Poa annua: An Annual Species?

A Dissertation Presented for the
Doctor of Philosophy
Degree
The University of Tennessee, Knoxville

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May 2022

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DEDICATION

This dissertation is dedicated my grandfather, D.V. (Vic) Carroll. His words of wisdom and support were influential in my decision to pursue this degree. I know he is proud that finally, one of the doctors in the family will have the last name Carroll!

ACKNOWLEDGEMENTS

The saying “It takes a village to raise a child” is also applicable to earning a Ph.D. The support, guidance, and wisdom of many others were essential to my degree completion. First and foremost, I am unendingly grateful for the mentorship of my advisor, Dr. Jim Brosnan. It has been an honor and privilege to be a part of the Brosnan lab during my studies. Dr. Brosnan is a transformational leader. He provided an environment for me to flourish and empowered me to achieve more than I thought possible for myself. His mentorship has been the key to my success. Similarly, I thank my graduate committee members Drs. Brandon Horvath, Robert Trigiano, Avat Shekoofa, and Thomas Mueller for their continued commitment to my education and support in a multitude of capacities on this journey. I also owe thanks to my M.S. advisor, Dr. Carrie Stephens, for being one of my biggest cheerleaders and for encouraging me to be a scientist in new ways. Additionally, I am appreciative of all members of the University of Tennessee Turfgrass Team for their contributions in conducting research and making my education possible. Special thanks are owed to Bill Czekai for managing the turfgrass research farm and members of the Brosnan lab including Greg Breeden, Javier Vargas, Ben Pritchard, Brynn Johnson, Josh Beau, and Corbin Estes for providing research assistance. Beyond academics, I have many people to thank for their emotional support and encouragement. Without my parents, Ken and Chris Carroll, I would not have achieved this degree or my previous successes. My brother Doug Carroll, grandmother Joan Carroll, and all my extended family were also influential in my ability to pursue this degree and I am grateful for their love and positivity. Additionally, Marcia

Quinby, Ben Pritchard, and Trinity Hamm are friends who have become family during this degree and their faith in me has been a pillar in my commitment to finish. Finally, I am incredibly thankful to Collin McCorkel for keeping me grounded and never letting me lose sight of my goals. These individuals have earned this degree with me, and I am fortunate to have such a large group of supporters guiding me through life.

ABSTRACT

Poa annua L. is ranked the most troublesome turfgrass weed but can also be a highly desirable turfgrass species. As the Latin name *annua* implies, the species is thought to persist via an annual life cycle; yet there are many reports in literature of *P. annua* persisting perennially. Considering that *P. annua* senescence patterns do not align with other true annual species such as *Triticum spp*. and *Zea mays* L., we hypothesized that *P. annua* is similar to other perennial, C_3, turfgrass species that are subject to a confluence of environmental factors in summer that can cause mortality. Four experiments were conducted in Knoxville, TN with the objective of determining environmental factors lethal to *P. annua*. A field monitoring study assessed 100 *P. annua* plants across ten micro-environments in golf course fairways and roughs. Forty plants survived the duration of 2020, establishing the existence of perennial *P. annua* ecotypes. Analysis of environmental factors at the time of plant death for ecotypes that did not survive perennially indicated soil moisture, soil temperature, and pathogenic infection were associated with mortality. A series of individual greenhouse or field experiments were conducted to investigate the effects of each factor on *P. annua* mortality. Neither soil moisture nor soil temperature were lethal to *P. annua*, except under extreme conditions not typical in the field (e.g., 37.8°C soil temperature for 12d under optimal soil moisture, or 35°C soil temperature with no irrigation for 19d). A field study assessed mortality from disease and observed that *P. annua* plants treated with fungicide throughout the summer on a 14-d interval survived year-round, whereas plants not receiving fungicide applications senesced. These findings support our hypothesis that the

name *P. annua* is a misnomer and that plants persist via a perennial life cycle unless environmental conditions are unfavorable.

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INTRODUCTION

CHAPTER I
CURRENT UNDERSTANDING OF THE *POA ANNUA* LIFE CYCLE

A version of this chapter was originally published online on 6 January 2021 and print in May 2021 by Devon E. Carroll, James T. Brosnan, Robert N. Trigiano, Brandon J. Horvath, Avat Shekoofa, and Thomas C. Mueller:

Carroll, D.E., Brosnan, J.T., Trigiano, R.N., Horvath, B.J., Shekoofa, A., & Mueller, T.C. (2021) Current understanding of the *Poa annua* life cycle. *Crop Science*, 61(3), 1527-1537. doi:10.002.csc2.20441

The publishing of this article involved an extensive peer-review process that improved the quality of the manuscript and allowed the authors to develop deeper hypotheses and arguments. The publishing of this article involved six authors from the University of Tennessee departments of Plant Sciences and Entomology and Plant Pathology. All authors made conceptual or technical contributions relative to their area of expertise. My primary contributions to this paper include (i) reading literature, (ii) writing the manuscript, and (iii) revising during the review process.

Abstract

Poa annua L. is a common component of turfgrass systems both as a weed and a desirable species. Since first classified by Carl von Linné in 1753, nearly 50 taxa of *P. annua* have been described, with delineations made on the basis of plant morphology and not life cycle. Yet, peer-reviewed turfgrass literature has recognized only two of these taxa over the past 50 yr, *P. annua* L. var. *annua* and *P. annua* var. *reptans*, categorizing var. *annua* ecotypes as upright growing annuals and var. *reptans* ecotypes as laterally growing perennials. Herein we provide a comprehensive review of peer-reviewed literature to highlight that these associations between life cycle and plant morphology are flawed given that morphology is plastic and strongly influenced by environment. We conclude by exploring knowledge gaps regarding *P. annua* perennation that, if addressed through research, would help turfgrass managers better understand the biology of the ecotypes they aim to eradicate or maintain.

Introduction

The *Poa* genus, comprised of ~575 species, is the largest grass genus (Gillespie & Soreng, 2005). The genus has been described as cosmopolitan because of the diversity in growth habit and geographic distribution among species (Anton & Connor, 1995). Species including Kentucky bluegrass (*P. pratensis* L.), rough bluegrass (*P. trivialis* L.), Canada bluegrass (*P. compressa* L.), wood bluegrass (*P. nemoralis* L.), and supina bluegrass (*P. supina* Schrad.) are turfgrasses commonly used on golf courses, sports fields, and lawns. These aforementioned species of *Poa* are considered perennial, cool-

season turfgrasses and are native to Europe, temperate Asia, or northern Africa (Bush, 2002; Freshour, 2013; Pecetti et al., 2010; Rajasekar, Fei, & Christians, 2005; United States Department of Agriculture, 2020). One of the most commonly observed *Poa* species in turfgrass is *P. annua*, a cool-season turfgrass often referred to as annual bluegrass in the United States; annual meadowgrass across Europe; wintergrass in Australia and New Zealand; or junegrass, suffolkgrass, speargrass, and walkgrass in other parts of the world (Gibeault, 1971). When present in swards of other turfgrass species, *P. annua* has been ranked the most troublesome weed of turfgrass (Van Wychen, 2020).

Poa annua is an allotetraploid species believed to have originated in the Mediterranean through a natural hybridization between the two diploid species *P. infirma* Kunth [syn. *P. exilis* (Tomm. ex Freyn) Murb.] and *P. supina* Schrad. (Nannfeldt, 1937; Tutin, 1957). There are only a few morphological and cytological characteristics in which *P. annua* is not an exact intermediate between the two putative parent species (Nannfeldt, 1937). However, the putative parent species described by Nannfeldt (1937) were disputed by Koshy (1968) who found the *P. annua* karyotype did not follow the expected multiples of chromosomes passed down from parents.

Koshy (1968) suggested that either the chromosome structure of *P. annua* had undergone changes since its origin or that only one of the putative parents previously reported was correct. Darmency and Gasquez (1997) found that *P. infirma* and *P. supina* produced diploid hybrids that morphologically resembled *P. annua* but were not allotetraploid. Despite the suggestions of Koshy (1968), *P. supina* and *P. infirma* have been long-considered putative parents to *P. annua*. Mao and Huff (2016) confirmed

that *P. infirma* was the female parent and *P. supina* the male parent to *P. annua* through phylogenetic analysis using nuclear and chloroplast gene sequences. Mao and Huff (2012) noted that findings of differing ploidy, such as those reported by Koshy (1968), may still be correct as it is likely that multiple hybridization events have occurred and altered the chromosome structure.

There is some evidence that the first hybridization resulting in *P. annua* was due to glacial movement that transported *P. supina* from Europe to the northern Mediterranean where *P. infirma* was present (Tutin, 1957). The most recent possibility of this mode of hybridization occurred about 10,000 yr ago with the Eurasian continental glacier of the Late Pleistocene era (Hobbs, 1946; Mao & Huff, 2012). If this theory is correct, the ability of a plant to colonize the world in the capacity *P. annua* has in such a short time-frame is striking. The swift spread of *P. annua* to a multitude of environments on all continents (Molina-Montenegro et al., 2014) is a testament to the high adaptability of the species. Part of the colonization ability of *P. annua* is due to the species' reproduction primarily through self-pollination (Hovin, 1957; Tutin, 1957). Ellis (1973) confirmed that *P. annua* reproduces sexually through self-pollination and that outcrossing occurs only 0–15% of the time and is dependent on environmental conditions.

Additionally, there is great adaptation potential in allotetraploid species such as *P. annua*. The two genomes present in allotetraploid species such as *P. annua* facilitate plant adaptation to a wider range of environments than either diploid parent species (Thompson & Lumaret, 1992). The diploid parents of *P. annua* have been characterized as an erect annual in *P. infirma* and a prostrate perennial in *P. supina* (Darmency, Berti,

Gasquez, & Matjicek, 1992). *Poa annua* encompasses adaptations inherited from both parents (Johnson, 1995). Despite the name annual bluegrass indicating the species persists via an annual life cycle, the differing life cycles of *P. infirma* and *P. supina* have allowed *P. annua* to colonize a wider range of environments across a continuum of perennation (Hovin, 1957). The ploidy and reproduction of *P. annua* leads the species to exhibit greater phenotypic and genotypic variability than most, if not all, other weeds (Hutchinson & Seymour, 1982; Mitich, 1998; Tutin, 1957). Such variability in *P. annua* biology and morphology is maintained through ecological pressure and management practices (McElroy, Walker, Wehtje, & van Santen, 2004).

Taxonomical Classification

Poa annua was first recorded by Carl von Linné (Carl Linnaeus) in 1753 after observing the species in Denmark; however, no taxonomical description was provided (Linné, 1753). What was the basis for classifying this species of *Poa* as '*annua*,' which is Latin for annual?

There have been efforts to categorize *P. annua* into subspecies, varieties, and forms as a result of the vast differences in observed morphology (Figure 1.1). Gibeault (1971) reported the existence of 48 *P. annua* varieties and provided 32 translated taxonomical descriptions. Varietal classification was primarily based on growth habit, seed-head color, inflorescence characteristics, plant size, leaf morphology, and edaphic or climatic conditional growth (Gibeault, 1971).

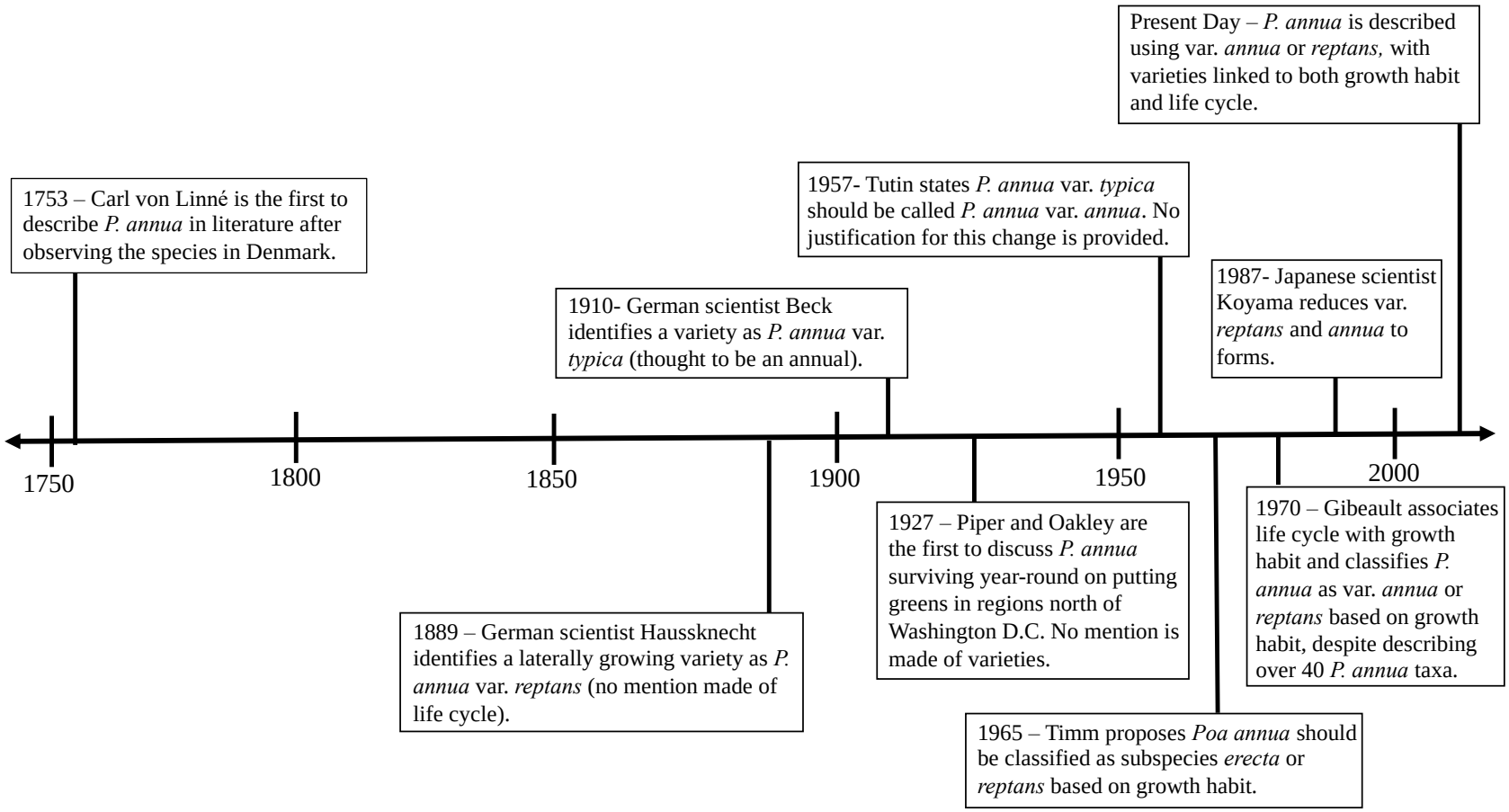


Figure 1.1. A timeline of the taxonomical descriptions of *Poa annua* L. in scientific literature.

The Missouri Botanical Garden Tropicos database lists taxonomical descriptions for eight subspecies, 27 varieties, and 11 forms of *P. annua* (Tropicos, 2020). Most recently, *P. annua* f. *pupurea* M.L. Grant was described by its flush, purple color (Grant, 2003). Despite the multitude of subdivision within *P. annua*, turfgrass literature primarily focuses on just two taxa: *P. annua* var. *reptans* (Hausskn.) T. Koyama and *P. annua* var. *annua*.

Poa annua var. *reptans* was described by German scientist Heinrich Carl Haussknecht in 1890 stating, "It is characterized by an elongated, heavily branched blade, which is largely procumbent on all sides and develops roots at the nodes. It primarily grows in slightly moist, sandy soils in lawn-like meadows" (Kurze & Regel, 1890). The selection was primarily characterized by its creeping growth habit and appropriately named *reptans*, meaning creeping in Latin. In modern turfgrass literature, *P. annua* var. *reptans* has come to synonymously mean perennial-type *P. annua* and is sometimes referred to as 'greens-type' (Mao, 2014). It is our contention that this is incorrect considering the original taxonomic description of *P. annua* var. *reptans* makes no mention of the plant life cycle and focuses exclusively on morphology (Kurze & Regel, 1890).

Similar discrepancies in the original taxonomic description and modern connotation exist in regard to *P. annua* var. *annua*. The original name of this variety is *P. annua* var. *typica* Beck and it was first described in Germany (Briquet, 1910) using the following verbiage: "Annual plant 10-to-30 cm tall. Loose panicle with spikelets with 1-to-5 florets and overlapping flowers, where the upper (generally female) are smaller (2-

to-2.5 mm) than the lower hemaphrodites (2.5-to-4 mm), and the terminal is twice as long as its pedicle; stamens with filaments do not reach the tip of the upper contiguous flower, and generally have anthers 0.6-to-0.8 mm long with linear-oblong loculi." Although this description delineates the species as annual, the report states that it is present year-round. What was the basis for classifying *P. annua* var. *typica* Beck as an annual plant? The varietal distinction "typica" suggests that this form of *P. annua* was the most typical of plants found in Germany. Tutin (1957) later suggested *P. annua* var. *typica* should instead be called *P. annua* var. *annua* but offered no reasoning for the change.

Over time, *P. annua* var. *annua* has been used to describe upright-growing *P. annua* plants assumed to have an annual life cycle (often referred to as annual-type *P. annua*). It is unclear how this association came to fruition considering the original taxonomic description of *P. annua* var. *annua* does not mention plant growth habit. In fact, Timm (1965) describes *P. annua* var. *typica* (*P. annua* var. *annua*) as a laterally growing, perennial variety and was the first to classify a distinct erect growing taxon termed *P. annua* subsp. *erecta*.

The presently assumed linkage between annual life cycle and upright growth is likely derived from the classification of *P. annua* subsp. *erecta*, and not *P. annua* var. *annua*. Timm (1965) assessed the life cycle of 6,466 *P. annua* plants harvested from sports fields, field edges, field paths, paved roads, parking lots, and public squares across Europe from Coimbra, Portugal to Apukka, Finland. She found that 42 of the studied plants exhibited an erect, bunch-type growth habit and that 37 (88%) of these 42 plants with upright growth were of an annual life cycle. Conversely, of the 6,424

plants that grew laterally, 21% survived as annuals. These findings indicate lateral rather than upright growth and a perennial life cycle is most common of *P. annua* plants. Although, Timm (1965) states that lifespan is "only loosely correlated with the two growth types", modern scientific literature has readily associated upright and lateral growth habit in *P. annua* with annuality and perenniality, respectively.

Timm (1965) proposed *P. annua* should be divided into two subspecies based on growth habit: *erecta* and *reptans* (Figure 1.2). *Poa annua* var. *reptans* was suggested to be further described by two varieties (*pauciflora* and *typica*) and four forms (*perennans*, *tenius*, *praecox*, and *serotina*) based on differing morphological characteristics beyond a lateral growth habit.

Given this streamlined classification model based on morphology, why does turfgrass literature continue to reference var. *reptans* and var. *annua*? In 1987, the *reptans* and *annua* varieties were reduced to forms, yet are incorrectly referenced as varieties in current literature (Koyama, 1987). Further, why is Timm referenced as the authority for *P. annua* var. *annua* when her 1965 report fails to make mention of such a variety?

Although the original taxonomical descriptions of *P. annua* focused on morphology rather than life cycle, over time the *reptans* and *annua* taxa have become the terminology used to describe selections that persist perennially or annually, respectively.

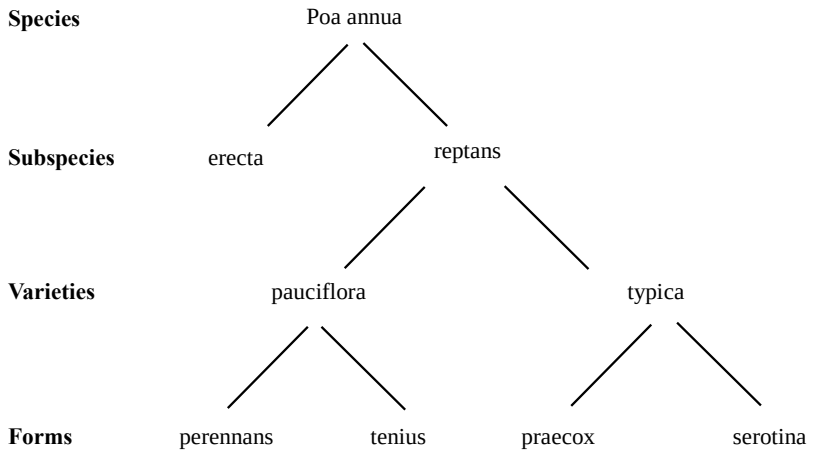


Figure 1.1. Classification of *Poa annua* L. based on morphology. Adapted from Timm (1965)

Given that original descriptions of *P. annua* taxa describe differences in morphology, why do scientists now classify selections as annual and perennial using these descriptions? Moreover, with at least 48 taxa of *P. annua* classified, why does contemporary turfgrass literature predominately use only var. *reptans* and var. *annua* to describe *P. annua*?

We contend that the life cycle of *P. annua* should not be represented by two taxonomical descriptions based on growth habit given that annual, perennial, short-lived perennial, and biennial ecotypes have been reported across a multitude of environments (McElroy, Walker, & van Santen, 2002; Mitich, 1998; Timm, 1965; Tutin, 1957; Warwick, 1979). Is *P. annua* an annual species that can survive perennially under certain environmental conditions? A similar phenomenon has been explored with goosegrass (*Eleusine indica* L.) and crabgrass (*Digitaria* spp.), annual weeds that can survive perennially in environments that facilitate year-long growth (Chauhan & Johnson, 2008; Nishimoto & Murdoch, 1999). Or is *P. annua* actually perennial with plant mortality occurring under certain environmental conditions, giving the appearance of an annual life cycle?

The objective of this review is to outline published research on conditions that could possibly affect *P. annua* life cycle and discuss knowledge gaps to be addressed with future research.

Previous Studies of *Poa annua* Ecotypes

Piper and Oakley (1927) were the first to report perennial ecotypes of *P. annua* on golf courses stating, “It (*P. annua*) vanishes completely by mid-summer, at least as far

north as Washington, D.C., but in Philadelphia and north-ward some plants may be found at any time during the summer." Since that time, turfgrass managers have desired the ability to discern between life cycles in order to inform management practices. Many researchers have aimed to associate the *P. annua* life cycle with morphological traits such as growth habit, plant size, leaf number, leaf length, leaf width, number of nodes per tiller, node branching, secondary tiller number, tiller length, number of flowering tillers, adventitious root number, inflorescence number, panicle length, and reproductive dry weight (Cline, 2001; Cordukes, 1977; Darmency et al., 1992; Gibeault, 1971; La Mantia & Huff, 2011; Till-Bottraud, Wu, & Harding, 1990). Biological processes, such as time to reproductive maturity, starch and sugar storage, seed production, and seed dormancy, have also been studied (Darmency et al., 1992; Gibeault, 1971; Sgambatti-Araujo, 1978; Till-Bottraud et al., 1990). This section will provide an overview of these efforts to elucidate how current paradigms about *P. annua* life cycle have developed over time.

Several researchers have associated growth habit with *P. annua* life cycle. Timm (1965) reported a "loose correlation" between *P. annua* life cycle and growth habit with annual types exhibiting upright growth vs. prostrate growth for perennials. However, 21% of laterally growing plants were annual. This correlative relationship has been used to define life cycle in numerous research efforts including Cline (2001), Gibeault (1971), Johnson (1995), Johnson and White (1997a), Kaminski and Dernoeden (2007), La Mantia and Huff (2011), Lush (1989), McElroy et al. (2002), Huff (2004), McElroy et al. (2004), Sgambatti-Araujo (1978), Standifer and Wilson (1988), and Stoy (2005). Differing *P. annua* growth habits reflect the parent species *P. infirma*, which displays an erect growth

habit, and *P. supina*, which grows in a prostrate or semi-prostrate form (Warwick & Briggs, 1978a). As *P. infirma* is annual and *P. supina* perennial (Tutin, 1957), the association of growth habit with life cycle may be related to observations of the parent species.

Research with prostrate growing perennial ecotypes of *P. annua* has focused on putting greens, which are closely and frequently mown to encourage lateral plant growth, leading to the assertion that growth habit and perennation are related (La Mantia & Huff, 2011). Considering that growth habit can be selected for via mowing, is prostrate growth associated with perennial ecotypes on putting greens simply a function of mowing rather than a characteristic of perennation? *Poa annua* harvested from a putting green was smaller in respect to number of nodes per tiller, as well as aboveground and total biomass, compared with rough and fairway ecotypes (Cline, 2001). Moreover, populations of *P. annua* harvested from putting greens produced more tillers than those obtained from roughs or fairways (Huff, 2004; Lush, 1989). These findings may again be attributed to perennation or conditions of low mowing height and high mowing frequency common to putting greens. Plants mown at low heights (< 3.2 mm) or in high frequency may display dwarfed growth to avoid mowing stress.

In addition to morphological traits, biological functions related to reproductive maturity have been associated with *P. annua* life cycle; however, such biological processes may also be influenced by management practices and have proven inconsistent in determining life cycle. Annual ecotypes have been reported to flower multiple times per year, whereas perennial selections flower only once in spring (Johnson, 1995),

potentially a result of vernalization requirements (Johnson & White, 1997a). However, La Mantia and Huff (2011) reported a perennial ecotype of *P. annua* flowered without any cold treatment. Differences in time to reproductive maturity between ecotypes have also been reported. Cline (2001) determined that an annual ecotype harvested from a putting green had a shorter pre-reproductive period (46 d) than perennial ecotypes harvested from roughs (56 d) and fairways (65 d). Stoy (2005) found no differences in days to flowering or inflorescence production between ecotypes harvested from putting greens and adjacent fairways. Such conflicting reports indicate the vernalization requirements and time to reproductive maturity of *P. annua* are variable and may be influenced by environment.

Several researchers have suggested that seed dormancy can discriminate between *P. annua* life cycles with perennial ecotypes lacking dormancy and annual ecotypes requiring 2 to 3 mo of dormancy (Gibeault, 1971; Hovin, 1957; Lush, 1989; Sgambatti-Araujo, 1978). Dormancy is thought to enhance establishment of annual ecotypes by delaying germination until growing conditions are favorable in autumn (Wu, Till-Bottraud, & Torres, 1987; Lush, 1989). Standifer and Wilson (1988) observed temperature-enforced dormancy in *P. annua* seeds harvested from Wisconsin, Maryland, and Louisiana. Seed from ecotypes harvested from Wisconsin and Maryland, which are cooler climates than Louisiana, were suspect perennial ecotypes that germinated readily, whereas the Louisiana ecotype, a suspect annual, did not germinate readily or following 2 wk of imbibed storage. Similarly, *P. annua* seeds harvested from frequently irrigated putting greens germinated at 12 and 25 °C, whereas germination of seeds obtained from

roughs and fairways was inhibited at 25 °C. This response indicates that dormancy may be temperature enforced and broken in situations where irrigation is frequent. Therefore, enhanced germinability of *P. annua* seeds from ecotypes found on putting greens may be related to management practices (particularly supplemental irrigation) and not related to life cycle.

Conditions required to break dormancy and stimulate germination in *P. annua* are not thoroughly understood. McElroy et al. (2004) found that *P. annua* of both suspect annual and perennial life cycles germinated across a wide range of diurnal temperatures from 10 to 39 °C and photoperiods from 0 and 24 h to 18 and 6 h of light and dark. Wu et al. (1987) studied the effects of storage temperature on *P. annua* germination and found that *P. annua* germination increased through altered storage temperature and indicated that previous research conducted with seeds stored at a single temperature may not be accurate. Sgambatti-Araujo (1978) suggested perennial ecotypes of *P. annua* are readily germinable, whereas dormancy for annual ecotypes may be broken through stratification, 1.5–2.5 mo of storage, or presence of light; however, studied *P. annua* ecotypes were obtained from Gibeault (1971) and identified via morphology. How much time, heat accumulation, chilling, or water is required to induce germination under field conditions? Could this information be used to develop a model to predict *P. annua* germination in the field?

It is not clear why morphology has been studied as a means of discriminating among *P. annua* life cycles as morphology is plastic and influenced by environmental changes such as mowing (Lush, 1989). La Mantia and Huff (2011) postulated somatic

reversions in life cycle may occur as environmental conditions change across generations of *P. annua*; however, their research only addressed changes in growth habit and did not quantify observed differences in the length of plant life.

We argue that efforts to compare *P. annua* var. *annua* with var. *reptans* have been inconclusive because they are foundationally flawed. The original taxonomical classifications of *P. annua* do not group plants according to life cycle. These taxa are based on morphology, yet *P. annua* var. *reptans* has been used to describe 'perennial *Poa*' in peer-reviewed turfgrass literature for nearly 50 yr. Are ecotypes with var. *reptans* morphology inherently perennial or do a combination of management practices and environmental factors yield this morphology and facilitate perennation?

Effects of Management Practices and Environment

Cline (2001) suggested that perennation of *P. annua* is likely driven through varying life history traits that lead *P. annua* ecotypes to display either *r* (annual) or *K* (perennial) selection characteristics. The *r* strategy is density-independent and common of ecotypes with high population growth and early reproduction, whereas the *K* strategy is density-dependent, and ecotypes display delayed reproduction and have greater competitive ability (Law, Bradshaw & Putwain, 1977). We contend that management and environmental factors affect *P. annua* density and reproduction and therefore could determine the degree to which ecotypes survive over years.

Phenotypic variation across locations on a golf course reflects the adaptability of *P. annua* based on environment (Mao, 2014). For example, Cline (2001) reported that *P. annua* variability was higher in roughs and fairways than on putting greens, which

receive the most intense and consistent management. This phenomenon indicates that a gradient of selection pressure may exist across a golf course. The ability of *P. annua* to change morphologically based on environmental cues can occur in distances < 3 m apart (Lush, 1989). Therefore, variation in perennation may arise across locations on golf courses as a result of differing intensity in management practices (Gibeault, 1971; La Mantia & Huff, 2011). The ability of *P. annua* ecotypes to survive perennially may be induced by intense management practices as perennial ecotypes are rarely found in unmanaged areas (Gibeault, 1971). Changes in management, such as a lack of mowing, irrigation, or fungicide treatment when pathogens are present, would likely facilitate the opposite survival strategy, equating to an annual life cycle.

Mowing

Mowing has been associated with perennation because perennial ecotypes have frequently been documented on putting greens under low heights of cut. Furthermore, mowing stress may be responsible for plants becoming perennial as an epigenetic mechanism to reduce growth in an effort to escape excessive wounding (LaMantia & Huff, 2011). Warwick and Briggs (1978b) reported that under a weekly clipping regime at 2 cm for 8 wk, prostrate *P. annua* plants grew and flowered more successfully than erect *P. annua* plants. Similarly, McNeilly (1981, 1984) established that *P. annua* populations can adapt to survive grazing or mowing and competitive stress.

Similar morphological changes resulting from environmental stresses have been observed in other crops, such as pasture grasses, that grow laterally under grazing pressure (Matches, 2013) and weed species that mimic crop phenology to avoid control

methods (Barrett, 1981). Ryegrass (*Lolium* spp.) populations with an increased ratio of tillers to flowering culms are better able to survive heat stress as less energy is used to flower and is instead allocated to tillering (Soper, 1958). *Poa annua* is thought to be unable to survive prolonged exposure to elevated temperatures because root systems deteriorate after carbohydrates are used during flowering, thus causing plants to decline (Petrovic, White, & Klingerman, 1985). If *P. annua* ecotypes shifted to increased tillering and reduced flowering, possibly because of mowing, they may be more likely to survive perennially.

Water availability

Irrigation is another management practice thought to influence *P. annua* perennation (Gibeault, 1971; Lush, 1989; Wu et al., 1987). *Poa annua* undergoes greater stress in warm temperatures and requires higher soil moisture for survival than other *Poa* species such as Kentucky bluegrass (*P. pratensis* L.) (Bogart, 1972). *Poa annua* ecotypes harvested from areas with little rainfall compared with adequate rainfall displayed smaller plant sizes and a low number of spikelets, indicating that *P. annua* may take on the annual form in situations where drought avoidance is necessary (Ellis, Lee, & Calder, 1971). Therefore, if adequate soil moisture is obtained through supplemental irrigation, *P. annua* may be able to survive perennially. Till-Bottraud et al. (1990) observed that total annuality was not achieved in suspect annual ecotypes harvested from golf course roughs when maintained in a greenhouse and supplied irrigation after flowering. These findings indicate that soil moisture may influence the ability of *P. annua* to assume a perennial life cycle.

Fungicide use

Fungicide use may also play a significant role in determining the life cycle of *P. annua*. Because of a lack of breeding (Inguagiato & Martin, 2015), the species is highly susceptible to numerous diseases including anthracnose basal rot (*Colletotrichum cereale*), summer patch (*Magnaporthe poae*), dollar spot (*Clarireedia* spp.), brown patch (*Rhizoctonia solani*), and Pythium blight (*Pythium* spp.), which can be lethal (Smiley, Dernoeden, & Clarke, 2005). These pathogens are most prevalent in summer months and may be exacerbated during times of *P. annua* heat and drought stress (Agnew, 2007; Inguagiato & Martin, 2015). Conditions in regions with warm climates are conducive to pathogenicity for an extended period of time and may compromise the ability of *P. annua* to survive perennially. For example, anthracnose is more likely to develop during periods of water stress, which occurs more frequently in situations of high air temperature, than when water is not limiting (Roberts, Inguagiato, Clarke, & Murphy, 2011). Additionally, models predicting dollar spot and brown patch indicate disease is most likely to develop during periods of warm air temperature and high relative humidity (Fidanza, Dernoeden, & Grybauskas, 1996; Smith et al., 2018). In cooler regions with a limited window for disease occurrence or areas of a golf course where fungicides are regularly applied, such as putting greens (Gelernter, Sotwell, Johnson, & Brown, 2016; Lyman, Johnson, Stacey, & Brown, 2012), *P. annua* may be more likely to be of a perennial life cycle.

Micro-environments

The intensity of mowing, irrigation, and fungicide use vary among regions of a golf course. Ecotypes of *P. annua* surviving perennially have been found in turfgrass swards subjected to frequent defoliation (Hovin, 1957; Tutin, 1957; Younger, 1959). Researchers in Pennsylvania reported that perennial ecotypes of *P. annua* grow almost exclusively in turfgrass swards maintained at 2.5 to 3.2 mm, common mowing heights used on putting greens, and termed these selections 'dwarf-type' or 'greens-type' (La Mantia & Huff, 2011; Mao, 2014). These terms are misleading considering that perennial ecotypes of *P. annua* have been identified in areas outside of putting greens as have selections that do not exhibit a dwarf-type growth habit.

A survey of *P. annua* in Washington and Oregon reported over one-half of the samples exhibited a perennial life cycle (a classification based on morphology rather than length of life observation), with many ecotypes displaying a semi-prostrate growth habit (Gibeault, 1971). Stoy (2005) performed a similar survey of 15 golf courses in Utah and found that the majority of ecotypes conformed to neither the perennial nor annual life cycle based on the ratio of reproductive and vegetative morphology. A study conducted in Minnesota assessed *P. annua* ecotypes harvested from three locations on a single golf course and concluded that putting green ecotypes displayed an annual life cycle, whereas fairway and rough ecotypes were perennial by assessing morphological characteristics associated with *r* and *K* selection strategies (Cline, 2001). This finding contradicts reports of by LaMantia and Huff (2011) that perennial ecotypes are exclusively found on putting surfaces.

Certain environmental conditions may facilitate *P. annua* survival regardless of growth habit. Cline (2001) claimed that all *P. annua* in the northern United States was perennial because mature *P. annua* could be found for the duration of the year across an array of mowing heights. In the transition zone and the southern United States, there may be micro-environments where soil moisture is not limiting, fungicides are applied, or soil temperature is reduced, which facilitate year-long survival. Lush (1989) found that seedlings of *P. annua* ecotypes from golf course putting greens, roughs, and fairways grown at 32 °C were short and erect with very few tillers compared with seedlings grown at 22 °C. Perhaps in climates with high temperatures, *P. annua* can survive perennially but exhibits different morphology than ecotypes surviving perennially in cooler climates that often grow laterally.

Knowledge Gaps and Future Directions

Many knowledge gaps need to be addressed in order to fully understand the life cycle of *P. annua*. Is *P. annua* an annual species that can survive perennially under a combination of management practices and environmental conditions? Or, is *P. annua* a perennial species that presents an annual life cycle when environmental conditions become lethal? What environmental conditions are either conducive or lethal to *P. annua* life? Is the life cycle of *P. annua* a binary system or is there a continuum of perennation?

Life cycle

Gibeault (1971) writes that *P. annua* has a "supposed annual life cycle." Likewise, Cordukes (1977) described *P. annua* as a species "with some degree of

perenniality," suggesting uncertainty exists regarding how long the species lives. The *P. annua* life cycle may not conform to the traditional distinctions of annual or perennial. The work of Lush (1987) creates skepticism that *P. annua* is perennial. A *P. annua* ecotype on a putting green in Australia was described as a perennial turfgrass because the selection was present year-round, comprised of continuously overlapping successions of annual plants rather than individual perennial plants. Similar numbers of seedling and mature *P. annua* were observed on the putting green in mid-summer, the number of tillered plants was stable, and there was no indication of seedling death (Lush, 1987). The researcher suggested that the stable number of tillered plants throughout the season indicated that the population was maintained through overlapping successions of annual plants rather than vegitatively growing perennial plants. Kaminski and Dernoeden (2007) observed continual, though at a declining rate, *P. annua* emergence in an observational study from fall to spring in Maryland. This work indicates that plants of varying levels of maturity are present throughout most of the year. Perhaps the window for *P. annua* seed germination is so great that annual populations are able to sustain life through constant succession in certain environments, thus mimicking a perennial life cycle as proposed by Lush (1987).

Similarly, La Mantia and Huff (2011) reported that some annual ecotypes of *P. annua* are not true annuals and may have tillers that do not flower and are able to sustain new growth for several years. If growth was sustained for successive years, why were these selections classified as annual? Till-Bottraud et al. (1990) demonstrated extended survival of annual ecotypes in a greenhouse with supplemental irrigation, signifying

that *P. annua* is highly affected by environment and may persist when conditions stimulate growth. Several researchers have defined perennial-type *P. annua* as a short-lived perennial, persisting for 2–3 yr (Mitich, 1998; Tutin, 1957; Warwick, 1979), with biennial ecotypes identified as well (Timm, 1965). These observations suggest *P. annua* exists across a continuum of perennation. Field monitoring studies similar to those conducted by Lush (1987) and Kaminski and Dernoeden (2007) observing life cycles of *P. annua* plants over time in areas other than putting greens could provide useful insight to the length of life typical of *P. annua* in various environments.

Conducting life-cycle monitoring studies is also a more appropriate method of life cycle characterization than use of morphological characteristics. For management of *P. annua* (of any life cycle) to be successful, turfgrass mangers must know and understand the biology of the grass they aim to manage. Classification based primarily on growth habit can potentially misidentify *P. annua* ecotypes. The association of a prostrate growth habit with a perennial life cycle has led many turfgrass managers to believe perennial ecotypes are exclusively found on putting greens. Is this creeping growth habit alone enough to delineate perennation, particularly given that perennial ecotypes without lateral growth have been documented in areas other than putting greens (Bogart, 1972; Cline, 2001; Gibeault, 1971; Stoy, 2005; Timm, 1965)? Similarly, perennial ecotypes are described as setting seed only once per year, often in spring, or not at all (Johnson & White, 1997b). Such a characteristic could also be influenced by management practices including mowing or plant growth regulator use and may not accurately indicate life

cycle. Ecotypes should be delineated as perennial only after plant life is observed over the course of a year regardless of morphology.

Genes and genetics

Gene flow is noted to be highest from greens to adjacent roughs as *P. annua* seed may be moved on equipment or foot traffic (Till-Bottraud et al., 1990; Wu et al., 1987). This could cause ecotypic variability to be greater in rough ecotypes compared with that of putting green ecotypes (Cline, 2001). Yet many turfgrass managers are uninformed that perennial *P. annua* ecotypes could exist outside of putting greens. Understanding this phenomenon is important for those aiming to control *P. annua* particularly in warm-season turfgrass stands via preemergence herbicide applications.

Similarly, many turfgrass managers in the transition zone and the southern United States are not aware that perennial ecotypes of *P. annua* could exist in their climate given that perennial ecotypes are thought to be confined to the northern United States where climatic conditions are more favorable for *P. annua*. However, McElroy et al. (2002) studied five suspected perennial ecotypes from Alabama and Georgia, which are considered to be warm climates, and concluded that a single putting green ecotype from Alabama was perennial. This finding indicates that our understanding of climates where perennial ecotypes of *P. annua* may exist is flawed. Hovin (1957) described that perennial ecotypes of *P. annua* may wither and die back in summer before resuming growth in the fall. Younger (1959) reported *P. annua* growing in subtropical regions may be present in dense patches throughout the summer in areas that are well irrigated (Younger, 1959). Much of the southeast is considered humid subtropical

(Britannica, 2018), and therefore may have a climate capable of sustaining *P. annua* life year-round if management practices create a conducive environment.

Advances in *P. annua* life cycle characterization may also be made through genetic study. Given that morphological characterization is not advisable, genetic tests may provide a more rapid means of understanding *P. annua* life cycle than year-long plant monitoring. Previous research efforts in this area have largely been inconclusive, in part because ecotypes classified for study were selected based on morphology (not life cycle) and the lack of a *P. annua* genome limits identification of diagnostic markers. For example, Gelernter et al. (2016) assessed small RNA profiles of annual and perennial *P. annua* ecotypes and found they were similar in length distribution, microRNA families, and expression levels, therefore rendering differentiation of life cycles inconclusive. Similarly, Cline (2001) evaluated random amplified polymorphic DNA and found little variation between ecotypes collected from putting greens, fairways, and roughs. Warwick and Briggs (1978a) investigated five enzyme families in an attempt to identify isozyme markers to distinguish life cycles and found none were diagnostic. Advances in next-generation sequencing methods, such as RNA sequencing, may be able to discriminate between annual and perennial ecotypes of *P. annua* considering that the technology has been used to determine genetic divergence in other turfgrass species. For example, Reasor et al. (2018) used genotyping-by-sequencing to assess variation in 72 selections of bermudagrass [*Cynodon dactylon* (L.) Pers.] × *C. transvaalensis* Burtt Davy (hybrid bermudagrass) and found that 11% of samples were genetically divergent.

Environment

Environmental factors such as temperature, moisture availability, and disease pressure affecting the life cycle of *P. annua* are not thoroughly understood. For example, current dogma among turfgrass managers is that *P. annua* does not persist under warm temperatures. This is supported by the work of Bogart (1972), who observed that *P. annua* did not survive in soil temperatures >26.7 °C without supplemental irrigation in Michigan. However, Cline (2001) found that of four *P. annua* ecotypes harvested from a single golf hole in Minnesota, a rough ecotype classified as perennial (based on having a creeping growth habit) was the most tolerant of six high-temperature regimes ranging from 30 to 45 °C, and growth was actually stimulated at temperatures of 30 and 35 °C. Similarly, McElroy et al. (2002) found that a suspect perennial *P. annua* ecotype germinated (23%) under diurnal temperature conditions of 39 and 29 °C. Such research indicates that perennial ecotypes of *P. annua* may persist under warm temperatures, traditionally thought to be unsuitable for *P. annua* life.

Perennation in *P. annua* may be driven by intense management practices such as mowing, fungicide use, or irrigation (Gibeault, 1971; La Mantia & Huff, 2011; Lush, 1987; Wu et al., 1987), all of which have become more intensive on golf courses over time (Gelernter et al., 2016; O'Brien, 2002; Zontek, 2004). These practices may impact *P. annua* individually or in combination with fungicide applications to suppress disease. A matrix of these variables (mowing, irrigation, and fungicide application) may aid in facilitating *P. annua* survival in environments thought to be unfavorable for perennation.

If management practices produce conditions that influence *P. annua* survival in unfavorable climates, understanding these effects would be important for turfgrass managers. For example, if increased mowing, irrigation, or fungicide use are conducive to *P. annua* survival, such practices could be implemented on other regions of golf courses to culture *P. annua* as a desirable species. Conversely, if *P. annua* is considered a weed, these practices could be augmented for use as a nonherbicidal means of control.

Conclusions

Despite *P. annua* being a heavily studied species of turfgrass systems, its life cycle is not well understood. The history of *P. annua* classification indicates taxonomists described varieties using morphology independent of life cycle. Rationale for the current binary classification of *P. annua* as either annual or perennial is unclear given that these distinctions are based on morphology. Why have the 40+ taxa of *P. annua* that have been classified largely been unacknowledged in turfgrass literature since 1971? Efforts should be made to be consistent and thoughtful in use of terminology surrounding *P. annua* life cycle. For example, Timm is readily listed as an authority for *P. annua* var. *annua*, yet her 1965 paper makes no mention of this variety and rather proposes *P. annua* should be classified as the subsp. *erecta* or *reptans*. Additionally, the synonymous use of greens-type, dwarf-type, and perennial *P. annua* is misleading given that morphological traits optimal for use on putting greens are unrelated to plant life cycle. Many knowledge gaps surrounding the *P. annua* life cycle exist. More research is needed to determine if *P. annua* is truly an annual species that can survive perennially under certain environmental

conditions or a perennial species that cannot withstand a different set of environmental conditions that result in plant mortality, giving the appearance of an annual life cycle.

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CHAPTER II
POA ANNUA: AN ANNUAL SPECIES?

A version of this chapter was submitted for publication to The Plant Journal on 21 Feb. 2022 by Devon E. Carroll, Brandon J. Horvath, Michael Prorock, Robert N. Trigiano, Avat Shekoofa, Thomas C. Mueller, and James T. Brosnan:

Carroll, D.E., Horvath, B.J., Prorock, M., Trigiano, R.N., Shekoofa, A., Mueller, T.C., & Brosnan, J.T. (2022) *Poa annua*: An annual species? *The Plant Journal*. In Review.

The publishing of this article involved six authors from the University of Tennessee departments of Plant Sciences and Entomology and Plant Pathology. A seventh author from mesur.io aided in statistical analysis. All authors made conceptual or technical contributions relative to their area of expertise. My primary contributions to this paper include (i) reading literature, (ii) developing methodology, (iii) conducting experiments, (iv) collecting and analyzing data, (v) writing the manuscript, and (vi) revising during the review process.

Abstract

As the Latin name *annua* implies, the species *Poa annua* L. is thought to have an annual life cycle. Yet, there are many reports in literature of *P. annua* persisting perennially. Considering that *P. annua* senescence patterns do not align with other true annual species, we hypothesized that *P. annua* is similar to other perennial, C_3 turfgrass species that are subject to a confluence of environmental factors that can cause mortality. Four experiments were conducted in Knoxville, TN with the objective of determining environmental factors lethal to *P. annua*. A field monitoring study assessed 100 *P. annua* plants across ten grassland micro-environments from May to October 2020. Forty plants survived the summer and confirmed the existence of perennial *P. annua* ecotypes. Analysis of environmental factors at the time of plant death indicated soil moisture, soil temperature, and pathogenic infection were associated with mortality. A series of glasshouse or field experiments were conducted to investigate the effects of each factor on *P. annua* mortality. Soil moisture and soil temperature were not lethal to *P. annua* in the glasshouse, except under extreme conditions not typical in the field. A field study assessed mortality of plants from pathogenic infection and indicated that *P. annua* plants treated with fungicide throughout the summer survived year-round, whereas plants not receiving fungicide applications senesced. These findings support our hypothesis that *P. annua* is of a perennial life cycle, which can be influenced by environmental conditions. We suggest that the name, *P. annua,* is likely a misnomer.

Introduction

Poa annua L. is a C_3 grass species and a common component of urban grasslands. The ubiquity of *P. annua* in terms of both distribution and abundance has been compared to that of *Homo sapiens* (Pate & Hopper, 1994). Although the species can be desirable, it is also recognized as the most troublesome weed of urban grasslands and second most troublesome weed of all grass crops (Van Wychen, 2020).

An annual species is defined by abrupt senescence following completion of a single reproductive cycle, even if growing conditions are optimal (Taiz and Zeiger, 2002). Grass crops such as *Triticum spp*. (wheat) and *Zea mays* L. (maize) are annuals that senesce after fruiting regardless of environmental surroundings (USDA, 2021a; USDA, 2021b). Despite its name meaning annual in Latin, *P. annua* does not follow the same pattern of absolute monocarpic senescence. Furthermore, reports of *P. annua* surviving perennially are abundant (Piper & Oakley, 1927; Tutin, 1957; Younger, 1959; Timm, 1965; Ellis et al., 1971; Gibeault, 1971; Bogart, 1972; Cordukes, 1977; Law et al., 1977; Warwick, 1979; Danneberger & Vargas, 1984a; Lush, 1988; Lush, 1989; Till-Bottraud et al., 1990; Darmency et al., 1992; Johnson & White, 1997a & 1997b; Mitich, 1998; Cline, 2001; McElroy et al., 2002; McElroy et al., 2004; Stoy, 2005; Kaminski & Dernoeden, 2007; La Mantia & Huff, 2011; Mao & Huff, 2016).

It is unclear why *P. annua* is considered an annual species. Carl von Linné (Carl Linnaeus) provided no criteria when naming the species; in 1753 he describes *P. annua* as, “Extended corn bluegrass with straight angles, with smoothed spikes, with a compressed, slanted top. Minor field tuberous grass. Greater red field tuberous grass. It

lives on the European paths." and in 1754 states only that he identified the plant in a field in Denmark (Linné, 1753; Linné, 1754). Perhaps the epithet *annua* is taken too literally in modern times and instead of meaning of an annual life cycle was intended to describe an annual event such as inflorescence production.

Because *P. annua* is known to copiously produce seed (Cordukes, 1977; Law et al., 1977; Law, 1981; Danneberger & Vargas, 1984a), the *annua* epithet may be related to observations of inflorescence characteristics. While high fecundity is frequently associated with an annual life cycle (Struik, 1965; Harper et al., 1970; Lush, 1988), other species common to urban grasslands such as *Taraxacum offiicinale* Weber ex. Wiggers (dandelion) and *Trifolium repens* L. (white clover) are prolific in inflorescence/seed production and survive perennially (Medeiros & Steiner, 2000; Honek et al., 2005). Additionally, *P. annua* can produce seed continuously (Tutin, 1957; Lush, 1988; Till-Bottraud et al., 1990) resulting in classification as a polycarpic perennial (Timm, 1965; Law et al., 1977; Campbell & Grime, 1992). By definition, a polycarpic species cannot be annual as seed is produced more than once and is not followed by plant death (Taiz and Zeiger, 2002).

Could *P. annua* be a perennial species? A large-scale study of nearly 7,000 *P. annua* plants harvested across Europe from Portugal to Finland showed the majority of plants (5,352; 79.3%) were perennial, whereas only 1,398 (20.7%) survived less than one year (Timm, 1965). This response aligns with theories of senescence and selection, which favor a perennial over annual life cycle (Williams, 1957; Silvertown, 1983; Iwasa & Cohen, 1989). For example, long- lived individuals produce more offspring over time

than those with short life spans causing natural selection to advance toward perenniality (Williams, 1957; Law, 1977). Therefore, reproductive characteristics indicate a perennial life cycle is superior for global establishment. Given that *P. annua* has successfully colonized all seven continents across a wide range of environments (Molina-Montenegro et al., 2014) in potentially as few as 10,000 years (Hobbs, 1946; Tutin, 1957; Mao & Huff, 2012), it is more likely the species is perennial.

Therefore, similar to Johnson (1995), we hypothesize that *P. annua* is comparable to other perennial, C_3 urban grassland species, which are subject to a confluence of environmental factors in summer that can result in mortality. Premature senescence, particularly due to pathogenic infection, of perennial plants in summer months after seed production may give the appearance of an annual life cycle. Such an occurrence is similar in crops of *Solanum lycopersicum* L. (tomato), *Solanum tuberosum* L. (potato), and *Gossypium* L. (cotton), which are perennial species that are cultivated as annuals due to premature senescence in climates with adverse temperatures (Sukumaran & Weiser, 1971; Nadeem et al., 2013; Yang et al., 2015).

While scientists have reported *P. annua* is highly sensitive to heat and drought stress (Younger, 1959; Timm, 1965; Gibeault, 1971; Cordukes, 1977; Warwick, 1979; Gaussoin 1989; J Till-Bottraud et al., 1990; Johnson, 1995; Slavens et al., 2011; Yang et al. 2014;), the influence of these factors has often been assessed individually, for short periods of time, and in greenhouses or growth chambers. In the field, fluctuations in temperature and moisture over time can affect disease incidence in susceptible hosts such as *P. annua* when pathogens are present.

Poa annua is susceptible to numerous diseases including dollar spot (*Clarireedia* spp.), anthracnose (*Colletotrichum cereale*), Pythium blight (*Pythium* spp.), and brown patch (*Rhizoctonia solani*) (Smiley et al., 2005). The pathology literature describes these diseases as most devastating, resulting in summer decline or plant death, when plant stress is induced via anthropogenic activity or unfavorable environmental conditions (Danneberger et al., 1984b; Walsh et al., 1999; Inguagiato et al., 2009; Roberts et al., 2011). Pathogenicity of the aforementioned diseases peaks with elevated air temperature and atmospheric humidity (Fidanza et al., 1996; Walsh et al., 1999; Inguagiato and Martin, 2015; Smith et al., 2018). Therefore, stressful environmental conditions (e.g., elevated air temperature, drought, etc.) may falsely appear to cause *P. annua* senescence when heightened pathogenetic activity is the actual cause of mortality.

Although the nature of *P. annua* transience in some situations remains uncertain, the literature indicates the species may be incorrectly botanically characterized. If any environmental factors are causing plant death rather than programmed senescence after inflorescence production, classification as an annual species does not follow the accepted definition of such a life cycle (Taiz and Zeiger, 2002). Given the world-wide presence of the species and difficulties related to management (Brosnan et al., 2020; Van Wychen, 2020), efforts to better understand *P. annua* life cycle are warranted. Therefore, the objective of this study was to determine environmental factors lethal to *P. annua.*

Materials and Methods

Life Cycle Observation

An observational field study was initiated on 11 May 2020 at three locations in Knoxville, TN. One hundred *P. annua* plants were monitored throughout the summer and fall until 27 Oct. 2020. Monitoring occurred in ten unique micro-environments across the three study locations (Table 2.1). Experimental areas within micro-environments measured 1 x 2 m. Ten *P. annua* plants were identified and monitored within each area. Metal rings (7.6 cm in diameter and 2.8 cm in length) were installed around each plant to allow monitoring of the same *P. annua* plants throughout the study. Herbicides were not applied during the observational period.

At study initiation and every three weeks thereafter, photographs were taken of each *P. annua* plant. Visually rated plant mortality was recorded on each rating date. A multifactor F-score analysis of climatic data related to death events was conducted using Python (Version 3.9.9, https://www.python.org/) programming language. Libraries used in analyses included 'pandas' (v. 1.3.4; https://pandas.pydata.org/) for data handling, 'NumPy' (v. 1.22.0; https://numpy.org/) for numerical transformations and math, 'scikit learn' (v. 1.0.1; https://scikit-learn.org/stable/) for modeling and related functions, 'SciPy' (v. 1.7.1; https://scipy.org/) for code modeling and curve fitting, 'matplotlib' (v. 3.5.0; https://matplotlib.org/) and seaborn (v. 0.11.2; https://seaborn.pydata.org/) for visualization, and 'xgboost' (v. 1.5.1; https://xgboost.readthedocs.io/en/stable/) for broad modeling to determine feature importance. All statistical analyses are available in a Google collaborative environment at:

https://colab.research.google.com/drive/16eIDgyFMX08K2szhc_LdXmbYavf982jY?usp=sharing.

Soil Temperature Evaluation

Ecotypes from micro-environments one and two (Table 2.1) in the aforementioned observational study were selected on the basis of observed length of life for inclusion in a repeated glasshouse experiment. The experiment was conducted in Knoxville, TN (35.946590, -83.939360) during early spring 2021. Mature *P. annua* plants were harvested from the field on 24 Feb. 2021. Harvest location one was comprised of plants that senesced during 2020, whereas harvest location two contained plants that survived the entire duration of our observational experiment in 2020. Plants were harvested within a 7.5 m^2 area as undisturbed soil cores (10.2 cm diameter) containing aboveground biomass and an intact root system. Plants of similar size, tiller number, and growth stage (e.g., no inflorescence) were selected for collection. Extracted soil cores were cut to a uniform depth of 7.6 cm, such that no roots were visibly protruding.

Plants were acclimated in a glasshouse for seven days before being placed in water baths to impose variable soil temperatures, which was similar to the design reported in Huang & Xu (2000) and Huang et al. (2001). Water baths in our experiment were constructed by mounting polyvinyl chloride (PVC) pipes (10.2 cm in length and diameter) in plastic containers (51 x 39 x 15 cm) (Figure 2.1). Pipes were affixed to the container using waterproof caulk atop drainage holes. Three *P. annua* plants from each micro-environment were installed in pipes by sliding the core into the pipe without

disturbance. Pipes were randomized within each water bath. Filter paper was placed in the bottom of each tube to prevent loss of soil while allowing for water movement.

Glasshouse conditions averaged 50% relative humidity and 22.8°C in air temperature. A 16/8 hour day/night artificial photoperiod was imposed via metal halide lights (1000 W; P.L Light Products, Beamsville, ON, Canada) with night occurring from 2200 to 0600 hours. At the beginning of the acclimation period, plants were supplied with complete fertilizer (20N-20P_2O_5-20K_2O; Southern Ag Triple Twenty with minors; Hendersonville, NC) to deliver 49 kg N ha^{-1} and immediately irrigated (5 mm) via an overhead sprinkler system. Five mm of irrigation was applied daily between 0700 and 0900 hours. Plants were clipped to a height of 2.5 cm by hand using scissors on days three and seven of acclimation.

After acclimation, plants were subjected to one of four soil temperatures for four weeks. Water baths were arranged in a split-plot design with three replications. The whole plot factor was soil temperature whereas the split-plot factor was *P. annua* ecotype (n = 72 plants; 2 ecotypes x 3 plant replications x 4 soil temperatures x 3 water bath replications). Two experimental runs were conducted simultaneously in separate glasshouse bays.

Aquarium heaters with digital thermometers (500 W; Hygger, Renton, WA) were used to manipulate water temperature in each bath to augment soil temperature. Each water bath was equipped with a 4 W circulation pump and a secondary liquid thermometer to monitor water temperatures. For the duration of this experiment, water bath temperatures were 21.1, 26.7, 32.2, or 37.8°C. Temperatures in this range were

chosen because previous studies assessing soil temperature effects on C_3 grasses (such as *P. annua*) consider 20°C and 35°C to be optimal and supraoptimal soil temperatures, respectively (Huang & Xu, 2000; Huang et al., 2001).

For water baths set to 26.7, 32.2, or 37.8°C, water was added outside of PVC pipes containing *P. annua* plants to a depth of 7.6 cm. Water baths set to these temperatures were placed atop a heating mat (iPower, Irwindale, CA) to prevent heat loss and ensure intended temperatures were achieved. The 21.1°C temperature was achieved using the same equipment devoid of water. Heat from water in baths was transferred to soil cores containing *P. annua* until soil temperature was in equilibrium with water temperature.

Soil temperatures were monitored in one replication of each water bath using external soil sensors (#3667; Spectrum Technologies Inc., Aurora, IL) programmed to record soil temperature every 15 min and transmitted to a micro station (WatchDog 1000 Series; Spectrum Technologies Inc., Aurora, IL). Micro stations also recorded air temperature and humidity every 15 minutes. Secondary digital soil thermometers (#6300; Spectrum Technologies Inc., Aurora, IL) were monitored daily in one replication of each plant ecotype in each water bath as well. In experimental run one, mean air temperature was 22.6°C and mean relative humidity was 49.4%. In experimental run two, mean air temperature was 22.5°C and mean relative humidity was 46.0%.

Soil temperature treatments were imposed for four weeks. During the treatment period, plants were clipped daily to a height of 2.5 cm by hand using scissors and water baths were refilled to the appropriate water level as needed. Plants were maintained at a

constant gravimetric moisture content via addition of supplemental water (15 to 23 mL) twice per week. After four weeks of exposure to soil temperature treatments, plants were subjected to ambient soil temperature for two weeks to assess the potential for regrowth. During this time, plants were not clipped, and supplemental irrigation (5 mm) was applied daily via an overhead misting system.

Plants were visually evaluated daily for mortality, rated as all aboveground tissue brown, with death confirmed via assessments of photochemical efficiency using a handheld fluorometer (Fv/Fm meter; Opti-Sciences, Inc., Hudson, NH) to measure Fv/Fm ratio. Two readings were taken from each plant once per week after randomly placing five- to- ten attached leaves into dark adaption chambers for 20 minutes. Plants were considered dead when the Fv/Fm ratio was zero. For most plant species, Fv/Fm ratios in the range of 0.79 to 0.84 are considered optimum, whereas lower values indicate plant stress (Maxwell & Johnson, 2000; Wu et al., 2021).

Data were subjected to analysis of variance using the SAS (University Edition, SAS, Cary, NC) mixed procedure. Experimental run, soil temperature, and ecotype interactions were tested. Analysis of variance revealed ecotype was not a significant factor for photochemical efficiency data. Figures were generated in Prism (Prism 9 for Mac, Graph-Pad software, La Jolla, CA).

Soil Water Evaluation

Mature *P. annua* plants that survived the entire experiment in 2020 were harvested from micro-environment two (Table 2.1) on 21 April 2021 for inclusion in a glasshouse experiment exploring effects of soil moisture on *P. annua* mortality at

different soil temperatures. Harvest methods were identical to those previously described. All harvested plants were of similar size and had inflorescences present at the time of collection. The collection area was treated with cyazofamid 24 hours prior to harvest (Segway; PBI Gordon Corporation, Shawnee, KS) at 1.1 kg ha^{-1} to prevent confounding effects of soil pathogens. Fungicide was immediately irrigated into the soil after application.

Soil cores containing *P. annua* were tightly wrapped with polyethylene plastic (0.15 mm thick) to prevent water loss via evaporation. *Poa annua* plants were carefully moved to protrude out of a 2.5 cm incision at the top of each core (Figure 2.2). Four drainage holes were created in the bottom of soil cores. Plastic wrapped soil cores were installed in PVC pipes (10.2 cm in length and diameter). Plants then underwent 7 d of acclimation in a glasshouse under conditions described previously for photoperiod, nutrient application, irrigation, and clipping. Ambient conditions during acclimation averaged 64.9% relative humidity and 22.2°C air temperature in experimental run one and 53.9% relative humidity and 22.1°C air temperature in experimental run two.

Soil cores were saturated to the point of water freely draining through drainage holes at the termination of the acclimation period. Water was allowed to drain overnight, resulting in soil cores at pot capacity at the onset of treatment, similar to Sheldon et al. (2021). A total of 36 *P. annua* plants in each experimental run were subjected to a 2 x 2 factorial of soil temperature and irrigation regime for six weeks. Two experimental runs were conducted concurrently in separate glasshouse bays. During treatment, plants

received no supplemental nutrients and were clipped daily to a 2.5 cm height using scissors.

Soil temperature was either ambient or elevated via a heating mat (iPower, Irwindale, CA). Irrigation treatments included well-watered (n = 12) or full drought (n = 24; Figure 2.3). Irrigation needs were determined by calculating daily plant transpiration similar to the methods of Sheldon et al. (2021). Individual PVC pipes were weighed at 1200 to 1300 hours daily. Daily transpiration was calculated as the difference in weight of each pot on successive days. The well-watered plants were irrigated by hand daily to maintain a weight of no less than 50 g of their initial weight. A syringe was inserted through the incision at the top of the core to facilitate controlled watering. Plants subject to full drought conditions received no supplemental irrigation throughout the entirety of the study.

Environmental conditions including air temperature, relative humidity, and soil temperature were monitored as previously described. In experimental run one, soil temperature averaged 26.8°C in cores subjected to ambient conditions and 34.6°C in cores subjected to elevated soil temperature. Mean air temperature was 26.6°C and mean relative humidity was 59.4%. In experimental run two, mean soil temperatures were 24.7°C and 32.0°C for cores subjected to ambient and elevated soil temperature, respectively. Mean air temperature was 25.7°C and mean relative humidity was 56.1.%.

Data collection and analyses were similar to that described in the soil temperature experiment. Plants were evaluated daily for mortality, which was confirmed via photochemical efficiency readings. *Poa annua* photochemical efficiency was evaluated

three times per week by measuring chlorophyll fluorescence using a handheld fluorometer (Fv/Fm meter; Opti-Sciences, Inc., Hudson, NH). Two readings were taken from each plant after randomly placing five- to- ten attached leaves into dark adaption chambers for 20 minutes. Volumetric water content (VWC) of soil was recorded at approximately a 5.0 cm depth on the day of mortality, or for those that survived, at the conclusion of the study by inserting a soil moisture sensor (ML3 ThetaProbe; Delta -T Devices, Cambridge, UK) into the center of each core and recording a single reading.

Data were subjected to analysis of variance using the SAS (University Edition, SAS, Cary, NC) mixed procedure. Experimental run, soil moisture, and soil temperature interactions were tested. Figures were generated in Prism (Prism 9 for Mac, Graph-Pad software, La Jolla, CA).

Disease Susceptibility Evaluation

A field experiment was conducted at the East Tennessee AgResearch and Education Center (Knoxville, TN) from 27 May to 28 October 2021 to assess the impacts of fungicide application on *P. annua* mortality. The experimental site was a mixed stand of *P. annua* and zoysiagrass (*Zoysia matrella* Merr. cv. 'Trinity') atop a Sequatchie silt loam soil with a pH of 6.2 and 2.9% organic matter. The area was mown twice per week at a 1.3 cm height of cut and watered four times per week via automatic irrigation. Slow-release fertilizer (Gal-Xe$^{ONE®}$; Simplot, Boise, ID) was applied to the entire area on 22 April 2021 to deliver 195 kg N ha^{-1} yr^{-1}. On 25 May 2021, the experimental area was treated with quinclorac (Drive XLR8; BASF Corporation, Research Triangle Park, NC) at 0.84 kg ha^{-1} + methylated seed oil (0.5% v/v) to control summer annual weeds.

The experiment was arranged as a randomized complete block design with 10 replications of two treatments applied to 1 m^2 plots. Treatments included either no fungicide application or fungicide mixtures including active ingredients from Fungicide Resistance Action Committee groups #2, 3, 5, 7, 11, and 21 intended to manage outbreaks of dollar spot, anthracnose, *Pythium* blight, and brown patch (Table 2.S1). Treatments were applied every 14 days from 26 May to 13 October 2021. Every two weeks, experimental units were visually assessed for *P. annua* mortality. An F-score analysis of climatic data associated with mortality was conducted using the same methods and packages as those used in the life cycle monitoring experiment.

Results & Discussion

Life Cycle Observation

In four of the 10 micro-environments (#2, #6, #7, and #9) all ten monitored *P. annua* plants survived the summer season, whereas in the other six micro-environments, all ten monitored plants senesced during the observational period. The ability of *P. annua* to survive year-round in certain environments in this study aligns with reports of perennial ecotypes (Piper & Oakley, 1927; Tutin, 1957; Younger, 1959; Timm, 1965; Ellis et al., 1971; Gibeault, 1971; Bogart, 1972; Cordukes, 1977; Law et al., 1977; Warwick, 1979; Danneberger & Vargas, 1984a; Lush, 1988; Lush, 1989; Till-Bottraud et al., 1990; Darmency et al., 1992; Johnson & White, 1997a & 1997b; Mitich, 1998; Cline, 2001; McElroy et al., 2002; McElroy et al., 2004; Stoy, 2005; Kaminski & Dernoeden, 2007; La Mantia & Huff, 2011; Mao & Huff, 2016).

Surviving plants exhibited a range of morphological characteristics. Locations two and six had fine-textured plants that displayed somewhat lateral growth and produced inflorescences periodically throughout summer (Figure 2.S1). Plants in locations seven and nine were coarse-textured with an upright growth habit and lacked inflorescences. Micro-environments conducive to *P. annua* survival were those where water was either not a limiting factor or where fungicides was applied. Analysis of environmental factors at the time of observed plant mortality corroborated these observations and indicated that sum precipitation, maximum soil temperature, and likelihood of pathogenic infection were significant factors related to plant death (Figure 2.4).

Soil Temperature Evaluation

Analysis of variance revealed only a significant treatment effect in photochemical efficiency data. Therefore, data were pooled across experimental runs and ecotypes. Considering that ecotypes in this experiment were harvested from distinct microenvironments where we observed mortality or continued survival in our observational study, the lack of an ecotype effect in photochemical efficiency provides evidence that *P. annua* mortality is dictated by environmental conditions rather than programmed senescence common of annual plants.

In both experimental runs, *P. annua* plants survived 28 days of exposure to soil temperatures of 21.1, 26.7, or 32.2°C and senesced when subjected to 37.8°C. Plants subjected to the 21.1°C soil temperature remained healthy for the duration of the experiment as photochemical efficiency was near optimum (Fv/Fm of 0.79), ranging from 0.75 to 0.78 (Figure 2.5). *Poa annua* exposed to the 26.7 or 32.2°C soil temperature

treatments did not senesce but were negatively affected by prolonged exposure to elevated soil temperatures. Both treatments numerically reduced photochemical efficiency from 21 to 42 days after treatment (DAT). For plants subjected to the 37.8°C soil temperature, photochemical efficiency was drastically reduced from 0.71 at 7 DAT to 0.33 by 14 DAT, remaining ≤ 0.02 thereafter.

Only the 37.8°C soil temperature treatment was lethal to *P. annua* plants. Death first occurred 12 DAT with the senescence of five *P. annua* plants in experimental run one and two plants in experimental run two. By 17 DAT, 35 of 36 *P. annua* plants exposed to the 37.8°C soil temperature across both runs had died, and by 28 DAT all plants at this temperature senesced in both experimental runs.

Reductions in *P. annua* plant health without death, except in extreme cases, when soil temperature is elevated supports other published reports (Cordukes, 1977; Yang et al. 2014). Cordukes (1977) exposed 115 *P. annua* selections to conditions of 47°C at 100% relative humidity in a growth chamber for six hours and observed that after 10 days of recovery, no plants had died, but 77% were negatively affected (< 50% recovery). Similarly, Yang et al. (2014) subjected *P. annua* to day/night air temperatures of 20/15, 30/25, or 40/35°C for eight days in growth chambers and found that the supraoptimal temperature of 40/35°C caused physiological damage to plants, although none senesced.

Survival of *P. annua* in these glasshouse experiments agrees with observations made in our life cycle monitoring experiment in the field. For example, peak soil temperature across all ten micro-environments was 30.8°C (The Earthstream Platform; mesur.io, Yanceyville, NC) in 2020 and average daily air temperature did not exceed

28.7°C. Given that some *P. annua* plants in the observational experiment senesced without soil temperatures exceeding 32.2°C, we concluded that elevated soil temperature alone does not result in *P. annua* death.

Soil Water Evaluation

Analysis of variance found a significant soil temperature x soil moisture x experimental run interaction. This interaction was expected to be significant because of differences in evaporative demand and subsequent plant stress response (Shekoofa et al., 2013). The significance of experimental run is likely due to differences in air temperature, light, and relative humidity between glasshouses, which were all higher in experimental run one compared with experimental run two.

Only 27 of 72 *P. annua* plants across both experimental runs succumbed to stress in this experiment. Senescence was similar across experimental runs with 13 and 14 plants succumbing to soil water stress in experimental run one and two, respectively. *Poa annua* was more likely to senesce under drought stressed conditions when soil temperatures were elevated; only 7 of the 27 plants senesced when subjected to drought and ambient soil temperature. The first death was observed 19 DAT; however, the majority (17 of 27) of senesced plants died on or after 35 DAT as evidenced by reductions in photochemical efficiency (Figure 2.6). Mean VWC at the time of death for drought stressed plants was 4.2 and 8.9% in experimental run one and two, respectively.

Of *P. annua* plants subjected to drought stressed conditions, 23 of 48 plants across both experimental runs survived 42 days without water. Although these plants did not senesce, stress was evident as photochemical efficiency was reduced significantly

(Figure 2.6). Mean VWC at the termination of the experiment for surviving plants was only slightly higher than values observed for senesced plants and averaged 6.2 and 10.5% in experimental run one and two, respectively.

The ability of *P. annua* to survive an extended period with low water availability has been documented (Slavens et al., 2011). These researchers did not observe *P. annua* senescence when plants were maintained in a sand-based medium with VWC from 4 to 12% for 95 days. While VWC treatments tested by Slavens et al. (2011) are similar to those achieved via drought stressed treatment in this study, air and soil temperature were not published in their report. Although a combination of full drought and elevated soil temperature was lethal to some *P. annua* plants in our experiment, the conditions required for death rarely occur in the field. For example, during our observational study (11 May to 27 Oct. 2020), the longest period without rainfall was only 9 d, which is less than the 19 d without water required for plant mortality in this glasshouse study. Additionally, managed urban grasslands where *P. annua* is widely distributed are often supplied supplemental irrigation, limiting extended periods without water. Overall, our data indicate that low soil moisture alone is not lethal to *P. annua* except in extreme situations, which are atypical of managed urban grasslands.

Disease Susceptibility Evaluation

Poa annua plants in plots not treated with fungicide did not survive the summer period. High individual plant mortality was observed in non-treated areas on 24 June 2021 with some plots completely devoid of *P. annua* beginning on 8 July 2021 (Figure 2.7). By 4 August 2021, no *P. annua* plants were alive in any non-treated plots.

Conversely, in plots treated with fungicide, some *P. annua* plants survived to the termination of the experiment.

The range of the anthracnose severity (Figure 2.7) in this experiment suggests that increases in zoysiagrass cover throughout the monitoring period (and a concomitant decline in *P. annua* plant size) may have affected our ability to document *P. annua* during summer. For example, zoysiagrass cover across the experimental area at study initiation was only 63%, whereas by 18 August 2021, zoysiagrass cover reached 88%. Zoysiagrass is a C_4 species adapted to the summer season in Knoxville, TN and *P. annua* is a C_3 species that is at a disadvantage during summer.

Therefore, we surmised that zoysiagrass outcompeted *P. annua* for resources and caused a natural population dynamic shift. A similar observation was made by Younger (1959) who found that inter-species competition with common bermudagrass (*Cynodon dactylon* Pers.) limited *P. annua* persistence in summer months. As zoysiagrass grew more dense, *P. annua* became more difficult to assess via visual ratings. Without the presence of an inflorescence to clearly identify *P. annua* plants, it is possible plants were not identified, but persisted throughout the season (Figure 2.8). For example, fungicide treated plots scored as not containing *P. annua* on 4 August had multi-tillered plants in autumn when temperatures cooled and indicated that plants had survived the summer and grew rapidly when environmental conditions were optimal.

Interestingly, several *P. annua* plants maintained vigorous growth throughout the experiment, producing inflorescences as early as 4 August 2021 (Figure 2.8). *Poa annua* emergence modeling developed by Taylor et al. (2021) in Knoxville, TN indicates peak

emergence typically occurs between the 40th and 43rd week of the calendar year when mean seven-day soil temperature lowers to ≤ 18.9ºC and seven day rainfall accumulation is ≥ 12.7 mm. Observation of *P. annua* inflorescence production in only the 32nd week of the year when soil temperatures were > 24 ºC and average weekly rainfall was < 4.75 mm (The Earthstream Platform; mesur.io, Yanceyville, NC) indicated that the observed plants were not newly germinated and had survived the summer season.

Results of this experiment indicate that fungal pathogens are the primary cause of *P. annua* mortality in maintained urban grasslands. Although not all plants in fungicide treated plots survived, *P. annua* plants treated with fungicide lived markedly longer than those not treated with fungicide (Figure 2.7). F-score analysis of climatic data at the time of plant mortality revealed risk of anthracnose and brown patch infection as significant factors associated with mortality (Figure 2.9). On the first date mortality was documented in non-treated plots (24 June 2020) anthracnose acervuli were present on necrotic foliage.

Summer decline of *P. annua* via fungal infection is thoroughly discussed in pathology literature (Smiley et al., 2005; Agnew, 2007; Inguagiato and Martin, 2015). Fungicide use on *P. annua* managed as a desirable species is a readily accepted for summer survival (Lyman et al., 2012; Gelernter et al., 2016). Some reports indicate *P. annua* cannot be commercially released until disease resistance is incorporated into the species because plants are so highly susceptible to pathogen attack (Younger, 1959). Therefore, we recommend that susceptibility to fungal pathogens be considered when assessing annual vs. perennial life cycle of *P. annua*.

Conclusions

Results of these experiments suggest *P. annua* perishes from fungal infection, which may be exacerbated by environmental and abiotic stress during summer months (Fidanza et al., 1996; Roberts et al., 2011**;** Smith et al., 2018). *Poa annua* seems to persist unless environmental conditions are unfavorable, often presenting as a polycarpic plant rather than succumbing to programmed senescence following seed production. This phenomenon is similar to that observed in other perennial, C_3 grass species such as Kentucky bluegrass (*Poa pratensis* L.) or tall fescue (*Festuca arundinacea* Schreb.), which may experience premature senescence in summer months, yet are still classified as perennial species. Annual species die following a single reproductive cycle regardless of environment conditions (Taiz and Zeiger, 2002); if any factor other than programmed senescence results in mortality, plants are not considered to be annual. Our results show that *P. annua* does not meet the definition of an annual species. Thus, observations made in this study and in literature present little evidence supporting an inherently annual life cycle in *P. annua.* This shift in our understanding of species biology will profoundly affect plant management, as life cycle is foundational information to management strategy.

Because *P. annua* is more likely a perennial than annual species, the epithet *annua* seems incorrect. While modern interpretation of "*annua*" is in terms of an annual life cycle, the original name may have been intended to represent the yearly observation of the species or its annual seed production instead of the species life cycle. Therefore, a more appropriate and descriptive name may be *P. typica* or *P. vulgari*, meaning 'typical'

and ‘common’ in Latin, respectively. These epithets preclude misinformation surrounding life cycle and indicate the species prevalence given that *P. annua* is found on all seven continents in a myriad of climates.

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Appendix

Table 2.1. Site characteristics for micro-environments monitored from 11 May to 27 October of 2020 to assess *Poa annua* survival.

Number[†]	GPS coordinates	Height of cut (cm)	Soil series	Soil texture	Soil pH	Soil organic matter (%)
1	36.091N, -83.845W	1.3	Urban land-Udorthents complex	Loam (42% sand, 46% silt, 12% clay)	5.5	5.6
2	36.091N, -83.845W	1.3	Urban land-Udorthents complex	Sand (90% sand, 4% silt, 6% clay)	6.1	2.3
3	36.091N, -83.845W	1.3	Urban land-Udorthents complex	Sandy loam (76% sand, 16% silt, 8% clay)	5.9	3.5
4	36.091N, -83.845W	1.3	Urban land-Udorthents complex	Loam (40% sand, 46% silt, 14% clay)	5.4	7.9
5	36.091N, -83.845W	1.3	Urban land-Udorthents complex	Silt loam (32% sand, 58% silt, 10% clay)	5.8	7.3
6	36.091N, -83.845W	1.3	Urban land-Udorthents complex	Sand (92% sand, 6% silt, 2% clay)	6.1	2.9
7	35.911N, -83.955W	1.6	Waynesboro loam	Silt loam (18% sand, 60% silt, 22% clay)	6.4	6.4
8	35.980N, -84.324W	1.4	Collegedale silt loam	Loamy sand (82% sand, 14% silt, 4% clay)	6.8	5.1
9	35.980N, -84.324W	6.4	Collegedale silt loam	Silt loam (40% sand, 50% silt, 10% clay)	7.2	4.2
10	35.980N, -84.324W	1.4	Collegedale silt loam	Loamy sand (84% sand, 14% silt, 2% clay)	6.6	3.4

[†] Micro-environments are assigned a number for clarification of results.

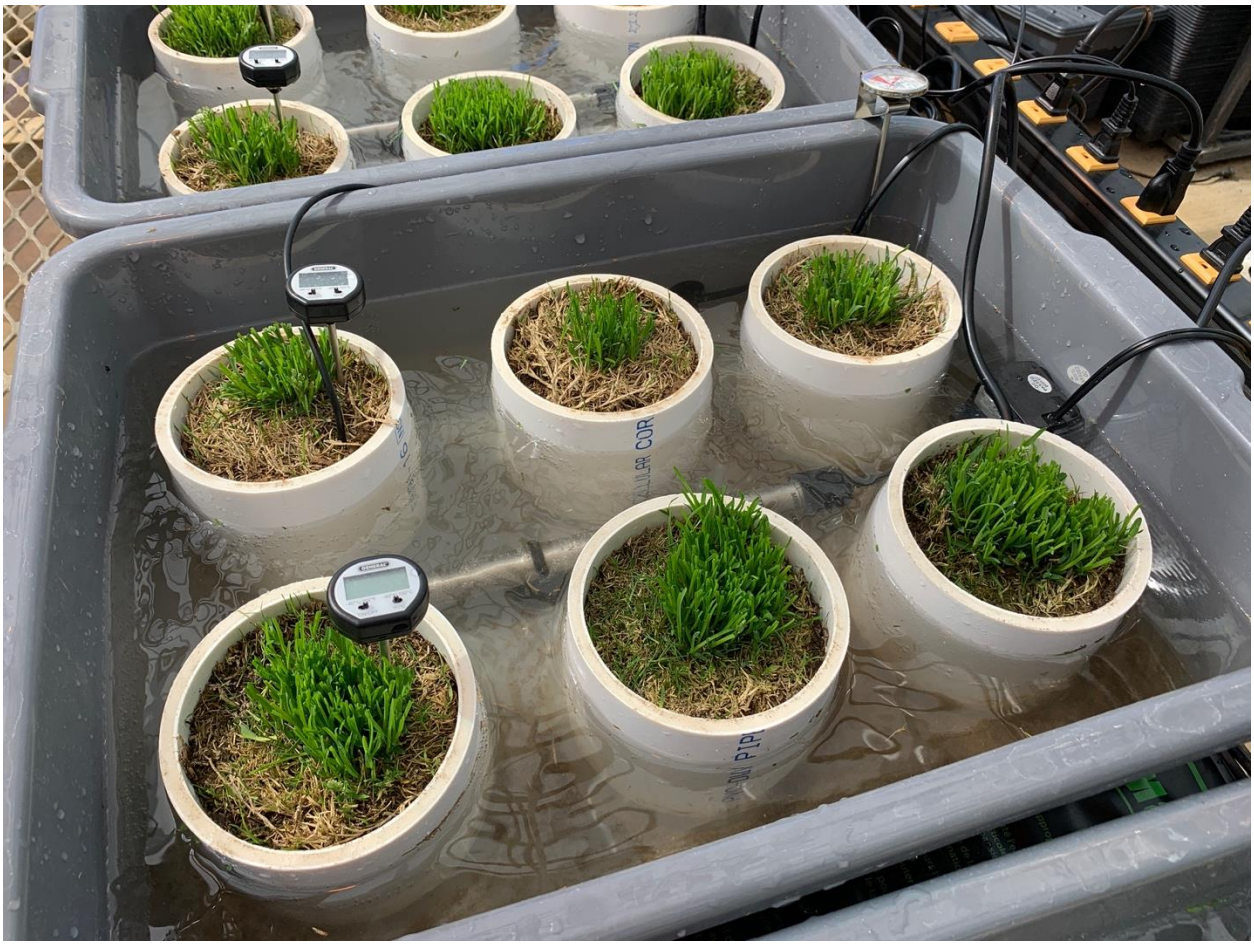


Figure 2.1. *Poa annua* plants harvested on 24 February 2021 in Knoxville, TN placed in polyvinyl chloride pipes (10.2 cm diameter and length) and mounted in plastic tubs. Water added outside of pipes was heated to 21.1, 26.7, 32.2, or 37.8°C to elevate soil temperatures.

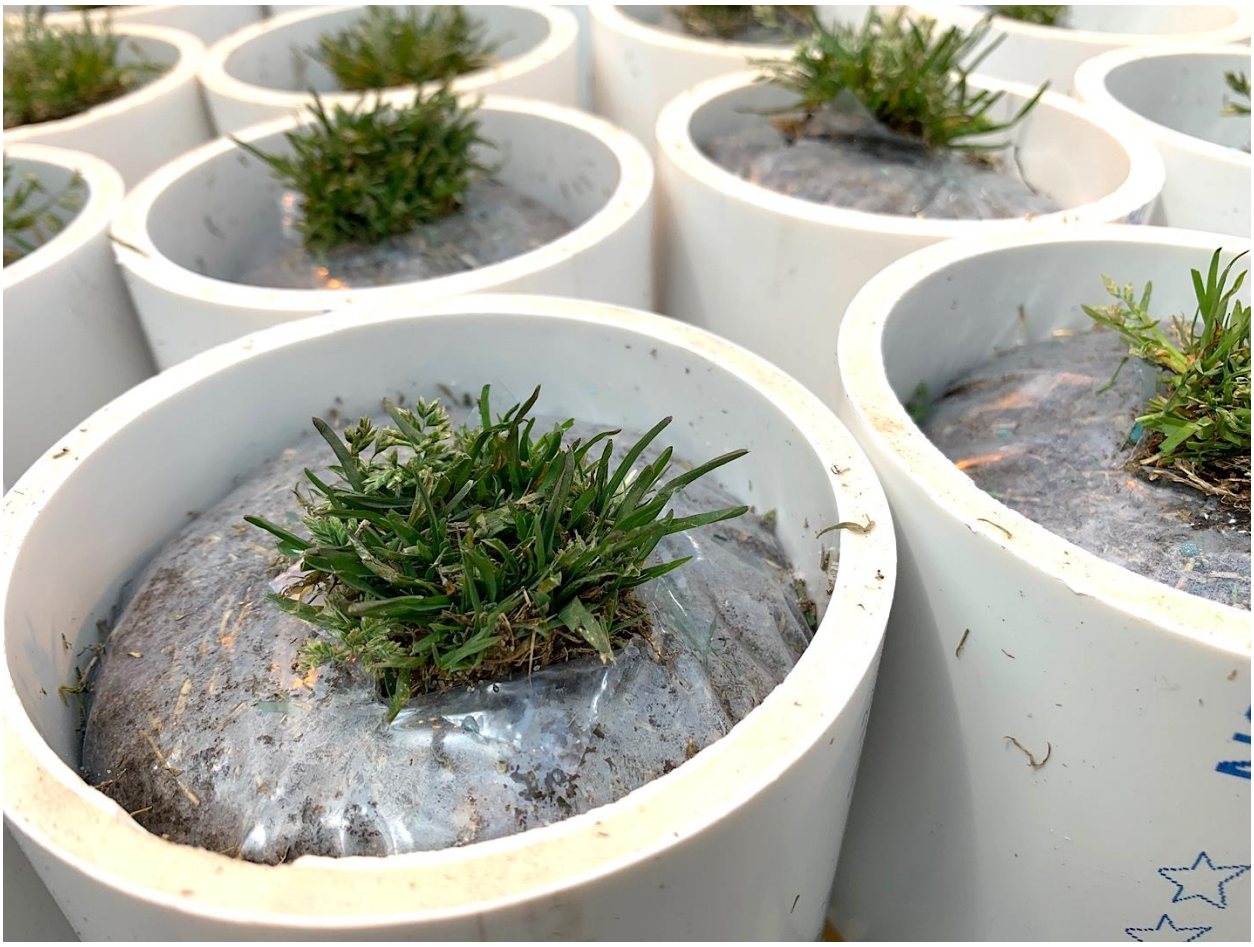


Figure 2.2. *Poa annua* plants harvested in soil cores (10.2 cm diameter) wrapped in plastic (0.15 mm) and installed in polyvinyl chloride pipes (10.2 cm diameter and length).

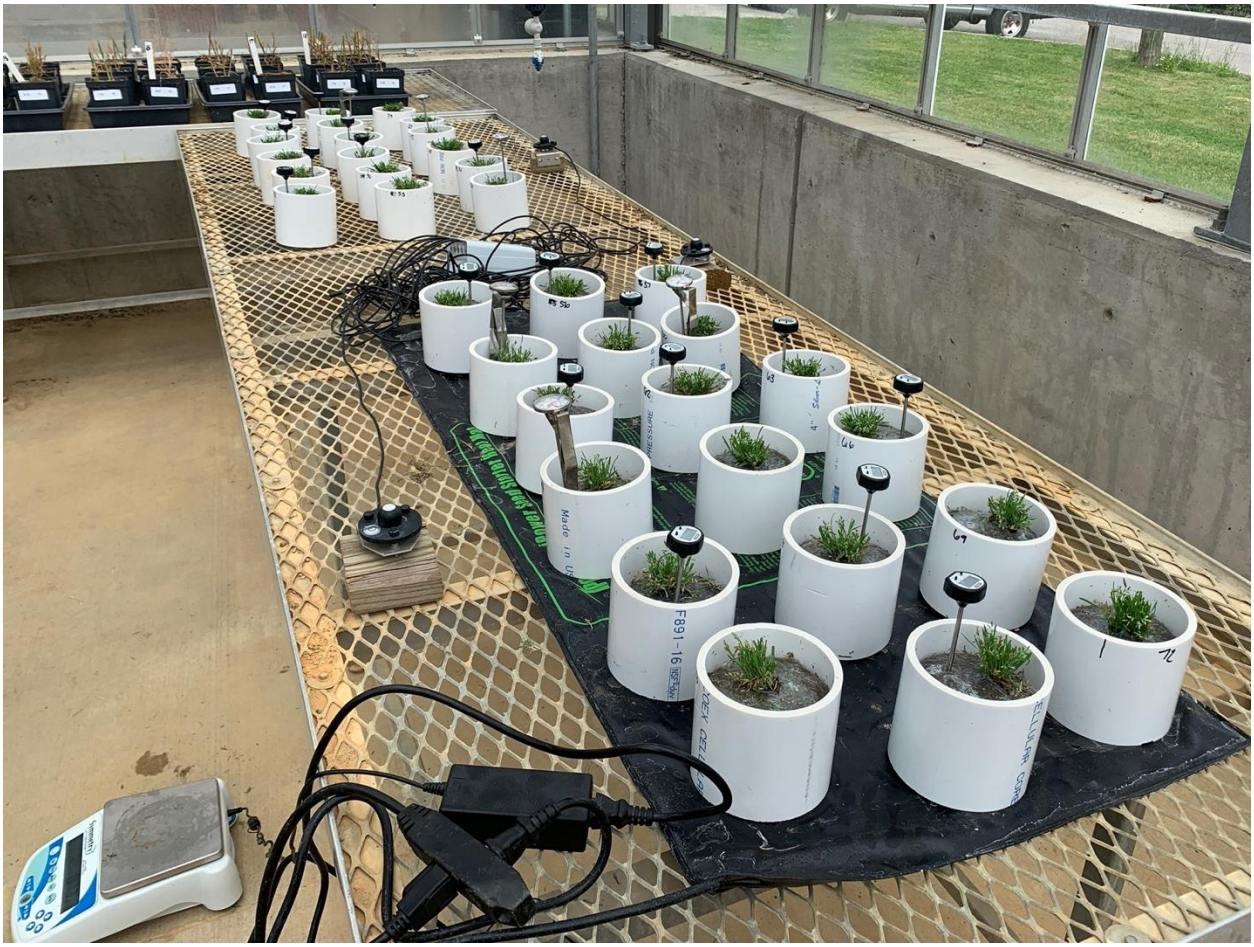


Figure 2.3. *Poa annua* plants harvested on 21 April 2021 in Knoxville, TN and wrapped in 0.15 mm polyethylene plastic to prevent soil water evaporation. Plants were subjected to either ambient or elevated soil temperature via a heating mat. Within each soil temperature treatment, water was either completely withheld or added to meet transpirational loss.

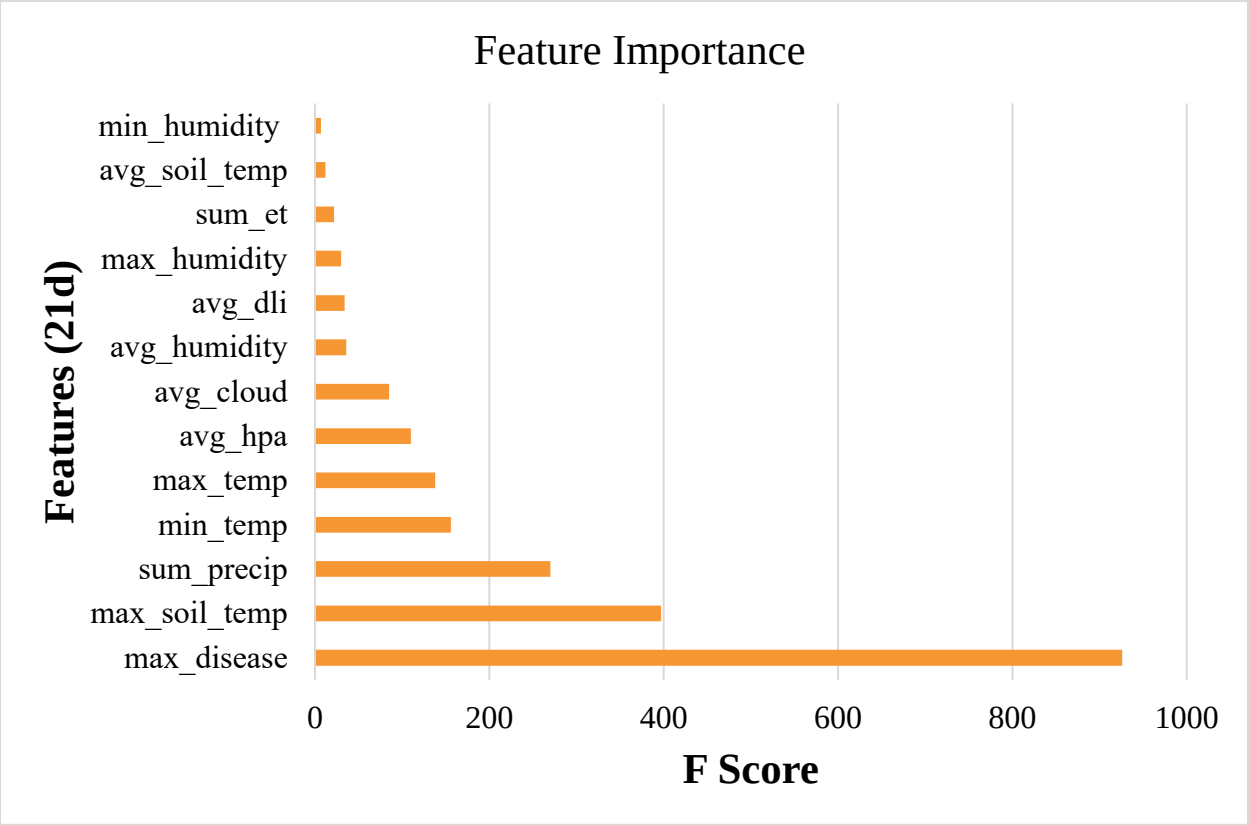


Figure 2.4. F-score analysis conducted in Python of climatic data related to death events of *Poa annua* monitored for length of life from May to October 2020 in ten micro-environments in Knoxville, TN.

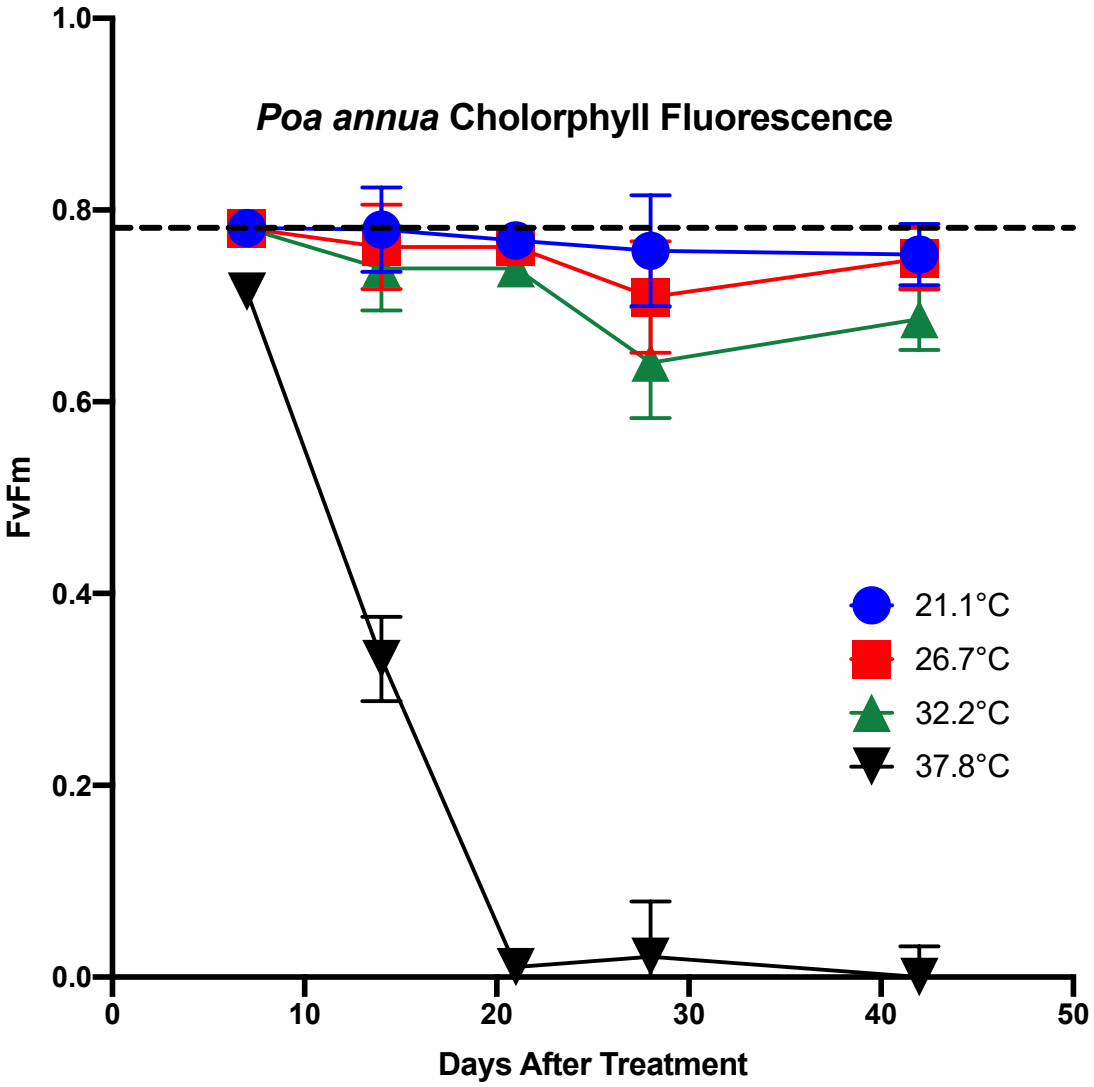


Figure 2.5. Photochemical efficiency was evaluated by measuring chlorophyll fluorescence (Fv/Fm) twice per *Poa annua* plant using a fluorometer. Measurements were taken once per week from plants subjected to soil temperatures of 21.1, 26.7, 32.2, or 37.8°C imposed via water baths. The dotted line indicates optimal chlorophyll fluorescence of 0.79.

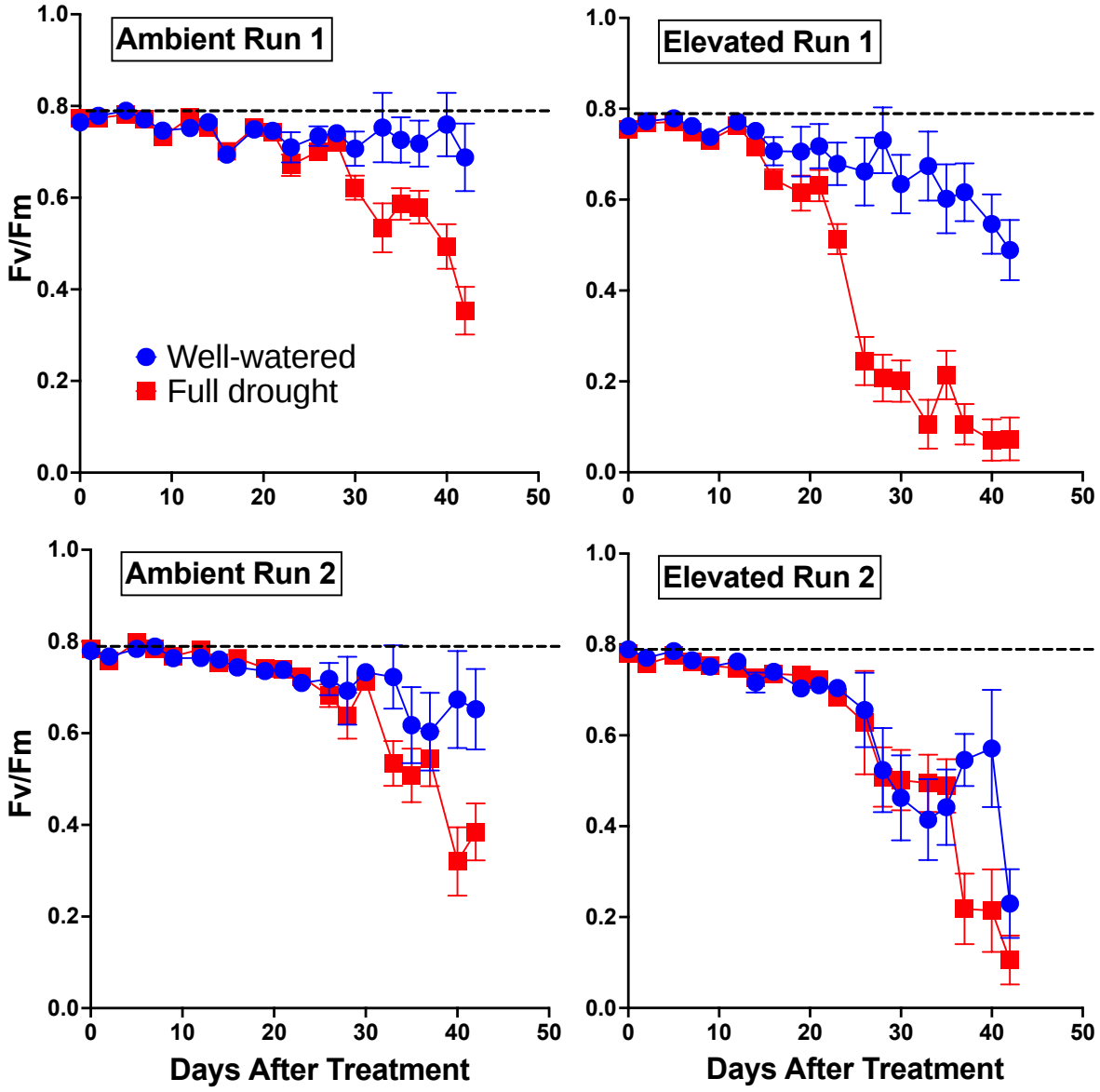


Figure 2.6. Photochemical efficiency (Fv/Fm) measured twice per *Poa annua* plant using a fluorometer. Three measurements were recorded per week from plants maintained at ambient or elevated soil temperatures and subjected to either well-watered or drought stressed conditions. The dotted line indicates optimal photochemical efficiency of 0.79.

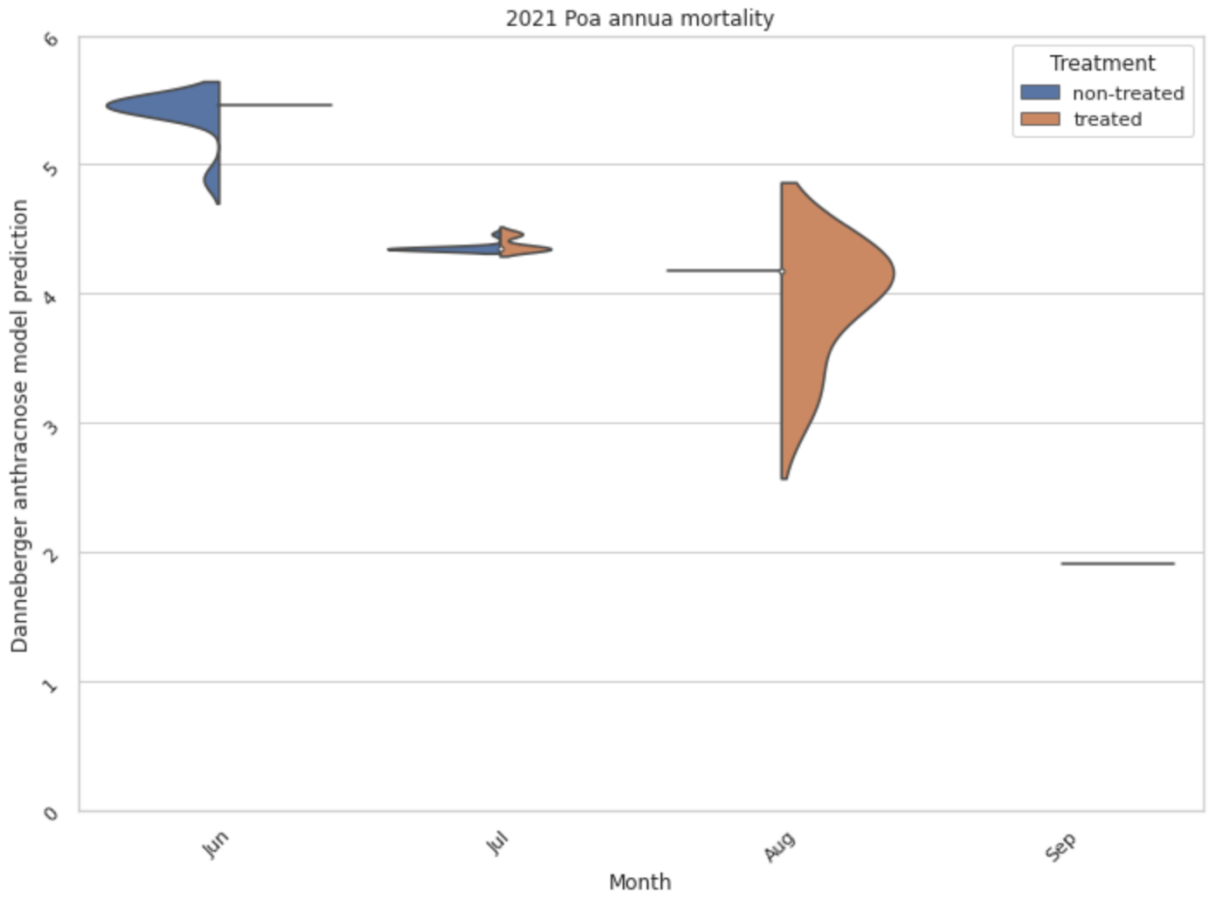


Figure 2.7. *Poa annua* mortality following either no fungicide treatment or fungicide treatment every 14 days from 27 May to 28 October 2021. The y-axis represents anthracnose severity index calculated using the Danneberger model (Danneberger et al., 1984b), where an anthracnose severity index of 2 is the minimum conditions for infection. The x-axis represents time, indicated by month of year. Within each month, *Poa annua* mortality is split by fungicide treatment with vertical space equating to the range of anthracnose severity across the month and horizontal space equating to the smoothed number of senescence observations corresponding to each treatment. The figure was created in Python using 'matplotlib' (v. 3.5.0; https://matplotlib.org/) and seaborn (v. 0.11.2).



Figure 2.8. *Poa annua* plants with inflorescences on 4 August 2021 following biweekly fungicide applications in a zoysiagrass (*Zoysia matrella* Merr. cv. 'Trinity') urban grassland located in Knoxville, TN. Without the presence of inflorescences, plants would likely be difficult to identify.

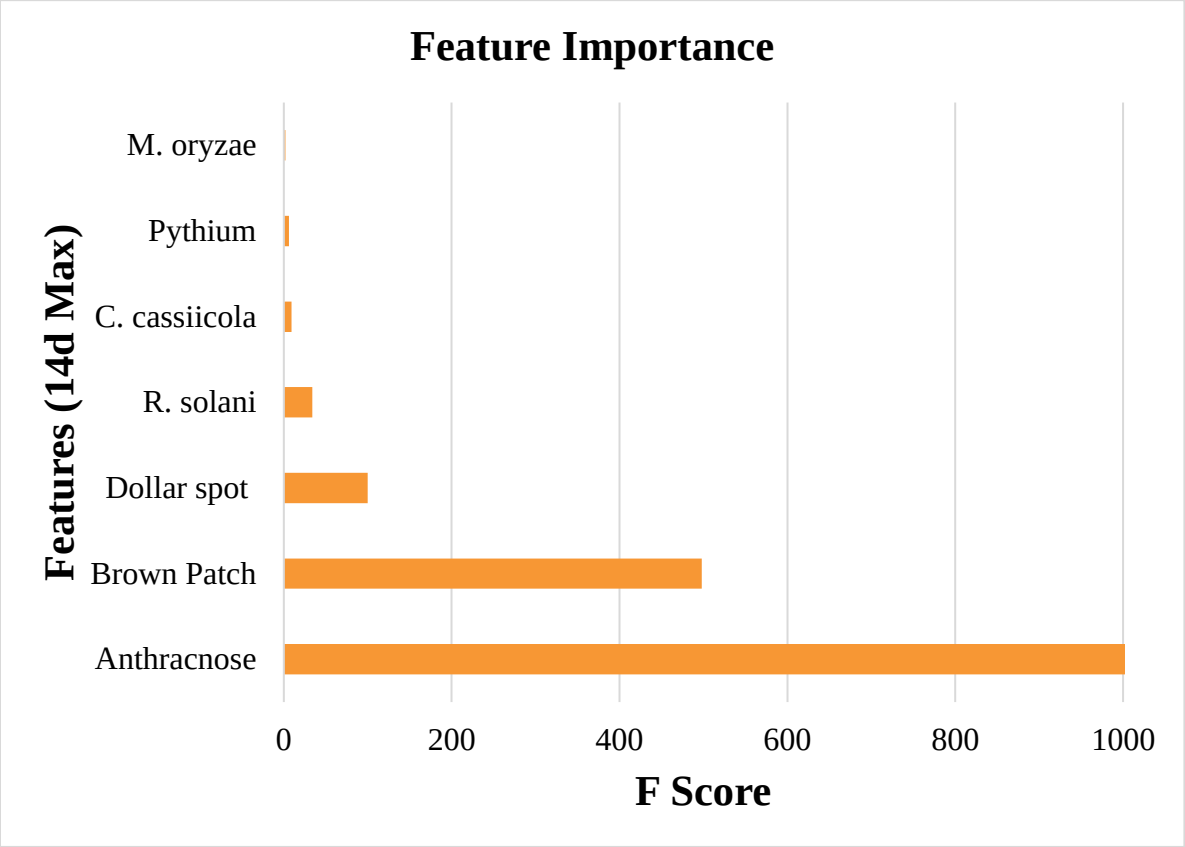


Figure 2.9. F-score analysis conducted in Python of climatic data related to death events of *Poa annua* plants monitored for length of life from May to October 2021 in a zoysiagrass (*Zoysia matrella* Merr., cv. 'Trinity') fairway. Plants were either left without treatment or treated with fungicide mixtures intended to control dollar spot (*Clarireedia* spp.), anthracnose (*Colletotrichum cereale*), Pythium blight (*Pythium* spp.), and brown patch (*Rhizoctonia solani*) every 14 days.

Supplementary Material

Table 2.S1. Fungicide applications made between May and October 2021 to control diseases common to *Poa annua*.

Application date	Fungicide trade name	Producing company (brand, city, state)	Active ingredient rate (ha^{-1})	FRAC† mode of action group number
27 May	Heritage Action	Syngenta, Research Triangle Park, NC	0.3 kg azoxystrobin	11
	Posterity	Syngenta, Research Triangle Park, NC	0.05 kg pydifluometofen	7
10 June	Maxtima	BASF Corporation, Research Triangle Park, NC	0.76 kg mefentrifluconazole	3
	Chipco Signature	Bayer Environmental Science, Cary, NC	9.8 kg aluminum tris	2
24 June	Daconil Ultrex	Syngenta, Research Triangle Park, NC	9.2 kg clorothalonil	5
	Segway	PBI Gordon Corporation, Shawnee, KS	0.72 kg cyazofamid	21
8 July	Insignia	BASF Corporation, Research Triangle Park, NC	0.71 kg pyraclostrobin	11
	Chipco Signature	Bayer Environmental Science, Cary, NC	9.8 kg aluminum tris	2
22 July	Ascernity	Syngenta, Research Triangle Park, NC	0.07 kg benzovindiflupyr + 0.25 kg difenoconazole	7
	Segway	PBI Gordon Corporation, Shawnee, KS	0.72 kg cyazofamid	21
4 Aug	Chipco Signature	Bayer Environmental Science, Cary, NC	9.8 kg aluminum tris	2
	Rayora	FMC, Philadelphia, PA	0.6 kg flutriafol	3
18 Aug	Heritage Action	Syngenta, Research Triangle, NC	0.3 kg azoxystrobin	11
	Daconil Ultrex	Syngenta, Research Triangle Park, NC	9.2 kg clorothalonil	5
	Segway	PBI Gordon Corporation, Shawnee, KS	0.72 kg cyazofamid	21
2 Sep	Maxtima	BASF Corporation, Research Triangle Park, NC	0.76 kg mefentrifluconazole	3
	Chipco Signature	Bayer Environmental Science, Cary, NC	9.8 kg aluminum tris	2
16 Sep	Insignia	BASF Corporation, Research Triangle Park, NC	0.71 kg pyraclostrobin	11
	Posterity	Syngenta, Research Triangle, NC	0.05 kg pydifluometofen	7
30 Sep	Maxtima	BASF Corporation, Research Triangle Park, NC	0.76 kg mefentrifluconazole	3
	Segway	PBI Gordon Corporation, Shawnee, KS	0.72 kg cyazofamid	21
13 Oct	Daconil Ultrex	Syngenta, Research Triangle Park, NC	9.2 kg clorothalonil	5
	Heritage Action	Syngenta, Research Triangle, NC	0.3 kg azoxystrobin	11

† Fungicide Resistance Action Committee (FRAC).

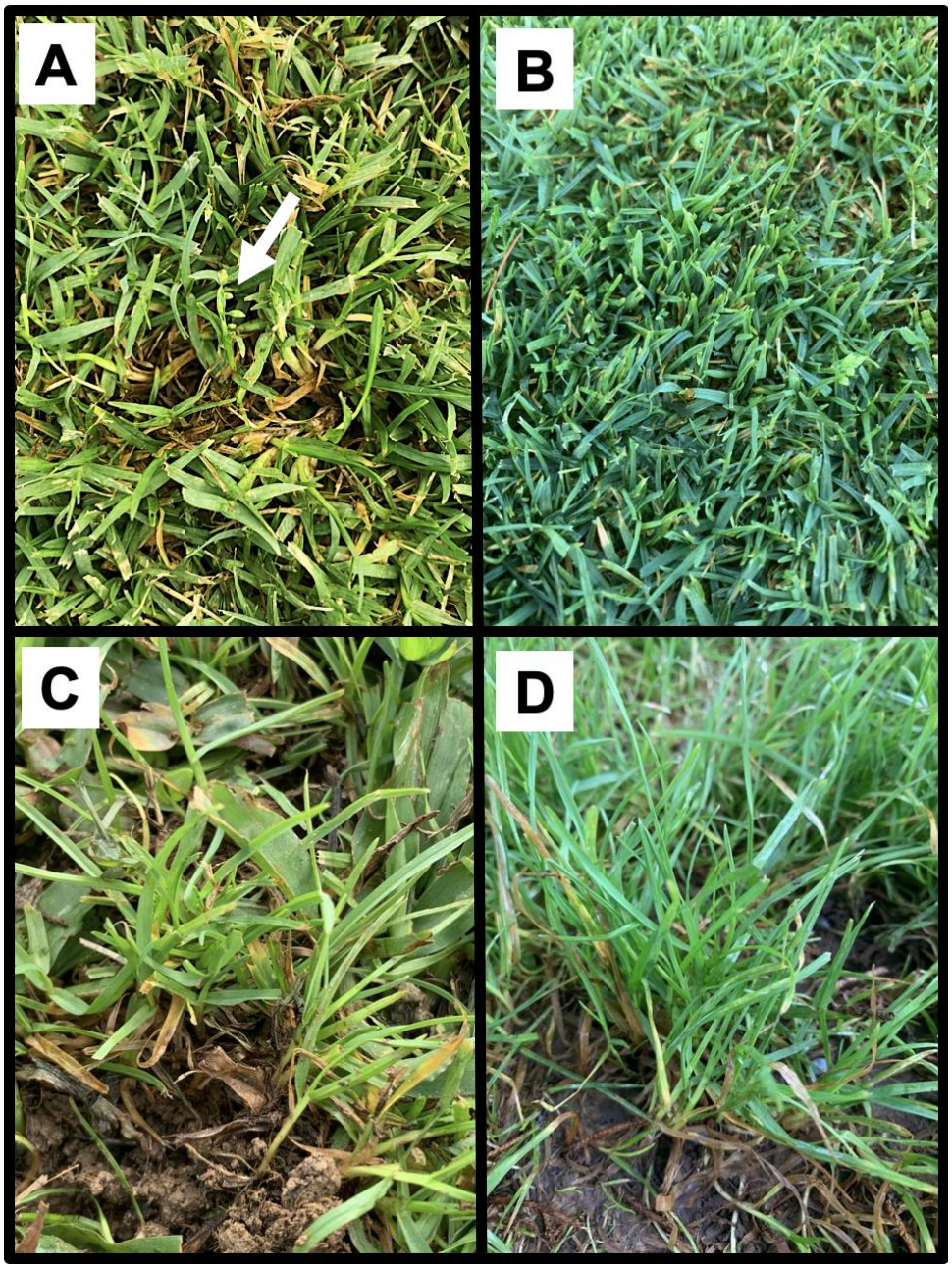


Figure 2.S1. ***Poa annua*** **L. plants of wide morphological variation in texture and growth habit observed to survive from 11 May to 27 October 2020 in Knoxville, TN. Images taken on 4 August 2020 where A = a fine texture, laterally growing plant with inflorescence in micro-environment two; B = a fine texture, laterally growing plant in micro-environment six; C = an upright growing, coarse textured plant in micro-environment seven; and D = an upright growing, coarse textured plant in micro-environment nine.**

CONCLUSION

An extensive review of literature including translation of original taxonomic documents and the findings of four greenhouse and field experiments indicate that *Poa annua* L. is of a perennial rather than annual life cycle.

A field monitoring study of 100 *P. annua* plants across ten unique micro-environments found that *P. annua* survived the summer period in four of ten environments, revealing that environmental factors allow *P. annua* to survive year-round or cause mortality not programmed senescence common of annual species. Analysis of environmental factors at the time of plant death for ecotypes that did not survive perennially indicated soil moisture, soil temperature, and pathogenic infection were associated with mortality. Greenhouse experiments assessing the effects of elevated soil temperature alone and in combination with limited soil moisture found that these factors were not lethal to *P. annua* except in extreme cases not common in the field. A field study assessed mortality from pathogenic infection and observed that *P. annua* plants treated with fungicide on a 14-d interval throughout the summer survived year-round, whereas plants not receiving fungicide applications senesced. Anthracnose basal rot (*Colletotrichum cereale*) was visually observed to be present at the time of *P. annua* mortality, aligning with peaks in anthracnose severity index calculated via published predictive models.

These findings support our hypothesis that *P. annua* plants persist via a perennial life cycle unless environmental conditions are unfavorable. This life cycle response aligns with that of other perennial, C_3 turfgrass species such as *Poa pratensis* L. (Kentucky

bluegrass) and *Festuca arundinacea* Schreber (tall fescue), which are subject to a confluence of environmental factors that can cause premature mortality.

Therefore, we contend the epithet “annua”, meaning annual in Latin, is a misnomer according to its current interpretation. Although “annual” can be associated with life cycle, the definition of the word is “occurring once every year”. Thus, the species epithet “annua” may have been awarded to *P. annua* to mark a yearly observation such as growth at the same location or yearly inflorescence production. Early taxonomic descriptions of *P. annua* provide no evidence of life cycle study at the time of naming, indicating the species name has been misconstrued in modern times. The shift in present understanding of *P. annua* as a perennial rather than the long purported annual species has major implications for plant management.

The conclusions of this research profoundly affect management of *P. annua* as both a problematic weed and desirable turfgrass species. *Poa annua* survival is likely increased in areas that are treated with fungicide or those with limited environmental stressors associated with pathogenic infection. Turfgrass managers should scout regularly in summer months to identify areas with conditions supporting *P. annua* survival, remembering that plants size will likely recede and may cause *P. annua* plants to be covered by the canopy of the desirable turfgrass species.

In situations where turfgrass managers aim to control *P. annua* and environmental conditions allow plants to survive year-over-year, changes in herbicide use must be made. Reliance on preemergence (PRE) herbicide chemistries alone will not result in control of *P. annua* if plants are pre-existing and not newly germinating.

Therefore, use of PRE and postemergence herbicides is necessary for control. Such herbicides mixtures are already recommended as a best management practice for herbicide resistance mitigation and their use better aligns with *P. annua* biology than PRE products alone.

Additionally, turfgrass managers should consider the influence of other management factors contributing to *P. annua* survival. For example, areas receiving fungicide application for summer disease are more likely to support *P. annua* than those not receiving fungicides. Therefore, fungicide overspray from putting greens into green surrounds may promote *P. annua* persistence and should be avoided. If *P. annua* is considered a weed, altering management practices such as limiting fungicide use and supplemental irrigation are warranted if possible. Conversely, if *P. annua* is the desired turfgrass species, turfgrass managers should limit abiotic plant stress via methods such as regular irrigation and traffic reduction while judiciously applying fungicides.

Communicating the findings of this work and conducting follow up experiments are necessary to furthering our knowledge of *P. annua* biology. Because the conclusion that *P. annua* is a perennial species challenges many years of alternative thinking, dissemination of this information and its supporting science on a broad scale is needed. Future work should address the prevalence of year-round *P. annua* survival across southern climates. A large-scale surveying study could be conducted in summer months to further identify environments supporting survival and communicate the wide-spread prevalence of *P. annua* surviving the summer. Additionally, future studies should further investigate the interface of *P. annua* in competition with C_4 turfgrasses and pathogenic

infection. Overcoming the introduction of such a profound change in turfgrass knowledge will require additional data to garner support from the turfgrass industry.

APPENDIX

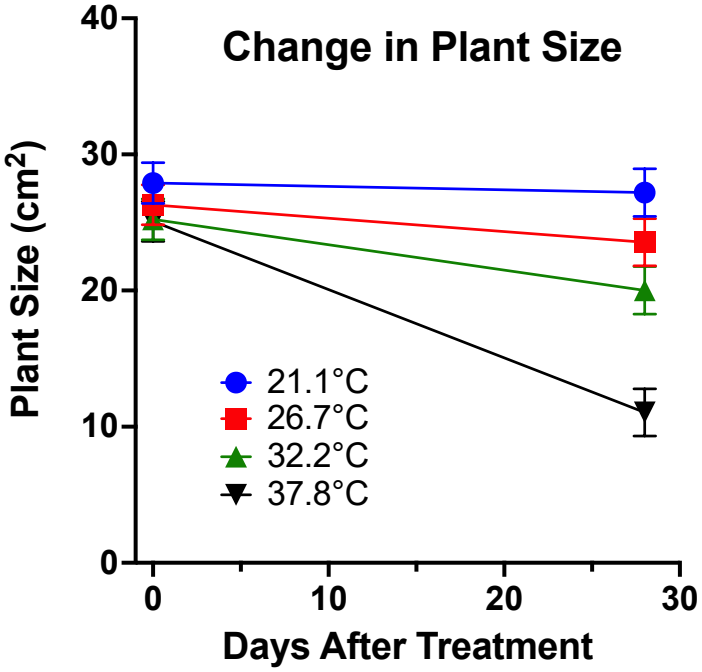


Figure A1. Change in *Poa annua* L. plant size after exposure to elevated soil temperatures of 21.1, 26.7, 32.2, or 37.8°C for four weeks. Plants were harvested from micro-environments in Knoxville, TN in February 2021. Plant size was determined by measuring perpendicular major and minor axes of each plant. These values were used to calculate the ellipse area of each plant using the formula $A = \Pi ab$ where A is area, a represents the major radius, and b represents the minor radius.

VITA

Devon Carroll was born on 24 June 1996 to Kenneth and Christine Carroll. She is originally from the town of Thornhurst, just outside of Scranton, Pennsylvania. Devon graduated from North Pocono High School in 2014 and went on to pursue a Bachelor of Science degree in Turfgrass Science from Penn State, which she completed in 2016. She chose to continue her graduate education at Penn State, earning a Master of Science in Agronomy focused in turfgrass in 2018 under the direction of Drs. John Kaminski and Pete Landschoot. In 2019, Devon began studying toward a Ph.D. in Plant, Soil, and Environmental Science at the University of Tennessee. Her degree program, advised by Dr. Jim Brosnan, was concentrated in turfgrass weed science. While at the University of Tennessee, Devon concurrently pursued a second Master of Science degree in Agricultural Leadership, Education and Communications under the mentorship of Dr. Carrie Stephens. Devon is grateful for the support and guidance provided by her faculty mentors and family throughout her education. She looks forward to serving the turfgrass industry in a research role after graduating.