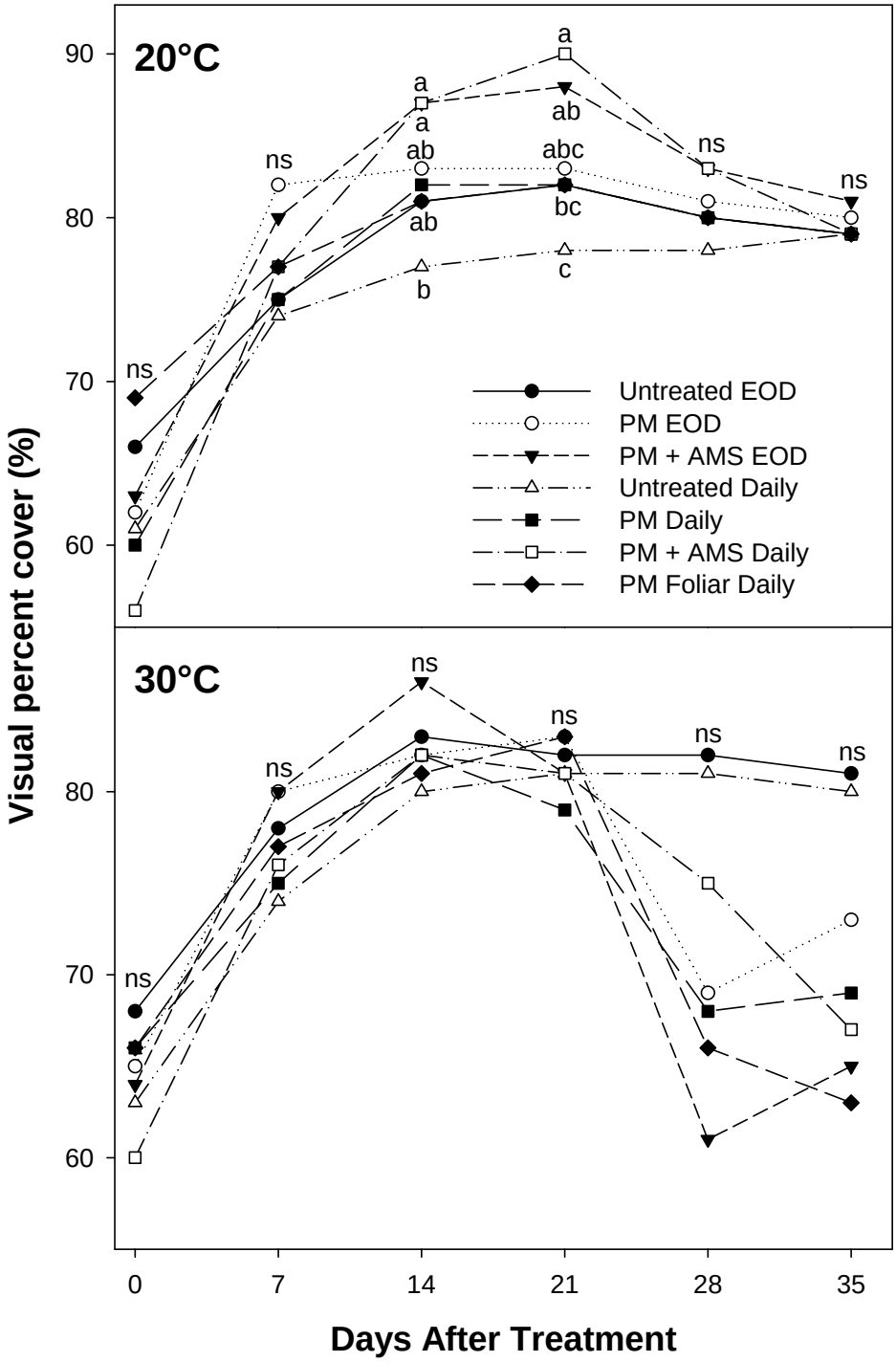

Figure 11. Growth chamber study 2 visual percent cover (%). Means followed by the same letter are not significantly different according to Tukey's HSD ($P = 0.05$).

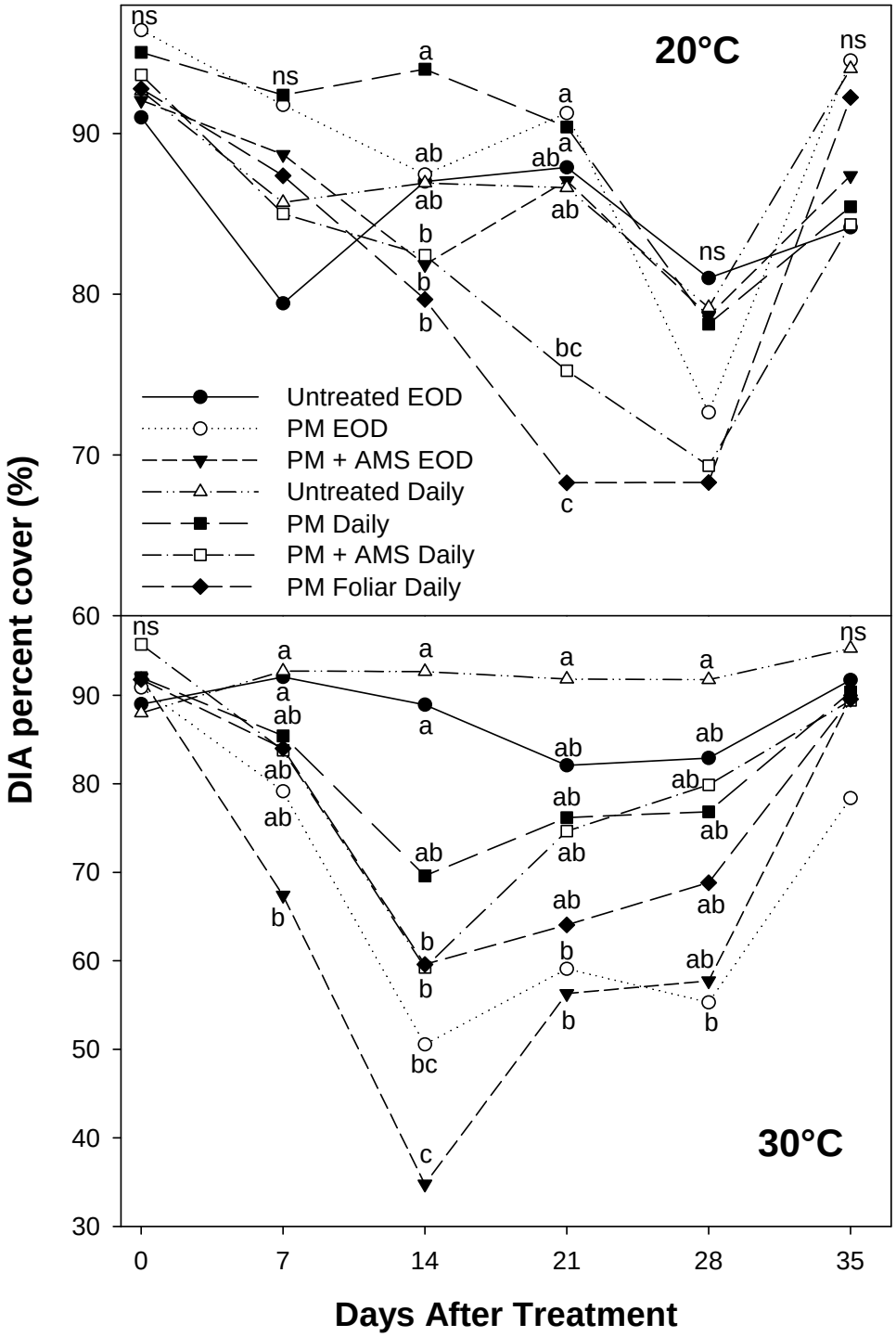

Figure 12. Growth chamber study 1 DIA percent cover (%). Means followed by the same letter are not significantly different according to Tukey's HSD ($P = 0.05$).

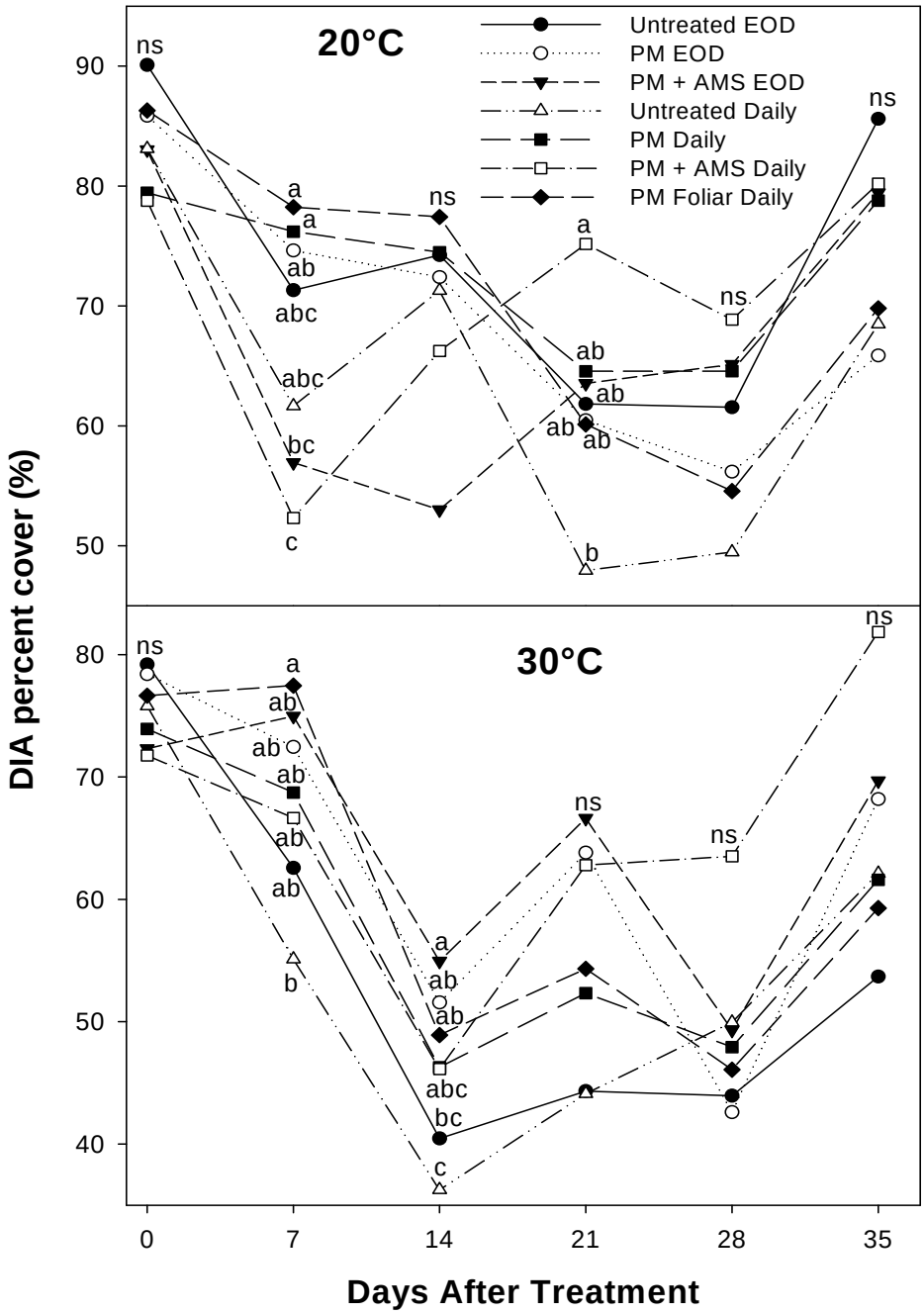

Figure 13. Growth chamber study 2 DIA percent cover (%). Means followed by the same letter are not significantly different according to Tukey's HSD ($P = 0.05$).

CHAPTER V

SUMMARY AND CONCLUSIONS

Summary

There are limited options for natural weed control in lawns. Many of the natural products available, which aren't many, lack selectivity. These products often kill or injure both weeds and grasses, including turfgrasses. PM is a fungus that shows herbicidal activity on many broadleaf weeds being evaluated as a natural herbicide for selective weed control for turf. Much of its development work has been conducted mainly in northern climates at temperatures between 15 to 25°C. Data is limited on the product's efficacy in warmer climates, where temperatures can be elevated. It is not known how well the product will maintain weed efficacy over a period of time at elevated temperatures, such as are common in Texas. Spectrum of weeds controlled is also another area of interest where information is lacking.

When products are being developed it is necessary to test the product on as many weeds as possible, in order to build a data set for developing a list of controlled weeds for the product label prior to release in to the market. PM has shown good efficacy on dandelions, Canada thistle, and other broadleaf weeds, but defining the scope of weeds controlled is an ongoing process. PM is a fungus, therefore a living organism that is providing control of the weeds. Fungi respond to different environmental factors, like temperature and moisture, which could impact the ability of the product to control weeds. It is also not fully understood how cultural practices, like fertilization, can affect

the efficacy of PM. These problems are what led to this research project, evaluating PM for weed control use in warm-season turfgrasses.

Approach

PM was evaluated in many different ways to gather as much data about the product as possible in a short amount of time. It was evaluated for control of slender aster in common bermudagrass, evaluating multiple application rates, in a field setting. Slender aster is a summer annual and is a problem weed in Texas, growing from a slender, succulent plant to a woody shrub like plant. When mowed, it adapts its growth habits and spreads out along the ground, helping it survive. It flowers and produces seeds in the late summer, but I have observed plants flowering and going to seed in early November. Testing PM in the field shows how the product works in real world situations, where the product will interact with heat, rain, and other soil microbes that it may have to compete against.

Evaluating the spectrum of weeds controlled was performed in a greenhouse on six weeds. Ten weeds were started, but germination problems led to four weeds being left out. Greenhouse testing allows for weed control to be evaluated on many weeds. It is easier to obtain seeds for the weeds and grow them in pots in a greenhouse than to find populations naturally occurring in turf. Multiple application rates were also evaluated during the greenhouse studies.

Growth chambers were used to evaluate the effects of different temperatures, moisture levels, and nitrogen fertilizer impact the efficacy of *P. macrostoma.* Growth chambers provide a controlled environment for precisely evaluating multiple factors.

Findings

PM performed well under field situations, with high application rates providing excellent control of slender aster under elevated temperatures, with air temperatures averaging 30°C, and max temperatures reaching 41°C. No injury was seen on common bermudagrass during the study. Higher application rates may be necessary to obtain good control. However, the product is still being optimized, so target application rates should be much lower than what provided good control in this study.

Although PM caused injury to most of the weeds evaluated, spectrum of weeds actually controlled by PM was variable, with PM working well on some weeds, but not on others. Therefore, PM may not have the broad spectrum weed control of some synthetic herbicides, at least using the application rates in this study. Dandelion has proven to be one of the weeds controlled by PM. In contrast, common mallow and common purslane showed high tolerance to PM, with little to no activity seen on either weed.

Differences were also observed between results of tests performed on similar weeds, but between the field and greenhouse. For example, control of slender aster in the greenhouse was much lower than what was seen in the field study. This was possibly due to the weeds in the greenhouse being larger and more mature than the weeds in the field at time of application. Weeds are easier to control when they are younger and less mature. Competition with turfgrass may have also helped increase the control of slender aster seen in the field.

Differences were also seen between studies with regard to control of similar weed species. For example, control of annual sowthistle was good in the first greenhouse study at high rates of PM, but poor in the second year. Growing conditions and weed stage were similar in both studies, so like other herbicides, one may realize good control one time after applying a herbicide, but the next time may not have the same results.

Other weeds such as California burclover had chlorosis and weed injury, but were able to survive and rebound from the PM rates applied, suggesting that higher application rates than those tested may be necessary to control California burclover. Using higher application rates may not be feasible, because the higher the application rate will require more product to treat an area. The preferred application rate is much lower than the rates that showed good activity, but is dictated by the concentration of macrocidins in the product.

The growth chamber studies demonstrated that increasing temperatures increase the activity of PM, leading to higher weed control. Similarly, the addition of ammonium sulfate also increased the activity of PM, resulting in greater weed control. This could possibly have been due to the nitrogen increasing fungal populations and activity, as well as increasing weed growth and the amount of macrocidins taken up by the weed. Increasing irrigation frequency proved to be detrimental to weed control, with less frequently irrigated (EOD) pots actually providing better dandelion control than those that were watered more frequently. This could be related to soil aerobic conditions that favored fungal activity under EOD watering.

Future Research

Further research is needed to follow up and build upon what was discovered in these studies. Field testing of weeds from the greenhouse study that saw good control should be done to see if those results translate to field settings. More greenhouse studies of the different weeds need to be done, to see if control can be improved on the weeds tested and possibly on other species as well. Applications could be made at different weed life cycles to see if weed control would be improved by treating smaller weeds, or also by incorporating nitrogen to see if control would be enhanced. Further research is definitely needed to better understand how environmental factors impact efficacy of PM. The study on dandelion should be repeated, as well as possibly looking at some of the weeds that showed marginal activity on, to see if any of these environmental factors would help increase weed control.

Conclusions

Based on this research, PM has shown that it could be a viable option for natural weed control of broadleaf weeds in turfgrass. It has shown that it can maintain efficacy in the field under high temperatures, and works on a number of different weeds, as well as not injuring any turf species tested. Higher temperatures increase the activity of the product, leading to increased weed control. Cultural practices like nitrogen fertilization may enhance PM activity. Like some synthetic herbicides, increased application rates may be needed to achieve acceptable control of some weeds. It should be noted that application rates evaluated in these studies might not be the optimum application rates, since the manufacturing process is still being streamlined to produce a more highly

concentrated product. Future application rates could be fractions of the rates evaluated if the macrocidin concentrations can be increased during production.

Given the tested range of application rates, PM may not offer the broad spectrum weed control of common three-way synthetic herbicides, but it does provide a broad enough weed control spectrum to make it a good candidate for natural weed control for turfgrass systems, an area of increasing future importance and consumer demand.

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