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BIOLOGY AND CONTROL OF COMMON PURSLANE (*PORTULACA OLERACEA* L.)

by

Christopher A. Proctor

A DISSERTATION

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BIOLOGY AND CONTROL OF COMMON PURSLANE (*PORTULACA OLERACA* L.)

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University of Nebraska, 2013

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Common purslane (*Portulaca oleracea* L.) is a summer annual with wide geographic and environmental distribution. Purslane is typically regarded as a weed in North America, but it is consumed as a vegetable in many parts of the world. One of the characteristics that make purslane difficult to control as a weed is its ability to vegetatively reproduce. Severed sections of purslane stem containing a node will produce adventitious roots from the cut end of the stem. Isoxaben and simazine were the only two effective preemergence herbicides for controlling purslane in our studies when applied at maximum or one-half maximum label rates. Of the 25 postemergence herbicides evaluated, fluroxypyr, triclopyr, dicamba, and metsulfuron-methyl were the most effective for controlling purslane. Purslane's ability to adapt to a broad range of environmental conditions is due, in large part, to its unique photosynthetic metabolism. Under well-watered conditions, purslane utilizes C_4 photosynthetic metabolism. However, purslane will shift to a CAM-like photosynthetic metabolism under droughty conditions, with nocturnal acid accumulation in the leaves and reduced CO_2 uptake. Phosphoenolpyruvate carboxylase (PEPC) catalyzes the initial step in photosynthetic fixation of atmospheric CO_2 in C_4 and CAM plants. Regulation of PEPC is primarily via phosphorylation by PEPC kinase (PEPCK). For well-watered purslane, PEPC and PEPCK transcript abundance are indicative of C_4 metabolism, but in water-stressed purslane, PEPCK transcripts accumulate at night suggesting a shift in the phosphorylation pattern of PEPC to CAM-like metabolism. By understanding purslane's unique

photosynthetic metabolism, we can gain insight into how it effectively adapts to water limiting environments.

For Kara, Naomi, Ezra, Jack, and Isla

My biggest supporters. I could not have done this without you.

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INTRODUCTION

Common purslane (*Portulaca oleracea* L.), a member of the Portulacacae family, is one of 25 genera of succulent herbs and shrubs in this family (Mitich, 1997).The genus *Portulaca* includes about 40 species of tropical and warm climate species.

Origin and Distribution

The origin of *P. orlercea* has not been universally agreed upon. Different reports list it as native to South America (Rydberg, 1932), North Africa (Chapman et al, 1974; Holm et al. 1977), and western Asia and Europe (Mitich, 1997). Matthews et al. (1993) traces the history of purslane's origins and lists the Old World (India), North Africa, the Sahara, and even Australia as possible locations. Although purslane has been described as imported by post-Columbian immigrants (Vengris et al., 1972), seeds and pollen have been found in the sediment of Crawford Lake, Ontario, dating from AD 1350, and seeds from southern Louisiana, Illinois, and Kentucky date from 1000 BC to AD 750 (Kaplan, 1973; Walker, 1936; Watson, 1969). Typically the species is thought to be introduced into North America from the Old World. However, Gray and Trumbull (1883) suggest that purslane may have pre-Columbian roots since Columbus has an entry in his diary reporting purslane in Cuba. Purslane was also reported in Missouri and Colorado before settlers could have transported it there from the east (Matthews et al., 1993).Within North America, the spread of purslane is attributed, in part, to American Indians (Chapman et al., 1974). Nevertheless, purslane has been cultivated as a vegetable crop since at least the middle of the 17th century and was reported in Massachusetts as early as 1672 (Salisbury, 1961; Montgomery, 1964).

Purslane is widely distributed around the world and can be found from sea level to 3835 m above sea level (Mitich, 1997). It is most common in tropical and temperate environments, though it is reported from 58° north latitude to 40° south latitude (Matthews et al., 1993). In a taxonomic study, Gorske et al. (1979) classified 44 ecotypes of purslane from 18 different countries based on 36 plant characteristics. The plants were differentiated into 4 major groups: cool temperate; warm temperate to wet dry sub-tropic; humid sub-tropic to tropic; and cultivated. The cultivated group was made up of robust forms used for culinary purposes. The non-cultivated groups were prostrate, increasing in size and growth habit from the cool temperate regions to maximum size in the sub-tropic and tropic group.

Vegetative and Reproductive Characteristics

Portulaca oleracea has been described in detail by several authors (Holm et al., 1977; Matthews et al., 1993; Mitich, 1997; Rydberg and Howe, 1932). In general, purslane is described as a succulent annual with predominately prostrate growth, reproducing by stem fragments and seed. Stems are glabrous, reddish in color, and branch radially from the central axis forming a mat up to 60 cm in diameter. Leaves are alternate, subalternate, or opposite; glabrous; succulent; sessile; and broad-rounded at the tips. Roots consist of a long thick taproot as well as many fibrous lateral roots. Yellow flowers open on hot sunny mornings, can self-pollinate, and are not apomictic (Zimmerman, 1969). While insects play little to no role in pollination, 5% outcrossing has been observed most likely due to wind. Vivipary has not been reported. The fruit is a many-seeded capsule that splits open around the middle. Seeds are tuberculate with a white scar at one end, brownish-black and shiny in color, and measure 0.5 to 0.8 mm in diameter. Seeds are spread by wind, water, and with the seeds of crops. As many as 10,000 seeds have been counted on a single plant and it has been stated that over an entire season

purslane can produce 101,625 to 242,540 seeds per plant (Holm et al., 1977; Matthews et al., 1993; Zimmerman, 1976). Seeds develop on the main branches first, followed by development on secondary branches throughout the growing season (Egley, 1974). An average of 72 seeds per capsule has been observed (Dunn, 1970). Seed dormancy is highly variable and some viability has been reported in seeds up to 40 years old. Egley (1974) found that dormancy was highly related to seed age and water content, with less mature seeds germinating more readily than more mature seeds with lower water content. Germination was 96% when seeds were exposed to 35/25°C day/night temperatures, but 25/10 °C day/night temperatures resulted in 15% germination (Miyanishi, 1979). Light is also important in seed germination as seeds at 10 to 40°C under light conditions had relatively high germination compared to seeds kept in darkness at the same temperature (Singh, 1973).

In the tropics and warmer regions, a complete life cycle may be two to three and a half months, and up to four months in cooler regions. After being soaked, seeds begin to germinate in 12 hours and at high temperature (30 to 40°C), emergence is complete in 24 hours. Rapid vegetative growth begins at 15 days and flowering begins after one month or 10 to 12 leaf stage (Holm et al., 1977). Overall, Matthews (1993) summarized the characteristics of purslane as a weed of open, disturbed habitats and almost world-wide adaptability and distribution. Due to its rapid growth, it produces flowers and large numbers of seeds within six weeks of germination. It can tolerate an extensive range of photoperiod, light intensity, temperature, moisture, and soil types. Finally, the self-compatible breeding system, seed dormancy, and the ability of seeds to withstand animal digestive processes and overwintering all help to ensure its survival and distribution. Hopen (1972) found germination decreased with seeding depth, plant growth increased with nutrient level, particularly phosphorus, and high soil temperatures were required

for optimum germination. While the descriptions given previously are generally true, there is some variation reported in the literature. For example, purslane is generally considered to be an annual, but Matthews (1993) reports that it may be perennial in the tropics. Holm (1977) suggests that purslane can either have an upright or prostrate growth habit depending on light conditions

Purslane can also reproduce from vegetative cuttings left in contact with the soil (Holm et al. 1977; Miyanishi and Cavers 1980; Muenscher 1980). Severed stems form adventitious roots from the cut end of the stem (Connard and Zimmerman, 1931) and can regrow from stem or intact root segments following rototilling or hoeing (Miyanishi and Cavers, 1981). Adventitious roots have been shown to form only from the cut end of a stem and not from nodes (Vengris et al., 1972).

Purslane as a Crop

Purslane has been studied for its relatively high salinity tolerance, especially as it pertains to its use as a vegetable crop (Teixeira and Carvalho, 2009). Purslane is also known to be high in antioxidants and is a rich source of essential fatty acids (18:2ω6 and 18:3ω3) and carotenes, which have been shown to have human health benefits (Guil-Guerrero and Rodriguez-Garcia, 1999). Of edible wild plants tested, purslane has the highest amount of α-linolenic acid as well as high concentrations of the antioxidants α-tocopherol, β-carotene, and glutathione (Simopoulos et al., 1992). In addition, melatonin has been discovered in relatively high amounts in purslane leaves (Simopoulos et al., 2005). The benefits of melatonin in human health and cancer growth prevention have been documented (Allegra et al., 2003; Black et al., 2002, 2004; Cuzzocrea et al., 1999; Lopez-Burillo et al., 2003; Rodriguez et al., 2004; Sauer et al., 2001; Simopoulos, 1999; Tan et al., 1993). The function of melatonin in plants is less well

understood. In addition to its antioxidative properties, it is thought to perform roles in diurnal regulation as a plant hormone-like molecule (Kolar and Machackova, 2005).

Purslane as a Weed

Purslane is an aggressive weed and is considered a major problem in many crops throughout the world. Coquillant (1951) is frequently referenced for reporting purslane as the eighth most common plant on earth (Holm et al., 1977; Matthews et al., 1993; Zimmerman, 1976). Singh and Singh (1967) cite purslane as one of the ten most noxious weeds in the Upper Gangetic Plain of India and it is listed as a noxious weed by the state of Arizona (USDA, 2010). Holm et al. (1977) reports that purslane is a weed of 45 crops in 81 different countries. To determine the "weediness" of purslane, Zimmerman (1976) compared it with two other species of the *Portulaca* genera. Part of purslane's characterization as a weed is due to the plant's quick response capability. Purslane thrives under a wide variety of photoperiods and capsule numbers are positively correlated with the amount of light received. Purslane can tolerate a wide range of light intensity, temperature regimes, soil types, and produces capsules over a broad range of these factors. When these factors occur at optimal levels, purslane rapidly produces large numbers of capsules (Zimmerman, 1976). Dunn (1970) studied the effects of light quality on purslane's growth and development, and found that increased light intensity and temperature stimulates growth, and yields are greatest under cool white light followed by green, blue, red, and yellow light respectively.

Non-pesticidal control

There have been several studies on non-pesticidal controls for purslane. Dhima et al. (2009) conducted a field study in northern Greece looking at mulch effects of seven annual and

three perennial aromatic plants as green manure on the emergence and growth of four weed species and maize. Green manure-treated plots reduced common purslane emergence by 12-59% compared to the green manure-free plots, while maize yield increased by 10-43%. The use of mustard (*Brassica* spp.) as a cover crop reduces purslane densities in both onion and lettuce crops (Bensen et al., 2009; Wang et al., 2008). In another study, purslane was grown in pots outdoors and significant control was achieved using 20% clove oil in spray volumes of 281 and 468 L ha^{-1} (Boyd and Brennan, 2006). Boyette et al. (2007) found conidial sprays made from an isolate of the fungus *Myrothecium verrucaria* provided 90-95% control of common purslane 7 d after treatment in tomato production. Juglone, aromatic organic compounds found in black walnut (*Juglans nigra*), resulted in 100% purslane seedling mortality or complete germination inhibition when applied at concentrations of 30% or greater (Shrestha, 2009). Some research has investigated the use of insects for biological control of purslane. The weevil *Ceutorhynchus portulacea* is natural enemy of purslane and could contribute to the reduction of purslane as a weed in field crops (Visalakshy, 2007). The purslane sawfly (*Schizocerella pilicornis*) has also been used as a biological control agent (Gorske and Hopen, 1978).

Light cultivation or hoeing has been effective in controlling purslane <3 wk old (Haar and Fennimore, 2003; Muenscher, 1980). Mature plants should be removed after uprooting to prevent severed stems from rooting or seed production continuing (Haar and Fennimore 2003; Holm et al. 1977; Miyanishi and Cavers 1980; Muenscher 1980). Flaming or glyphosate were effective in reducing rooting and seed production in uprooted purslane plants compared with untreated control (Haar and Fennimore, 2003).

Chemical Control

In addition to cultural controls, herbicides have been used to control purslane. Stacewicz-Sapuncakis et al. (1973) found that purslane was sensitive to dicamba, but the lethal dose rate depended on the age of the plant. Some dicamba was exuded by the roots; however, there was no sign of dicamba degradation by purslane 8 d following treatment. In a study using mesotrione for post-emergent weed control in maize (*Zea mays* L.), Pannacci and Covarelli (2009) found it was ineffective against purslane even at maximum labeled dose (0.15 kg a.i. ha^{-1}). In a greenhouse study, bentazon 0.6 kg ai ha^{-1}, paraquat 0.14 kg ai ha^{-1}, and bentazon 0.6 kg ai ha^{-1} plus paraquat 0.14 kg ai ha^{-1} were effective in controlling purslane 10 days after treatment (Bellinder et al., 1997). Preemergence (PRE) herbicides diethatyl 4.5 kg ha^{-1}, diphenamid 4 kg ha^{-1}, and diethatyl plus diphenamid all resulted in significantly lower purslane density 4 weeks after planting of *Capsicum annuum* under plastic mulch (Cavero et al., 1996). Imazethapyr applied both PRE and postemergence (POST) at 0.067 kg ai ha^{-1} in lettuce provided >80% PRE control and >85% POST control of purslane (Dusky and Stall, 1996). Both nitrofen and oxyfluorfen have been shown to increase membrane permeability resulting in stomatal closure, membrane disruption, ethylene synthesis, and ultimately leaf abscission in purslane (Gorske and Hopen, 1978). The soil fumigant methyl iodide was shown to be as effective as methyl bromide in reducing purslane seed germination rates in a laboratory study for the two highest rates tested (Ohr et al., 1996). An evaluation of PRE herbicides for weed control in pumpkin found that 21 days after treatment, sulfentrazone 0.28 kg ha^{-1}, dimethenamid 2.24 kg ha^{-1}, and clomazon + sulfentrazone 0.37 + 0.28 kg ha^{-1} resulted in 74-94%, 81-100%, and 82-98% control, respectively (Brown and Masiunas, 2002). The use of bensulide and pronamide PRE reduced purslane density in lettuce and wide banding was more effective than narrow banding of the

herbicides (Haar and Fennimore, 2003). Preemergence control of purslane in leafy greens with S-metolachlor 0.45 and 0.67 kg ha^{-1}, and pendimethalin 0.43 and 0.86 kg ha^{-1} resulted in >84% control while bensulide 2.24 kg ha^{-1} and dimethenamid 0.31 and 0.63 kg ha^{-1} resulted in <78% control. All POST treatments with phenmedipham and clopyralid resulted in <45% control of purslane at harvest of leafy greens (Norsworthy and Smith, 2005). Finally, resistance to linuron and atrazine has been noted in some bio-types of purslane (Masabni and Zandstra, 1999).

Control of purslane with herbicides is difficult and inconsistent. Leaf morphology, epicuticular wax in particular, can influence the effectiveness of herbicide control measures. The leaf surface of purslane has no glands or trichomes and has relatively few stomata (Sanyal et al., 2006). Purslane has more epicuticular wax than velvetleaf (*Abutilon theophrasti* Medik), but less than common lambsquarter (*Chenopodium album* L.). In a lab test, the spread of a primisulfuron droplet was greatest when combined with an organosilicone wetting agent compared to a nonionic surfactant. An inverse relationship between the amount of epicuticular wax and the spread of the spray droplet was also noted with more wax resulting in decreased droplet spread (Sanyal et al., 2006). Falk et al. (1994) suggested that component differences found within leaf epicuticular waxes of diverse weed species may account for some of the variation seen between weed species and surfactant type on leaf tissue damage.

Purslane physiology

Most plants can be classified based on their photosynthetic pathway as C_3, C_4, or Crassulacean acid metabolism (CAM) (Ehleringer and Monson, 1993). In C_4 plants, mesophyll and bundle sheath cells spatially separate the C_3 and C_4 processes of the photosynthetic cycle, concentrating CO_2 around ribulose-1,5-bisphosphate carboxylase oxygenase (rubisco). CAM plants also concentrate CO_2 around rubisco, but the processes are separated temporally instead

of spatially. In order to conserve water, the stomates of CAM plants are closed during the day and open at night. When the stomates are open CAM plants fix CO_2 into C_4 acids and store them in the vacuole. These C_4 acids are then decarboxylated during the day when stomates are closed and the CO_2 is used by rubisco in the Calvin-Benson cycle. There is variation in CAM function between plants, ranging from obligate CAM plants to environmentally induced shifts from C_3 to CAM (Ting, 1985).

Few plants are known to possess both C_4 and CAM metabolism and all are in the *Portulaca* genus. These plants, namely, common purslane (*Portulaca oleracea* L.*),* rose moss *(Portulaca grandiflora* Hook*)*, and kiss me quick (*Portulaca mundula I.M. Johnst.*) exhibit CAM characteristics when subjected to drought-stress or short photoperiods (Koch and Kennedy, 1980, 1982; Ku et al., 1981; Guralnick and Jackson, 1993; Kraybill and Martin, 1996; Mazen, 1996, 2000; Lara et al., 2003, 2004). This shift from C_4 to CAM has primarily been characterized by malic acid fluctuations and gas exchange measurements. In addition to leaf acidity and gas exchange, differing phosph*enol*pyruvate (PEP) carboxylase (PEPC, EC 4.1.1.31) levels and activity have been reported in purslane in response to drought (Lara et al., 2003; Mazen, 1996, 2000). Lara et al. (2003) presented evidence with 2-D SDS PAGE, native IEF, and southern blots that indicate multiple gene isoforms of PEPC are present in purslane. This would suggest different PEPC genes are more active under C_4 or CAM-like metabolism.

Phospho*enol*pyruvate carboxylase (PEPC) is an enzyme that catalyzes the formation of oxaloacetate (OAA) from phospho*enol*pyruvate (PEP) and HCO_3^- as the first step in C_4 and CAM photosynthesis (Bowsher et al., 2008). Numerous PEPC isoforms have been found in various plant tissues (Brulfert and Queiroz, 1982; Brulfert et al., 1982a; Brulfert et al., 1982b; Gehrig, et al., 2001; Hofner et al., 1989; Müller and Kluge, 1983; von Willert et al., 1976). Some isoforms

perform anaplerotic functions in the leaves and non-photosynthetic tissues and are referred to as “housekeeping” isoforms since they are not directly involved in the photosynthetic pathway. These anaplerotic functions include the replenishment of tricarboxylic acid cycle intermediates, NADPH regeneration, and recapture of respired CO_2 (Andreo et al., 1987). PEPC also has been found to have specialized functions in stomatal guard cells (Kopka el al., 1997), in root nodules of legumes (Hata et al., 1997; Pathirana et al., 1997), in the development and germination of seeds (Golombek et al., 1999), lengthening of cotton fibers (Smart et al., 1998), and in the ripening of fruit (Guillet et al., 2002). Photosynthetic isoforms of PEPC exist in the leaves of C_4 and CAM plants and are noted by their elevated mRNA and protein expression in leaf tissue of C_4 (Cretin et al. 1991; Schaffner and Sheen 1992; Lepiniec et al. 1994; Rao et al. 2002) and CAM plants (Cushman and Bohnert 1999; Gehrig et al. 1995, 1998). Among CAM species, up to seven PEPC isoforms have been described for a given plant and CAM specific isoforms have been described in four species (Cushman et al, 1989; Gehrig et al., 1995, 2001, 2005). Phospho*enol*pyruvate carboxylase is an allosteric enzyme, inhibited by L-malate and activated by glucose 6-phosphate (Chollet et al., 1996; Vidal and Chollet, 1997). Regulation of PEPC occurs at the transcriptional level and post-translational level through phosphorylation of a single highly conserved serine residue near the N-terminal end of the polypeptide (Jiao and Chollet, 1991; Jiao et al., 1991). When phosphorylated, PEPC is less sensitive to inhibition by malate and more sensitive to activation by glucose 6-phosphate (Lepiniec et al., 1994; Ting, 1985; Jiao and Chollet 1991). The calcium insensitive Ser/Thr kinase, PEPC kinase (PEPCK) primarily controls the phosphorylation state of PEPC and is highly regulated at the transcriptional level (Hartwell et al, 1996). The C_4 PEPCK is up-regulated during the day and down-regulated at night (Li and Chollet 1993), whereas the CAM PEPCK is controlled by circadian rhythm with increased transcript abundance at night (Li and Chollet, 1994). The down regulation and dephosphorylation of PEPC

is catalyzed by a constitutive mammalian type protein phosphatase 2A and is not regulated by the same mechanisms that control PEPCK activity (Carter et al., 1990, 1991).

Purslane is a unique plant that has the ability to adapt to many diverse environments. It is a problematic weed in turfgrass and other crops, but is also grown as food crop. Furthermore, a better understanding of purslane's unique C_4-CAM physiology could allow harnessing this trait for crop improvement. Therefore, the objectives of my study were to 1) identify effective and reliable methods for vegetatively reproducing common purslane and to determine the survival and new leaf growth potential of cuttings from various plant tissues under controlled greenhouse conditions, 2) conduct herbicide screens to determine the efficacy of PRE or POST herbicides labeled for turfgrass to control purslane, and 3) identify shifts to CAM-like photosynthetic metabolism by measuring the transcript abundance of PEPC and PEPCK.

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CHAPTER 1

VEGETATIVE REPRODUCTION POTENTIAL OF COMMON PURSLANE (*PORTULACA OLERACEA*)

Proctor, C.A., R. Gaussion, and Z. Reicher. 2011. Vegetative reproduction potential of common purslane (*Portulaca oleracea*). Weed Technol. 25:694—697.

Abstract

Common purslane is a widely distributed summer annual weed. It can vegetatively reproduce from stem cuttings by forming adventitious roots from the cut end of the stem. Apart from large stem cuttings, it is unclear if purslane cuttings of various plant tissues differ in their ability to asexually reproduce. The objective of the study was to determine the survival and asexual reproductive capacity of purslane cuttings. A greenhouse study evaluated three cuttings from two stem locations and a leaf from one stem location for their survival and new leaf growth after 21 days. Cuttings included a stem node with either leaves attached or removed, stem internode, all from proximal and distal stem locations relative to the root crown and a leaf from a proximal stem node. Stem node cuttings had > 78% survival whereas internodes had 0% survival. Nodes with leaves attached further increased survival by > 20%. The location of the cutting on the main stem did not affect survival. Only noded cuttings produced new leaves, and cuttings with leaves attached produced the most new leaves. For purslane to vegetatively reproduce, nodes on stem cuttings are required and the presence of leaves on the cutting improves the survival and new leaf growth of cuttings. Therefore, mechanical methods of weed control that chop and spread purslane leaves and stems may not be effective means of control and could ultimately increase weed populations.

Introduction

Common purslane (*Portulaca oleracea* L.) (purslane) is a succulent summer annual weed. The origin of purslane is uncertain but has been reported as native to South America (Rydberg, 1932), North Africa (Holm et al., 1977), Western Asia, and Europe (Mitich, 1997). Although purslane was thought to have been imported by post-Columbian immigrants, (Vengris et al., 1972) seeds and pollen have been found in the sediment of Crawford Lake, Ontario from 1350 A.D., and seeds from southern Louisiana, Illinois, and Kentucky dating from 1000 B.C. to 750 A.D. (Kaplan, 1973; Walker, 1936; Watson, 1969).Within North America the spread of purslane is attributed in part to American Indians (Chapman et al., 1974).

Purslane has a prostrate growth habit, a thick taproot, and abundant fibrous secondary roots. Leaves are alternate, often clustered around the branch tip, succulent, with smooth margins; stems are glabrous, succulent, often reddish with primary and secondary branching, forming a mat up to 60 cm in diameter. Stems and leaves both contain a watery sap. Purslane produces small yellow-petaled flowers which only open on sunny mornings producing spherical many seeded capsules that open around the middle with the top coming off like a lid (Mitich, 1997; Miyanishi and Cavers, 1980). Purslane produces rapid vegetative growth under long-day photoperiod, flowers within a month of germination, and seeds ripen within two weeks of flowering (Holm, 1977; Miyanishi and Cavers, 1980). A single purslane plant can produce over 100,000 seeds in a growing season (Holm, 1977; Matthews, 1993; Zimmerman, 1976). Purslane will continue flowering under favorable conditions until the first killing frost in fall (Vengris et al., 1972) and plants growing in the tropics will senesce naturally after 3 to 4 months (Singh, 1973). Optimal seed germination occurs at air temperatures > 30°C and poor germination at

temperatures < 24°C (Miyanishi and Cavers, 1980; Zimmerman, 1976). Purslane is highly adaptive, growing in temperate and tropical environments from 58° north latitude in Alberta to 40° south latitude (Matthews et al., 1993) and has been found on 6 continents and in 81 different countries (Holm et al., 1977). Gorske et al. (1979) classified 44 ecotypes of purslane from 18 different countries based on 36 plant characteristics, as a result the plants were differentiated into 4 major groups: cool temperate, warm temperate to wet dry sub-tropic, humid sub-tropic to tropic, and cultivated. Purslane is a unique C_4 plant which is able to induce Crassulacean acid metabolism (CAM) photosynthesis under drought stress conditions (Koch and Kennedy, 1980; Lara et al., 2003). Historically, purslane has been used medicinally, as a blue dye, cultivated as a vegetable or potherb, and more recently fed as fodder for hogs (Mitich, 1997). As a weed, purslane is commonly found in bare soil areas along sidewalks, planting beds, marginal lands, or recently tilled soil for agricultural and horticultural crop production or turfgrass establishment (Holm et al., 1977; Hopen, 1972; Zimmerman, 1976).

In addition to reproducing by seed, purslane will reproduce vegetatively from cut stems left in contact with the soil (Holm et al., 1977; Miyanishi and Cavers, 1980; Muenscher, 1980). Control of purslane using hoeing or light cultivation has been effective for plants less than 3 weeks old (Haar and Fennimore, 2003; Muenscher, 1980). It is recommended that mature plants are removed after uprooting to prevent stems from rooting or seed production from occurring (Haar and Fennimore, 2003; Holm et al., 1977; Miyanishi and Cavers, 1980; Muenscher, 1980) Severed purslane stems produce adventitious roots from the cut end of the stem (Connard and Zimmerman, 1931) and can re-grow from stem and intact root segments following hoeing or rototilling (Miyanishi and Cavers, 1981). Adventitious roots will only form from the cut end of the stem but not from nodes of a buried section of an attached stem (Vengris et al., 1972).

While whole purslane stems have been shown to vegetatively reproduce, it is unclear if cuttings of individual plant parts (e.g. node, internode, leaf) will reproduce similarly. This study was part of a larger project examining the biology and control of common purslane. The objectives were to identify effective and reliable methods for vegetatively reproducing common purslane and to determine the survival and new leaf growth potential of cuttings from various plant tissues under controlled greenhouse conditions.

Materials and Methods

A greenhouse study was conducted in 2010 at the University of Nebraska in Lincoln, NE. Vegetative plant material used in this study was grown from purslane seed purchased from England (Herbiseed, New Farm, Mire Lane, West End, Twyford, England, RG100NJ). The seed source was a non-cultivated variety, and showed no obvious phenotypic differences when compared to local wild-type plants. To produce plant material for two runs of the study, seed was sown 26 May and 8 June in 6.35 cm square pots with approximately 5 to 10 seeds pot^{-1} and thinned to one plant pot^{-1} after emergence. Potting soil consisted of 35% peatmoss, 33% vermiculite, 9% soil and, 12% sand by volume with 2.3 mg cm^{-3} dolomitic lime and 9.8 mg cm^{-3} micronutrient fertilizer (Micromax micronutrients, The Scotts Company. Marysville, OH). Pots were placed on a greenhouse bench and misted for 10 s every 6 min from dawn to dusk. Purslane plants from the 8 June seeding were treated for aphids on 15 July with 0.01 g a.i. pot^{-1} imidacloprid (Marathon®, OHP Inc. Mainland, PA).

The study began on 25 June (run 1) and was repeated beginning 19 July (run 2). Plant cuttings were harvested from purslane plants with main stem length ranging from 7.5 to 20 cm

and 8 to 12 nodes. Since plants used to harvest cuttings were started from seed natural variation in size between plants existed. Based on results from a pilot study (Proctor and Reicher, 2011), cuttings from four vegetative tissues at two stem locations relative to the root crown were selected, plus seeded purslane was included as a control. The cuttings included internode from proximal (IN-P) and distal (IN-D) locations on the stem, node with leaves attached from proximal (NLA-P) and distal (NLA-D) locations on the stem, node with leaves removed from proximal (NLR-P) and distal (NLR-D) locations on the stem and a leaf from a proximal (L-P) node (Fig. 1.1). Two whole plants of similar size were used to collect cuttings so the same node location was used for both NLA and NLR. The proximal end of each cutting was inserted into the soil. The seeded control was sown, as described earlier, on the same day vegetative cuttings were planted. Identical pots, growing media, and irrigation were used as those to establish plant material. Pots were fertilized weekly with 20-20-20 N, P_2O_5, K_2O plus trace elements (Jack's Professional, J.R. Peters Inc., Allentown, PA) at a rate of 100 kg N ha^{-1} with a pressurized sprayer (RL Flo-Master, Lowell, MI). Air temperature, relative humidity, and photosynthetically active radiation (PAR) were measured in two locations at bench height every hour during the study with a data logger (Hobo data logger, model U12-012, Onset Computer Corp., Bourne, MA) (Table 1.1). After 21 d, data were collected to calculate the percent survival. For cuttings that survived, increase in leaf number (Δleaf) was also collected.

Survival data were binomial and defined by the presence of both roots and pigmented turgid plant tissue. Each run was arranged as a randomized complete block with 48 replications. Cuttings within a block were harvested from plants of the same size. Survival data were fit using a generalized linear model. Analysis of variance (ANOVA) was performed in SAS using PROC GLIMMIX and the logit link function (SAS, 2009). Treatment comparisons were made using

Fisher's LSD ($P \leq 0.05$). Treatment estimates for the binomial data were converted back to the scale of measure using the ilink function (SAS, 2009). The Δleaf data were normally distributed and analyzed separately from survival data. ANOVA was performed with PROC GLIMMIX and treatment comparisons made using Fisher's LSD. The run × treatment interaction was not significant for either survival or Δleaf, therefore data were pooled across runs.

Results and Discussion

Cuttings with the highest survival were NLA-P and NLA-D at 98 and 97%, respectively (Table 1.2). Comparatively, seeded plants resulted in 89% survival while L-P, IN-P, and IN-D resulted in ≤ 7% survival. Percent survival for seeded treatments was the percent that germinated and survived. The 7% of the L-P cuttings that survived were likely a result of the petiole being cut too close the stem and part of the node was removed along with the leaf. On the cuttings where this occurred, the node tissue resulted in the formation of adventitious roots and survival of the cutting. If the L-P cuttings had only consisted of leaf tissue we expect that survival would not have occurred as was the case for 93% of the L-P cuttings. For noded cuttings, presence of leaves increased survival by 21 to 27%. Location of the cutting on the main stem had no effect on percent survival in our study. However, Vengris et al. (1972) found that purslane stem cuttings from the distal end of the stem did not survive while mature cuttings from the proximal end of the stem did survive when rooting hormones were not used. Purslane has previously been shown to survive if severed by hand pulling, hoeing, or cultivation and left in contact with the soil (Miyanishi and Cavers, 1981; Vengris et al., 1972). Furthermore, Connard and Zimmerman (1931) describe the formation of adventitious roots originating from the cut

end of purslane stem cuttings parallel to the main axis of the stem resulting in the survival of the cuttings. Our study agrees with their research that severed purslane stems form adventitious roots from the cut end of the stem. Additionally, we noted that the presence of a node on the stem was necessary for root formation and survival. Similarly, *Portulaca grandiflora* Hook. requires leaves on stem cuttings for the formation of adventitious roots, and new leaf growth increases with the number of original leaves on the cutting (Yamdagni and Sen, 1973).

In addition to survival, both noded cuttings with or without leaves attached had a higher Δleaf than the seeded plants. However, nodes with leaves attached had at least 2 times higher Δleaf than all other cuttings (Table 1.2). While leaves on the cutting were not required to form new leaves, a node was required since the IN-P, IN-D or L-P cuttings did not form new leaves (Table 1.2).

The growth of purslane has been shown to increase with nutrient levels, and soil phosphorus plays a significant role during establishment (Hopen, 1972; Miyanishi and Cavers, 1980). The fertilization during this study likely positively affected growth of surviving plants.

We confirmed that purslane is able to vegetatively reproduce from cuttings under greenhouse conditions in our study. Though naturally occurring ecotypes of purslane may respond slightly differently under field conditions, we found that vegetative reproduction from stem cuttings only occurs when nodes are present. We substantiated that control methods resulting in severing and distribution of mature stem cuttings (> 3 weeks old) such as mowing, or cultivation may not be an effective means of control when left in contact with the soil, particularly if nodes and leaves remain intact on the stem.

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Table 1.1. Greenhouse air temperature, relative humidity and PAR data for run 1 and run 2 of purslane vegetative reproduction study.

	Air Temperature[a]		Relative Humidity[b]	PAR[c]
	Maximum	Minimum		
	----------°C----------		%	W m^{-2}
Run 1	39.6	22.3	73.6	26.3
Run 2	39.9	21.4	69.6	27.9

[a]Mean daily maxiumum and minimum air temperature from 25 June to 16 July (run 1) and 19 July to 12 Aug. (run 2)
[b]Mean relative humidity from 25 June to 16 July (run 1) and 19 July to 12 Aug. (run 2)
[c]Mean photosynthetically active radiation (PAR) from 25 June to 16 July (run 1) and 19 July to 12 Aug. (run 2)

Table 1.2. Percent survival and change in leaf number of purslane when vegetatively reproduced from different plant cuttings or seed.

Treatments[a]	Stem Location	Leaf Attached	Node included	Survival[b]	Δleaf[c]
				%	# of leaves
IN-D	Distal	No	No	0 e[d]	0.1 d
IN-P	Proximal	No	No	0 e	0.0 d
NLA-D	Distal	Yes	Yes	97 a	41.3 a
NLA-P	Proximal	Yes	Yes	98 a	41.0 a
NLR-D	Distal	No	Yes	70 c	13.7 b
NLR-P	Proximal	No	Yes	77 c	16.4 b
L-P	Distal	Yes	No	7 d	-0.9 d
Seed	-	-	-	89 b	6.8 d

ANOVA

	----------*P*-value----------	
Treatment (T)	<0.0001	<0.0001
Run (R)[e]	0.9779	0.5770
T × R	0.1176	0.3192

[a]Internode at distal stem location (IN-D), internode at proximal stem location (IN-P), node with leaf attached at distal stem location (NLA-D), node with leaf attached at proximal stem location (NLA-P), node with leaf removed at distal stem location (NLR-D), node with leaf removed at proximal stem location (NLR-P), leaf from proximal node (L-P)

[b]Percentage of cuttings with adventitious roots and pigmented turgid tissue after 21 days.

[c]Increase in leaf number after 21 days.

[d]Means followed by the same letter are not significantly different ($P \leq 0.05$) using Fisher's LSD.

[e] The study was conducted twice beginning on 25 June and again on 19 July

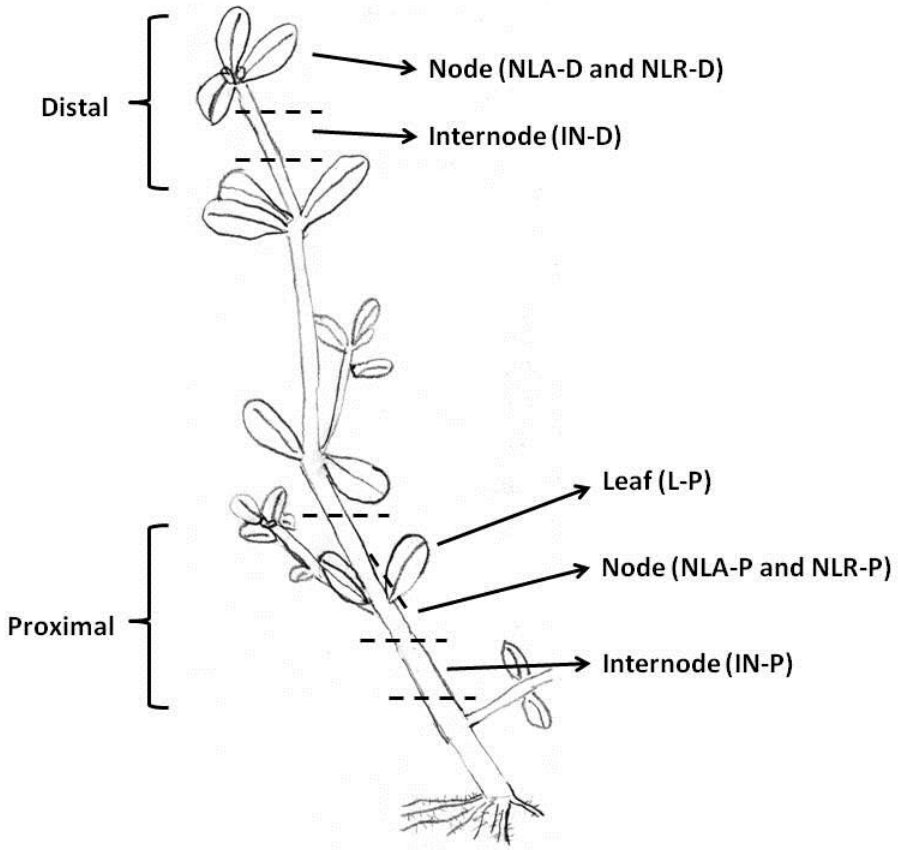

Figure 1.1. Location of cuttings on stem for purslane vegetative reproduction. Node leaf attached distal stem location (NLA-D), node leaf removed distal stem location (NLR-D), internode distal stem location (IN-D), leaf proximal stem location (L-P), node leaf attached proximal stem location (NLA-P), node leaf removed proximal stem location (NLR-P), and internode proximal stem location (IN-P).

CHAPTER 2

EFFICACY OF PREEMERGENCE AND POSTEMERGENCE HERBICIDES FOR CONTROLLING COMMON PURSLANE

Proctor, C.A. and Z. J. Reicher. Efficacy of preemergence and postemergence herbicides for controlling common purslane. HortSci. 48:902-905.

Abstract

Common purslane (*Portulaca oleracea* L.) can be problematic in thin turf, along sidewalks and drives, and especially during turfgrass establishment. Little published research exists evaluating herbicides that will control purslane and are also labeled for turfgrass. Thus our objective was to evaluate the efficacy of preemergence (PRE) or postemergence (POST) herbicides labeled for use in turf for controlling purslane. Experiments were conducted once in 2011 and twice in 2012 to evaluate nine PRE herbicides at one-half maximum and maximum label rates applied over immature perennial ryegrass (*Lolium perenne* L.). The PRE herbicides isoxaben and simazine consistently resulted in the best purslane control for all three PRE experiments. Experiments in 2011 and 2012 evaluated 25 POST herbicides at full label rates applied to mature purslane plants. The POST herbicides fluroxypyr, triclopyr, and metsulfuron-methyl were most effective in controlling purslane.

Introduction

Turfgrasses that establish quickly can resist weed colonization, and stands with high turf density may reduce weed competition (Busey, 2003). Conversely, stresses that thin turf and exposes the soil to sunlight could allow annual weed infestation (Busey, 2003). Common purslane (*Portulaca oleracea* L.) is a warm-season summer annual weed often found in thin turf, bare soil areas (Matthews et al., 1993), or during turfgrass establishment.

For spring establishment of turfgrass or in thin turf along sidewalks and drives, herbicide control of purslane may be necessary. Labels of many turfgrass herbicides list purslane as a weed species controlled, but limited published research is available on herbicide control of purslane in turf. Several studies in crops other than turfgrass have evaluated purslane control with herbicides, but often only report limited purslane data because it was not the primary target and/or because this weed appeared inconsistently during the studies. Of the research reported, few of the mentioned herbicides are labeled for use in turf while others are not labeled for use in the United States.

Among the products labeled for use in turf, Stacewicz-Sapuncakis et al. (1973) found that purslane was sensitive to the postemergence (POST) herbicide dicamba, but the lethal rate depended on plant age. Postemergence applications of clopyralid resulted in <45% control of purslane at harvest of leafy greens (Norsworthy and Smith, 2005), but clopyralid is no longer labeled for use on residential lawns. An evaluation of preemergence (PRE) herbicides for weed control in pumpkin (*Cucurbita* spp.), found sulfentrazone or dimethenamid resulted in ≥74% control of purslane 21 days after treatment (Brown and Masiunas, 2002). Bensulide or pronamide applied PRE, reduced purslane density in lettuce (*Lactaca sativa*) by 52 to 98% (Haar and Fennimore, 2003). Preemergence application to control purslane in leafy greens with

pendimethalin resulted in >84% control, while bensulide plus dimethenamid resulted in <78% control (Norsworthy and Smith, 2005).

Among herbicides for controlling purslane that are not labeled in turfgrass or not for use in the United States, POST herbicides nitrofen and oxyfluorfen increase membrane permeability resulting in stomatal closure, membrane disruption, ethylene synthesis, and ultimately leaf abscission in purslane (Gorske and Hopen, 1978). Doohan and Felix (2012) report between 11 and 95% purslane control in green onion with oxyfluorfen applied at three labeled rates. Postemergence treatments with phenmedipham resulted in <45% control of purslane at harvest of leafy greens (Norsworthy and Smith, 2005). The PRE herbicides diethatyl, diphenamid, diethatyl plus diphenamid or S-metolachlor reduced purslane in vegetable crops (Cavero et al., 1996; Norsworthy and Smith, 2005). Imazethapyr applied either PRE or POST in lettuce provided >80% PRE control and >85% POST control of purslane (Dusky and Stall, 1996). The soil fumigant methyl iodide was as effective as methyl bromide in reducing purslane seed germination rates in a laboratory study for the two highest rates tested (Ohr et al., 1996). Since many previously researched herbicides are not currently labeled for use in turf, the objective of our study was to conduct herbicide screens to determine the efficacy of PRE or POST herbicides labeled for turfgrass to control purslane.

Materials and Methods

Preemergence and POST herbicide studies were conducted in 2011 and 2012 at the University of Nebraska-Lincoln's John Seaton Anderson research facility near Mead, NE. Soil was a Tomek silt loam (Fine, smectitic, mesic Pachic Argiudolls) with pH 6.8 and 3.1% organic matter.

Experimental areas were tilled in July the year prior to herbicide treatment to encourage purslane establishment.

Preemergence experiments were conducted in 2011 (PRE2011) and twice in 2012 (PRE2012a and PRE2012b). Plot areas were seeded with 195 kg ha^{-1} of perennial ryegrass (*Lolium perenne* L.) in late September preceding herbicide application the following spring. The perennial ryegrass was killed with glyphosate at 3.1 kg a.i. ha^{-1} 7 (±3) d after experimental treatments were applied to limit competition and encourage purslane. Herbicide treatments for PRE2011 were applied April 20, 2011. Due to an unusually warm spring, herbicide treatments for PRE2012a were applied on 29 March at a similar growing degree day accumulation (300 ±50 GDD base 10°C) as PRE2011 and a third experiment, PRE2012b, was applied on 24 April 2012 (Fig. 2.1). For each experiment, nine PRE herbicides were applied once at maximum and half maximum label rates (Table 2.1) and watered in immediately following treatment with 2.5 mm irrigation. Herbicides were applied to plots measuring 1.5 by 1.5 m. using a CO_2 pressurized sprayer with three flat fan nozzles (LF8002, TeeJet Spraying Systems, Wheaton, IL), at 817 L ha^{-1} and 207 kPA. Purslane emergence was first noted on 2 June for PRE2011, 2 May for PRE2012a, and 14 May for PRE2012b. Percent purslane cover was visually estimated at 6, 8, and 10 weeks after treatment (WAT). Prior to analysis percent of the control was calculated as:

$$1 - (\text{treated percent cover} / \text{untreated percent cover}) * 100 \qquad \text{Eq. [2.1]}$$

for each of the rating periods. Plot areas were watered with 7.6 mm irrigation once every 2 wk. Chlorantraniliprole at 0.28 kg ha^{-1} was applied on 21 June 2011 and 22 May and 14 June 2012 to prevent white-line sphinx (*Hyles lineata)* infestation.

Postemergence experiments were conducted in 2011 (POST2011) and 2012 (POST2012). Plot areas were tilled in late April to control winter annuals or early germinating summer annuals and purslane was encouraged to naturally invade plot areas with light watering and

hand-weeding of non-purslane weed species. On 15 June 2011, and 4 June 2012, 25 different herbicides were applied at the high label rate to purslane plants with ≥ 10-cm main stem length (Table 2 and 3). Eight of the herbicides were applied both with and without the label-recommended surfactants, and the surfactants were also applied alone as standards. Herbicides were applied to plots measuring 1.5 by 1.5 m. using a CO_2 pressurized sprayer with three flat fan nozzles, at 817 L ha^{-1} and 207 kPA. The PRE herbicide isoxaben was applied at 1 kg ha^{-1} on 17 June 2011 and 5 June 2012 over all treatments to limit emergence of new purslane after POST application. Percent purslane cover was visually determined at 1, 2, 3, and 4 WAT. Percent of the control was calculated using Eq. [1] as described above. Plot areas were watered with 7.6 mm irrigation once every 2 wk. Chlorantraniliprole at 0.28 kg ha^{-1} was applied on 21 June 2011 and 1 June 2012 to prevent white-line sphinx infestation.

Treatments in all research trials were arranged as a randomized complete block with three replications. Preemergence study was a 9 x 3 x 2 factorial with nine herbicide treatments, three experimental runs, and two herbicide rates. The POST study had two factors, herbicide treatment and experimental run. Data were analyzed as a general linear model, which assumes homogeneous variance, however, variance for some factors such as year or experiment tends to be heterogeneous. Therefore, within each study (PRE or POST) variance for each experiment was modeled separately using PROC GLIMMIX in SAS (SAS, 2009) to account for heterogeneous variance and data for experiments were combined in one analysis (Littell et al., 2006). Mean separation was performed using Fisher's LSD at $P \leq 0.05$.

Results and Discussion

Preemergence Study. At 6 WAT, there were no difference in purslane control between treatments, and all treatments had a mean value of < 2% purslane cover (data not shown). For all remaining rating dates, there was a significant experiment by herbicide interaction, therefore data are presented by experiment (Table 2.1).

In PRE2011, isoxaben and simazine provided highest purslane control at 8 WAT (Table 2.1). On 18 June 2011, feeding from white-line sphinx caterpillars was found in PRE2011 and damage was inconsistent across the plot area. Ratings after 8 WAT for PRE2011 were confounded and thus not reported. Six of the nine or two of the nine herbicides provided best purslane control at 10 WAT in PRE2012a and 2012b, respectively. For experiments in 2012, isoxaben, simazine, and prodiamine were most effective in controlling purslane cover at both 8 and 10 WAT. Norsworth and Smith (2005) reported slightly better control (>84% control) with pendimethalin than was found in our experiments. Brown and Masiunas (2002) and Norsworth and Smith (2005) also noted poor control with dimethenamid as we found in our study. Although we attempted to synchronize our PRE herbicide applications between years by either GDD or calendar date, purslane cover for the control ranged from 5 to 53% when rated at 8 WAT across the three experiments. Discrepancy in purslane cover between the control plots was primarily due to environmental differences despite our effort to adjust for it. Natural variation exists in the amount and dormancy of purslane seed in the soil seed bank, which also could have affected the purslane cover (Egley, 1974). Regardless, we identified two herbicides, isoxaben and simazine, that were always in the statistical group that resulted in the best purslane control across all three PRE experiments (Table 2.1).

Postemergence study. Statistical analysis showed no difference in purslane control between herbicides applied with or without label-recommended surfactants, or between the isoxaben-treated or untreated controls, thus only treatments including the label-recommended surfactant and the isoxaben-treated control were included in the final analysis. Like the PRE experiments, there was a significant experiment by herbicide interaction for purslane control in the POST study, thus data are presented by experiment (Table 2.2 and 2.3). Though many treatments reduced purslane cover compared to the control during the two experiments, fluroxypyr and triclopyr were the only two herbicides that resulted in the highest control at each rating date in both experiments (Table 2.2 and 2.3). By 4 WAT in both experiments, fluroxypyr, metsulfuron-methyl, and triclopyr resulted in the best purslane control, although control from several other herbicides was statistically similar to these treatments in POST2011. Fluroxypyr at 0.3 kg a.i. ha^{-1} also provided 100% purslane control in sorghum (Love, 1993), whereas Durr (2012) documents > 60% purslane control with metsulfuron-methyl applied at 4.2 g ha^{-1}. Similar to our data, others note clopyralid and mesotrione applied POST result in poor purslane control (Norsworth and Smith, 2005; Pannacci and Covarelli, 2009).

There was some variation in herbicide performance among the POST experiments, which may be due to maturity of the plants at application. Stacewicz-Sapuncakis et al. (1973) found that efficacy of dicamba decreased with increased age and size of purslane. In our study, percent cover of purslane was 33-60% at the time of application in POST2011 compared to 88-95% cover in POST2012 (data not shown), as a result purslane plants were more mature when herbicides were applied in POST2012 compared to POST2011. This is despite that POST2012 herbicide applications were made earlier to account for earlier plant maturity in 2012 (Fig. 2.1). There were more herbicides in the statistically best-performing group at every rating date in POST2011 (Table 2) than in POST2012 (Table 3). This may be attributed to smaller and more

susceptible plants in POST 2011 compared to POST2012. In 2011, 11 of 25 herbicides were in the statistically best-performing group (≤15% cover) by 4 WAT compared to 95% cover in the control. However, in 2012, only three herbicides were in the best performing group (<17% cover) in 2012 at 4 WAT compared to 97% in the control. Only the most effective herbicides reduced purslane cover when applied to the more mature plants in POST2012, reinforcing the findings of Stacewicz-Sapuncakis et al. (1973) of herbicides becoming less effective when applied to more mature purslane.

The effect of purslane maturity at time of herbicide application is particularly important during turfgrass establishment where early herbicide applications could be more effective in controlling purslane, but applications often must be delayed to limit damage to turfgrass seedlings. The herbicide label for dicamba, fluroxypyr, and triclopyr all require two to three mowings (4 weeks or more depending on turfgrass species) before applying over newly-seeded cool-season turfgrass and metsulfuron-methyl requires one year before application to cool-season turf (Anonymous 2007, 2008, 2010a, 2010b). However, carfentrazone can be applied as early as seven days after emergence for most turfgrass and 14 days after emergence for zoysiagrass (*Zoysia spp.*)(Anonymous, 2009). Despite inconsistency from year to year in our study, carfentrazone resulted in 96 % purslane control 1 WAT for PRE 2011 and is one of the safest herbicide options at turfgrass establishment. If applied to young purslane or potentially in multiple applications, carfentrazone may be useful for situations where turfgrass seedling safety is a concern, to manage purslane until a more effective herbicide can be applied.

Results indicate fluroxypyr, triclopyr, and metsulfuron-methyl applied POST are most effective for controlling purslane in turfgrass stands. For extended purslane control, isoxaben or simazine applied PRE were shown to be effective and could be combined with any of the effective POST herbicides to ensure control of escaped purslane. Under the conditions tested,

not all herbicides were effective in controlling purslane and purslane maturity at time of application appears to influence the efficacy of many POST herbicides. Future work evaluating the effect of herbicide application timing, multiple herbicide applications, or use of combination products would be helpful in developing more complete control recommendations.

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Table 2.1. Percent purslane control by experiment following preemergence herbicide treatments.

Herbicide			Purslane control				
	½ maximum	Maximum	PRE201[a]	PRE2012a		PRE2012b	
Common Name	label rate	label rate	8 WAT[b]	8 WAT	10 WAT	8 WAT	10 WAT
	--------kg ai ha^{-1}--------		----------% ----------				
Dimethenamid	0.8	1.6	18 de[c]	37 b	29 b	46 cd	20 c
Dithiopyr	0.3	0.6	41 bcd	36 b	20 b	41 cd	10 c
Ethofumesate	1.7	3.4	61 b	89 a	80 a	32 d	13 c
Isoxaben	1.0	2.0	98 a	65 ab	62 ab	96 a	82 ab
Mesotrione	0.1	0.2	14 e	37 b	29 b	31 d	2 c
Pendimethalin	1.0	2.1	24 cde	55 ab	34 ab	63 bc	19 c
Prodiamine	0.8	1.6	45 bc	66 ab	63 ab	87 ab	63 b
Siduron	27.4	54.8	18 de	41 ab	40 ab	28 d	3 c
Simazine	1.4	2.8	99 a	57 ab	40 ab	98 a	92 a
			Cover				
			----------%----------				
Untreated[d]	-	-	53	5	14	28	48

[a] Herbicides for PRE2011, PRE2012a, and PRE2012b were applied on 20 April 2011, 29 March 2012, and 24 April 2011, respectively

[b] WAT weeks after treatment

[c] Means of 2 herbicide rates and 3 replications. Means with a column followed by the same letter are not significantly different according to Fisher's LSD at $P \leq 0.05$.

[d] Untreated means show percent purslane cover used to calculated purslane control.

Table 2.2. Percent purslane control in 2011 following postemergence herbicide treatments

Herbicide[a]		Purslane control							
Common name	Rate	1 WAT[b]		2 WAT		3 WAT		4 WAT	
	kg ai ha^{-1}	----------% ----------							
Fluroxypyr	0.31	98[c]	a	100	a	100	a	100	a
Metsulfuron methyl + NIS[d]	0.04	88	ab	100	a	100	a	100	a
Triclopyr	1.55	95	a	100	a	100	a	100	a
Dicamba	1.36	74	abcd	98	ab	99	a	99	a
Glyphosate	4.16	98	a	100	a	99	a	99	a
Glufosinate	1.12	98	a	100	a	100	a	98	a
MCPA	2.52	76	abcd	98	ab	97	ab	94	a
Flazasulfuron +NIS	0.06	92	a	99	ab	97	ab	93	a
Aminocyclopyrachlor	0.08	33	fg	67	defg	83	abcd	90	ab
Clopyralid	0.56	35	fg	62	fgh	76	cd	88	ab
Ethofumesate	0.86	75	abcd	98	ab	96	abc	85	ab
Carfentrazone	0.03	96	a	95	abc	86	abcd	74	bc
2,4-D	2.51	51	defg	80	bcdef	78	bcd	66	c
Formasulfuron +MSO[e]	1.05	59	bcdef	81	bcde	66	de	39	e
Diquat dibromide + NIS	0.06	87	ab	82	abcd	52	ef	40	e
Sulfentrazone	0.28	83	abc	78	cdef	51	ef	35	f
Sulfosulfuron +NIS	0.07	61	bcdef	75	defg	37	fg	29	fg
Dithiopyr	0.57	57	cdef	63	efgh	37	fg	22	fgh
Pyraflufen ethyl	0.01	81	abc	69	defg	30	fg	17	ghi
Simazine	2.24	46	efg	57	gh	17	ghi	10	hij
Fenoxaprop-P-ethyl	0.13	28	gh	16	ij	8	hi	5	hij
Penoxsulam	0.04	49	defg	45	h	24	gh	5	hij
Quinclorac + MSO	0.37	31	fg	23	i	8	hi	3	hij
MSMA +NIS	2.52	-3	hi	-3	j	1	i	0	ij
Mesotrione +NIS	0.28	-7	i	7	ij	2	i	-2	j
		Cover							
		----------%----------							
Untreated[f]	-	67		87		97		97	

[a] Herbicides were applied on 15 June 2011.Isoxaben was applied at 1 kg ha^{-1} over the top of all herbicide treatments 1 to 2 d after treatment.
[b] WAT weeks after treatment.
[c] Means with a column followed by the same letter are not significantly different according to Fisher's LSD at $P \leq 0.05$.
[d] NIS non-ionic surfactant.
[e] MSO methylated seed oil.
[f] Percent purslane cover of untreated means shown for reference only.

Table 2.3. Percent purslane control in 2012 following postemergence herbicide treatments

Herbicide[a]		Purslane control							
Common name	Rate	1 WAT[b]		2 WAT		3 WAT		4 WAT	
	kg ai ha^{-1}	----------% ----------							
Fluroxypyr	0.31	44[c]	a	79	a	99	a	100	a
Metsulfuron methyl + NIS[d]	0.04	-3	f	59	b	96	a	100	a
Triclopyr	1.55	40	ab	77	a	96	a	100	a
Dicamba	1.36	12	def	34	cd	49	b	83	b
Glyphosate	4.16	29	abc	45	c	81	a	79	b
MCPA	2.52	23	bcd	24	de	32	bc	38	c
Simazine	2.24	12	def	10	fgh	15	cdef	37	c
Penoxsulam	0.04	20	cde	8	fgh	4	def	21	d
Aminocyclopyrachlor	0.08	-1	f	1	gh	24	cd	14	de
2,4-D	0.56	13	de	14	efg	17	cdef	14	de
Clopyralid	2.51	-3	f	-1	h	3	ef	14	de
Sulfosulfuron +NIS	0.07	11	def	2	gh	3	ef	14	def
Glufosinate	1.12	32	abc	38	c	23	cde	9	def
Ethofumesate	0.86	3	f	15	ef	15	cdef	8	def
Formasulfuron +MSO[e]	0.06	7	e f	12	efgh	3	ef	5	def
Flazasulfuron +NIS	0.06	5	ef	10	fg h	2	f	3	ef
MSMA +NIS	0.03	11	def	5	fgh	1	f	3	ef
Carfentrazone	1.05	11	def	3	fgh	0	f	3	ef
Diquat dibromide + NIS	2.52	18	cde	-1	h	-1	f	3	ef
Quinclorac + MSO	0.37	-1	f	2	g h	-1	f	3	ef
Sulfentrazone	0.28	3	f	-3	h	0	f	0	ef
Pyraflufen ethyl	0.01	-1	f	0	h	-1	f	0	ef
Dithiopyr	0.57	-2	f	-1	h	-1	f	-2	ef
Mesotrione +NIS	0.28	-2	f	0	h	0	f	-2	ef
Fenoxaprop-P-ethyl	0.13	0	f	-1	h	-1	f	-4	f
		Cover							
		----------%----------							
Untreated[f]	-	92		97		98		94	

[a] Herbicides were applied 4 June 2012. Isoxaben was applied at 1 kg ha^{-1} over the top of all herbicide treatments 1 to 2 d after treatment.
[b] WAT weeks after treatment.
[c] Means with a column followed by the same letter are not significantly different according to Fisher's LSD at $P \leq 0.05$.
[d] NIS non-ionic surfactant.
[e] MSO methylated seed oil.
[f] Percent purslane cover of untreated means shown for reference only.

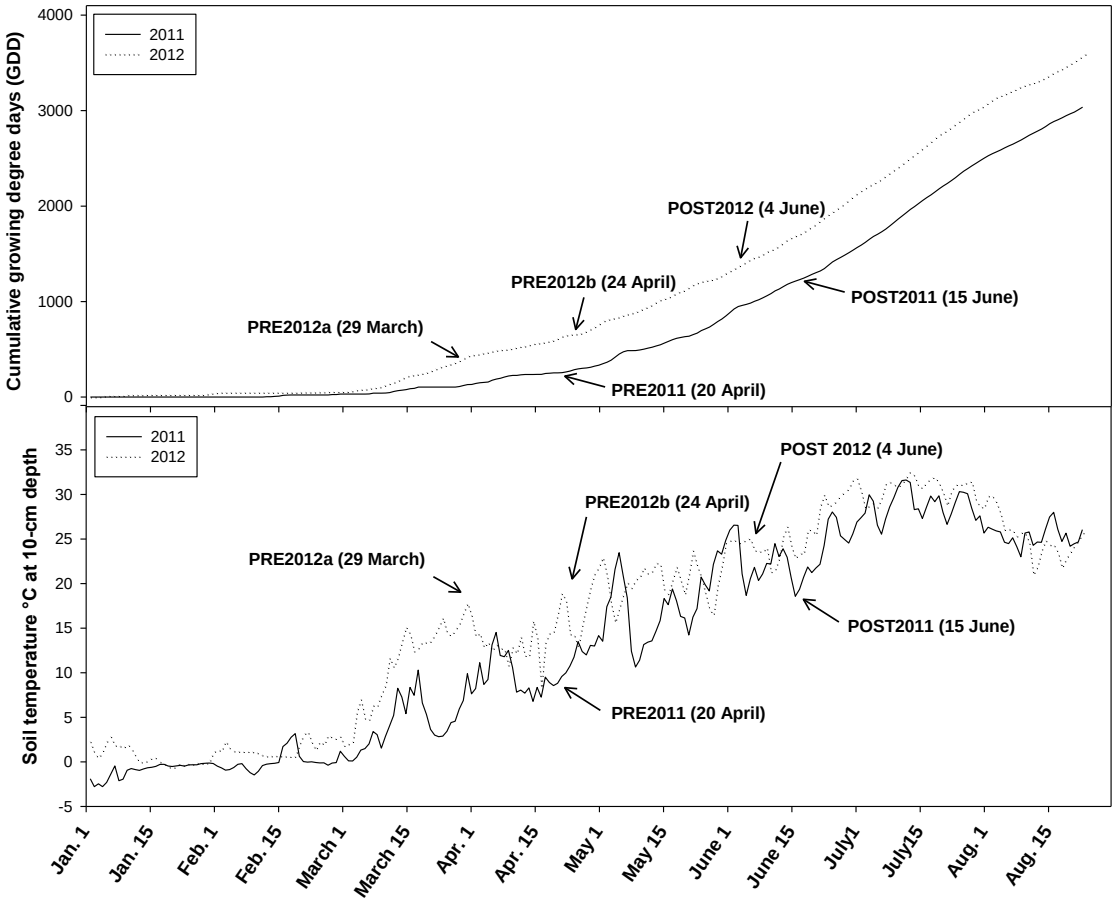

Figure 2.1. Cumulative growing degree days (GDD) using base 10°C model and soil temperatures recorded at 10-cm depth from 1 January to 31 August in 2011 and 2012 at the UNL John Seaton Anderson turfgrass research facility near Mead, NE. Herbicide application dates for the PRE2011, PRE2012a, PRE2012b, POST2011, and POST2012 studies are indicated.

CHAPTER 3

DROUGHT INDUCED CRASSULACEAN ACID METABOLISM IN COMMON PURSLANE

Abstract

Phospho*enol*pyruvate carboxylase (PEPC) catalyzes the photosynthetic fixation of CO_2 in C_4 and Crassulacean acid metabolism (CAM) plants. Phospho*enol*pyruvate carboxylase is activated by phosphorylation via phospho*enol*pyruvate carboxylase kinase (PEPCK), which is highly regulated at the transcript level. The study objective was to evaluate the induction of CAM-like metabolism from drought in common purslane by changes in transcript abundance for PEPC and PEPCK. Purslane plants were established from seed and grown in a greenhouse for six weeks before being divided into two groups, well-watered and water-stressed. Gas exchange results measured from water-stressed leaf samples were similar to previously reported values for CAM plants. After 1 d and 5 d following drought initiation, leaf samples were collected at 2:00 and 14:00 h. Titratable acidity measured from water-stressed leaf samples were similar to previously reported values for CAM plants. Reverse transcription-PCR was performed to evaluate transcript abundance of PEPC and PEPCK in well-watered and water-stressed purslane. Transcript abundance for PEPC was higher during the day than at night for well-water and water-stressed plants. Protein abundance, determined by Western blots, for PEPC was similar between day and night, but water-stressed plants had slightly lower levels of PEPC than well-watered plants. Transcript abundance for PEPCK 5 d after drought invitation indicated higher daytime expression for well-watered plants compared to night, but the inverse was found for water-stressed plants with higher nighttime expression. The expression data for PEPCK suggest the phosphorylation status of PEPC changes from day to night for the well-watered and water-

stressed samples, respectively. We were able to demonstrate, using PEPC and PEPCK transcript abundance, an apparent shift in the photosynthetic metabolism of purslane from C_4 to CAM, which is consistent with previous research reports.

Introduction

The C_4 photosynthetic pathway in plants is distinct from the C_3 pathway by initial fixation of atmospheric CO_2 into four-carbon organic acids in the C_4 pathway (Black 1973; Edwards and Walker, 1983). The leaves of C_4 plants possess Kranz anatomy, where mesophyll and bundle sheath cells are differentiated by form and function (Edwards and Walker, 1983). These biochemical and anatomical features result in higher net CO_2 fixation and reduced photorespiration in C_4 plants compared to C_3 plants, even under environmental stresses like drought. Crassulacean acid metabolism (CAM) plants also initially fix atmospheric CO_2 into four-carbon acids, but this occurs at night because stomata are closed during the day to limit water loss (Kluge and Ting, 1978; Winter 1985). The primary distinction between C_4 and CAM plants is that the initial fixation of atmospheric CO_2 and subsequent CO_2 reduction via ribulose-1, 5-bisphosphate carboxylase oxygenase (Rubisco) is spatially separated in C_4 plants, while it is temporally separated in CAM plants.

Most facultative CAM plants utilize C_3 metabolism under ideal conditions and shift to CAM when stressed. Few plants are known to possess both C_4 and CAM metabolism, and all are in the *Portulaca* genus. These plants, namely, common purslane (*Portulaca oleracea* L.*),* rose moss *(Portulaca grandiflora* Hook*)*, and kiss me quick (*Portulaca mundula* I.M. Johnst.) exhibit CAM characteristics when subjected to drought-stress or short photoperiods (Guralnick and Jackson, 1993; Koch and Kennedy, 1980, 1982; Kraybill and Martin, 1996; Ku et al., 1981; Lara et

al., 2003, 2004; Mazen, 1996, 2000). This shift from C_4 to CAM has primarily been characterized by leaf acidity fluctuations and gas exchange measurements. In addition to leaf acidity and gas exchange, differing phosph*enol*pyruvate (PEP) carboxylase (PEPC, EC 4.1.1.31) levels and activity have been reported in purslane in response to drought (Lara et al., 2003; Mazen, 1996, 2000). Lara et al. (2003) presented evidence with 2-D SDS PAGE, native IEF, and southern blots indicating multiple gene isoforms of PEPC are present in purslane. Suggesting different PEPC genes are expressed under C_4 or CAM.

In C_4 and CAM plants, PEPC plays an important role in photosynthetic carbon fixation, where it catalyzes the carboxylation of PEP to yield oxaloacetate and P_i. Phospho*enol*pyruvate carboxylase is an allosteric enzyme, inhibited by L-malate and activated by glucose 6-phosphate (Chollet et al., 1996; Vidal and Chollet, 1997). Regulation of PEPC occurs at the transcriptional level and the post-translational level through phosphorylation of a single highly conserved serine residue near the N-terminal end of the polypeptide (Jiao and Chollet, 1991; Jiao et al., 1991). When phosphorylated, PEPC is less sensitive to inhibition by malate and more sensitive to activation by glucose 6-phosphate (Jiao and Chollet 1991; Lepiniec et al., 1994; Ting, 1985). The calcium insensitive Ser/Thr kinase, PEPC kinase (PEPCK) primarily controls the phosphorylation state of PEPC and is highly regulated at the transcriptional level (Hartwell et al, 1996). The C_4 PEPCK is up-regulated during the day and down-regulated at night (Li and Chollet 1993), whereas the CAM PEPCK is controlled by circadian rhythm with increased transcript abundance at night (Li and Chollet, 1994). The down regulation and dephosphorylation of PEPC is catalyzed by a constitutive protein phosphatase 2A and is not regulated by the same mechanisms that control PEPCK activity (Carter et al., 1990, 1991).

Better understanding C_4 plant species with inducible CAM photosynthetic mechanisms will increase our understanding of how these plants adapt their photosynthetic pathways to accommodate stressful conditions. The facultative C_3-CAM plant, common ice plant (*Mesembryanthemum crystallinum* L.), has been extensively investigated as a model to understand the regulatory mechanism of CAM photosynthesis (Cushman et al., 2008a,2008b; Winter, 1985; Winter and Holtum, 2007) and in particular, PEPC and PEPCK transcription and PEPCK post-translational regulation have been measured (Taybi et al., 2004). We are unaware of research evaluating CAM in purslane using PEPC or PEPCK transcript abundance. The objective of our research is to identify drought-induced shifts to CAM-like photosynthetic metabolism by measuring the transcript abundance of PEPC and PEPCK.

Materials and Methods

Plant material

Purslane plants were grown from seed in 10 cm pots and clay-loam soil in a greenhouse with a 34/24°C day/night temperature, and a 16 h light photoperiod with supplemental lighting at the beginning and end of the photoperiod with a mean peak-light-irradiance of 1000 W m^{-2}. Six weeks after emergence, plants were divided in two groups: a water-stressed group from which water was withheld and a well-watered (control) group that was watered daily. Leaf samples were collected mid-photoperiod at 2:00 and 14:00 h, 1 and 5 d after treatment initiation from each group. After collection, the leaves were immediately frozen in liquid N_2 and stored at -80°C prior to analysis. In the greenhouse, treatments were arranged in a 3-factor factorial randomized complete block with five replications with water regime, stress duration, and

photoperiod being the three factors. The greenhouse study was conducted twice. Analysis of variance was performed on all quantitative data, followed by mean separation with Fisher's LSD at $P \leq 0.05$ using SAS (SAS, 2009).

Determination of titratable acidity

Total titratable acidity was determined for leaves harvested from well-watered and water-stressed plants at 2:00 and 14:00 h, 1 d and 5 d following water-stress initiation. The primary acid present in purslane leaf material is malic acid (Krabill and Martin, 1996; Lara et al., 2003), which is a water-soluble, dicarboxylic acid with pK_as of 3.5 and 5.1. Although there are two dissociable protons in this acid, their pK_as are too close together to produce two distinct inflection points in the titration. The pH at the equivalence point is slightly on the alkaline side of neutrality due to the hydrolysis of the weak base, sodium malate, present in the titration solution after neutralization (Eq 3.1). Consequently, phenolphthalein, with its color transition pH beginning at about pH 8.2, is ideal for indicating the endpoint of this titration.

$$C_4H_6O_5\ (aq) + 2\ NaOH\ (aq) \rightarrow Na_2C_4H_4O_5\ (aq) + 2\ H_2O\ (l) \qquad \text{[Eq. 3.1]}$$

The following procedure was used to determine titratable acidity of fresh purslane leaf material: 50 mg $\pm$ 5 mg of frozen (-80°C) leaf material were ground in 2 mL centrifuge tubes using a tissue lyser (Qiagen, Germantown, MD). Samples were kept frozen during the grinding process by the use of chilled components. Immediately following grinding, 1500 µL freshly boiled distilled water were added to the ground samples, and the tubes vortexed (~ 45 s) until a homogeneous suspension was obtained. Samples were then incubated at 95°C for 10 minutes in a dry-bath incubator (Fisher Scientific, Waltham, MA) to extract the leaf tissue acids. The extracts were then centrifuged at 17,000 g for 2 minutes and 750 µL aliquots of supernatant

were then transferred to 2 mL autosampler vials, and 6 µL of 0.08% phenolphthalein (in methanol) and a micro-stir bar were added to the vials. Extracts were titrated with 10 mmolar sodium hydroxide (0.010 µeq/µL NaOH) on a magnetic stirring plate to the pink phenolphthalein endpoint. A method blank required 7 µL of 10 mmolar NaOH to titrate 6 µL phenolphthalein in 750 µL H2O to its colorimetric endpoint (due to residual carbonate interference and titration of the phenolphthalein indicator itself). The 6 µL of 0.08 % phenolphthalein was found necessary to indicate a sufficiently distinct endpoint in the stressed plant extracts which were tinged brown obscuring less pronounced endpoints. This blank volume was subtracted from the sample titrant volumes to correct for this background acidity. Titratable acidity was calculated using equation 3.2.

$$Titratable\ Acidity\ (\mu eq\ g^{-1}FW) = \frac{(Titrant\ Volume,\mu L - 7\mu L)(0.010\ \mu eq/\mu L\ )(1.50\ mL)}{(Leaf\ Sample\ Mass,g)(0.750\ mL)} \qquad \text{[Eq 3.2]}$$

Leaf Gas Exchange

Stomatal conductance and photosynthetic rates were logged every 10 min over 5 d following water-stress initiation using portable photosynthesis systems (model 6400, LI-COR, Lincoln, NE). Using two photosynthesis systems, measurements were simultaneously recorded from one attached leaf of a randomly selected purslane plant from the water-stressed and the well-watered groups. The chamber flow rate was 500 µmol s^{-1} and ambient temperature, humidity, and CO_2 concentrations were used. A clear top leaf chamber was used, so irradiance within the chamber was similar to that available for the whole plant. Leaf area was determined using a leaf area meter (3100c, LI-COR, Lincoln, NE). The leaf used for gas exchange measurements was harvested and trimmed to the inside dimension of the chamber before leaf area was determined. Although purslane leaves have 3 dimensional structure, leaves were

assumed to be flat for leaf area measurements as we were interested in relative differences between plants not absolute gas exchange values. Gas exchange experiments were conducted at least two times. The photosynthesis system used to collect measurements for the well-watered or water-stressed group was then used on the alternate group for the subsequent experiment.

RNA isolation and cDNA synthesis

Total RNA was extracted from leaf tissue using a sodium dodecyl sulfate (SDS) Trizol method described by Holding et al. (2007) and subsequently treated with DNAse1 (Thermo Scientific, Pittsburg, PA) and re-purified with RNeasy cleanup columns (Qiagen, Valencia, CA), both according to the manufacturer's instructions. The RNA pellet was dissolved in diethylpyrocarbonate (DEPC) treated distilled water and stored at -80°C. RNA quality and concentration were determined by spectrophotometry (model NanoDrop 2000c, Thermo Scientific, Pittsburg, PA). Using 2 µg RNA, cDNA was synthesized using the High Capacity cDNA Reverse Transcription kit with random primers according to manufacturer instructions (Applied Biosystems, Foster City, CA, USA).

PCR Amplification

Transcript abundance of PEPC and PEPCK were assessed in leaves collected during the middle of the day and night photoperiods from well-watered and water stressed plants 1 and 5 d after water-stress initiation. Semi-quantitative RT-PCR was performed using 10 ng cDNA in a reaction mixture (20 µL) containing 0.5 µM of forward and reverse primers and 1U Phusion DNA polymerase. Two degenerate PEPC primers [PEPCfwd: TC(CTA)GA(TC)TC(CAT)GG(AC)AA(AG)GA(TC)GC; PEPCrev:

GC(GAT)GC(GAT)AT(GCA)CC(CT)TTCAT(GT)G] (Gehrig et al., 2001) and PEPCK primers [PEPCKfwd: TGCGAGGAGATCGGCCG(AT)G; PEPCKrev ACCTCCGGCGCCACGTA(AG)TAC] (Taybi et al., 2004) were used to amplify gene sequence for purslane. PCR products were gel-extracted using GeneJet gel extraction kit (Thermo Scientific, Pittsburg, PA) and cloned into pGEM-Teasy vector system (Promega, Madison, WI) according to manufacturer instructions. Plasmids were purified with the GeneJet miniprep kit (Thermo Scientific, Pittsburg, PA). Positive clones were sequenced from both directions. The resulting sequences were loaded into the National Center for Biotechnology Information (http:// www.ncbi.nlm.nih.gov) tool, BLAST 2.0 and compared with other sequences in the data base. Purslane sequences had the highest homology with PEPC genes from several other species. Gene-specific primers were designed using a Primer3 program (http://primer3.ut.ee/) [PEPCfwd: ACCGAACCATACCGTGTCAT; PEPCrev: GCCCAAAAGTGGAAACTTGA] and [PEPCKfwd: CGGAACTGTCTCTCCTCCTC; PEPCKrev: CGCTGCATCTCCTTGTCTTC]. Ubiquitin was used as a positive control [UBIQfwd:TACAACAT(CT)CAGAAGGAGTC; UBIQrev: CC(CT)TC(CT)TTGTC(CT)TG(AG)ATCTT]. The PCR conditions were 35 cycles, a 98°C denaturing cycle for 10 s and touchdown protocol decreasing 1°C per cycle for 10 cycles starting at 65°C for 15s followed by 25 cycles at 55°C for 15 s and a 72°C extension for 30 s per cycle.

1-D SDS-PAGE and Western Blot

Total protein was extracted by phenol extraction followed by ammonium acetate precipitation (Hurkman and Tanaka 1986). Protein content was estimated according to Bradford (1976) using bovine serum albumin (BSA) as the standard. Thirty micrograms of extracted leaf protein was loaded on a 4% (w/v) stacking and 10% (w/v) resolving SDS polyacrylamide gel. Proteins were visualized with Coomassie blue G-250 or electroblotted onto nitrocellulose. Anti-

PEPC antiserum (1/3,000 fold diluted rabbit anti-maize, provided by Dr. Gautam Sarath, USDA-ARS) was used for detection and bound antibodies were located by linking AP-conjugated mouse anti-rabbit IgG (Sigma-Aldrich, St. Louis, MO). Western blot and SDS-PAGE were repeated three times with consistent results and the representative data are shown.

Results and Discussion

There was no fluctuation in titratable acidity in leaves between the day and night photoperiods in the well-watered plants at 1 or 5 d as would be expected for C_4 plants (Figure 3.1). Titratable acidity was nearly threefold higher at night compared to the day in water-stressed plants at both 1 and 5 d after drought initiation which is similar to patterns exhibited by CAM plants. Lara et al. (2003) reported Δ titratable acidity of 14.7 µequivalents g^{-1} FW between day and night for water-stressed purslane, similar to results from Koch and Kennedy (1980) for water-stressed purslane under long photoperiods. Kraybill and Martin (1996) found Δ titratable acidity of 120 mmol kg^{-1} DW for drought-stressed purslane, which is similar to values reported for constitutive CAM plants. The Δ titratable acidity for water-stressed purslane in our study is similar to previous reports and suggestive of CAM-like metabolism (Figure 3.1).

Well-watered purslane plants had net CO_2 uptake during the day and a slight CO_2 release during the beginning of the night photoperiod (Figure 3.2), which is consistent with CO_2 exchange patterns typical for C_4 plants. Water-stressed purslane initially had CO_2 uptake similar to the control, but CO_2 exchange was close to zero by the end of the first diurnal cycle (Figure 3.3). Crassulacean acid metabolism plants typically limit CO_2 uptake during the day and slow uptake rates in the dark (Andrè el al., 1979). Significant variation is reported for CO_2 exchange of

purslane plants exhibiting CAM-like metabolism, ranging from net CO_2 uptake in the dark to reduced daytime uptake or even release of CO_2 (Kraybill and Martin, 1996; Lara et al., 2003; Mazen, 1996). Variable methods have been used for CO_2 exchange measurements of purslane, which may explain some of the variation reported above. Lara et al. (2003) recorded measurements on detached leaves harvested 5 h into the light or dark period. Krabill and Martin (1996) measured CO_2 exchange of intact shoots for 2 d. Koch and Kennedy (1980, 1982) determined CO_2 exchange rates of intact plants enclosed inside a Plexiglas chamber. In our study, we measured CO_2 exchange of attached leaves for 5 d using a portable photosynthesis system. Results were most similar to those reported by Krabill and Martin (1996) and Koch and Kennedy (1980, 1982), which showed no net CO_2 exchange of water-stressed plants. When considering our acid fluctuation data with the CO_2 exchange data for water-stressed plants (Figure 3.1 and 3.3), they suggest that CAM-idling may be occurring. CAM-idling is stress-induced and results in stomatal closure during the entire day and night, but respired CO_2 is refixed at night resulting in increased acidity (Cushman, 2001). Stomatal conductance data (not shown) followed a similar pattern as the CO_2 exchange data and would suggest CAM-idling is occurring as little to no stomatal conductance was observed for water-stressed plants during the day or night.

Phospho*enol*pyruvate carboxylase transcript abundance was higher for well-watered purslane compared to water-stressed plants (Figure 3.4). For well-watered and water-stressed plants alike, transcript levels of PEPC were higher during the day than at night, although differences between day and night appear greater for 1 d samples compared to 5 d samples. The higher abundance of PEPC transcripts during the day compared with the night is consistent with observations for three CAM performing *Clusia* species (Taybi et al., 2004). Changes in PEPC

transcript abundance between day and night were not reflected in PEPC protein abundance (Figure 3.4), as protein turnover occurs at a slower rate and PEPC is post-translationally regulated by phosphorylation (Chollet et al., 1996; Taybi et al, 2004; Vidal and Chollet, 1997). Phospho*enol*pyruvate carboxylase kinase transcript abundance was higher during the day than at night for both well-watered and water-stressed plants after 1 d (Figure 3.4). In well-watered purslane, PEPCK transcript abundance was similar for 1 d and 5 d samples with higher abundance during the day compared to night. In water-stressed purslane, however, transcript abundance was similar to well-watered purslane for 1d samples, but transcript abundance was higher at night compared to the day for 5 d samples (Figure 3.4). In C_4 plants, PEPC phosphorylation is regulated primarily by changes in PEPCK activity which is activated in the light by photosynthesis (Vidal and Chollet, 1997). The activity of C_4 PEPCK is a result of *de novo* synthesis of mRNA and protein (Hartwell et al., 1996, 1999b). For CAM plants, PEPCK activity is regulated by a circadian oscillator that prevents futile cycling of CO_2 by Rubisco and PEPC carboxylation reactions (Nimmo, 1998, 2000). The facultative CAM, common ice plant, has an 8-fold increase in day versus night activity of PEPCK when CAM is induced (Li and Chollet, 1994). The constitutive CAM plant, lavender scallops (*Kalanchoe fedtschenkoi* Raym.-Hamet & H. Perrier), also exhibits higher PEPCK activity at night than during the day (Hartwell 1996, 1999a). An investigation of *Clusia* species that range from constitutive C_3, inducible CAM, to constitutive CAM plants show differing PEPCK expression. The C_3 plants had consistent PEPCK expression across the day and night whereas the CAM plants had higher PEPCK expression at night compared to the day (Taybi et al., 2000).

Under drought conditions purslane appears to shift to CAM-like metabolism with nocturnal accumulation of leaf acidity, reduced CO_2 uptake, and change in the PEPCK expression

pattern. Understanding the drought-induced signaling pathways responsible for the shift from C_4 to CAM photosynthetic metabolism has potential for improving drought tolerance in crop plants grown in water-limiting environments. In our study, PEPCK shifts from higher daytime expression to higher nighttime expression in well-watered and water-stressed plants, respectively. Based on these data, it appears that PEPC changes from daytime phosphorylation status to nighttime phosphorylation, which could be evidence for the induction of CAM under drought stress. It would be helpful to directly evaluate the phosphorylation of PEPC between well-watered and water-stressed plants to further support our PEPCK expression data. We are currently conducting PEPC phosphorylation and RNA-seq experiments to further determine the drought induced effects on transcriptional and post-translational regulation of enzymes important for C_4 and CAM photosynthetic metabolism. Determining if photosynthetic isoforms of PEPC and PEPCK exist in purslane would provide further evidence that CAM-like metabolism is induced under drought. Our data coupled with previous investigations on the drought response of purslane provide mounting evidence for the induction of CAM photosynthesis from C_4 metabolism.

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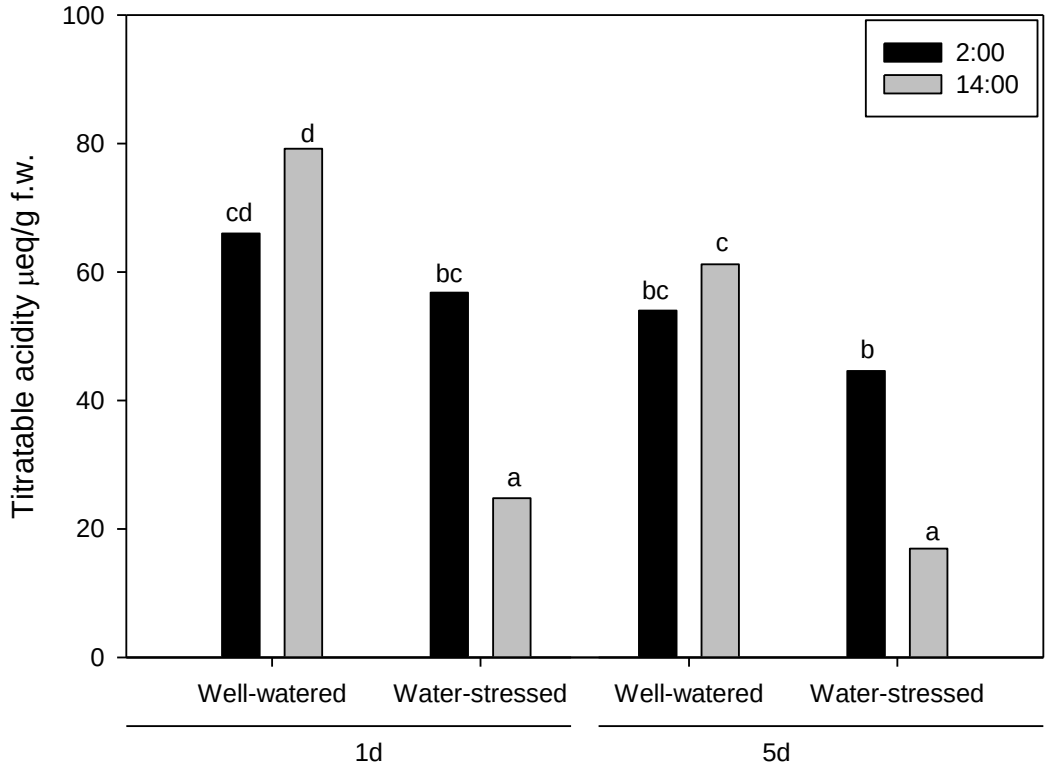

Figure 3.1. Titratable acidity of purslane leaves harvested at 2:00 and 14:00 h from well-watered and water-stressed plants at 1 d and 5 d following drought initiation. Each bar represents means from two experiments and five replications. Bars with the same letter are not significantly different according to Fisher's LSD at $P \leq 0.05$.

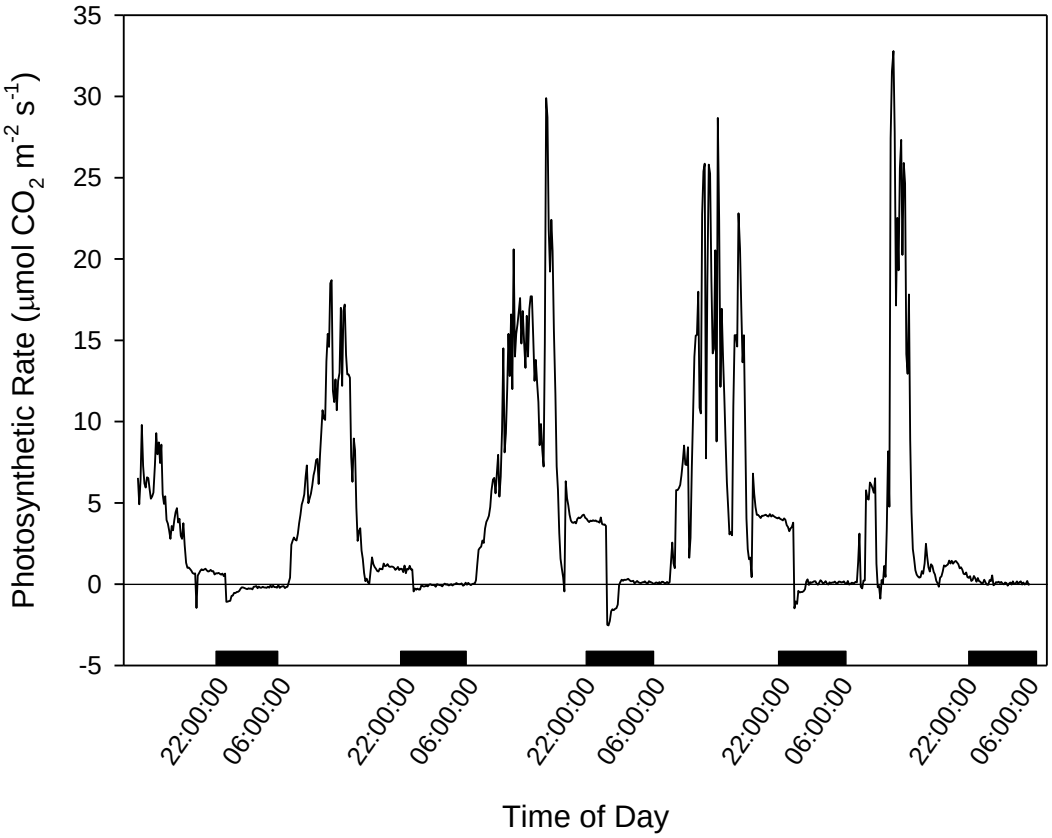

Figure 3.2. Photosynthetic rate for well-watered purslane measured every 10 min over 5 d on a single attached leaf. Data were recorded simultaneously for water-stressed purslane. Data was collected with a closed chamber infrared gas analyzer photosynthesis system using ambient light, temperature, and humidity. Leaf area within the chamber was measured with a leaf area meter. Black bars on the horizontal axis indicate the dark photoperiod.

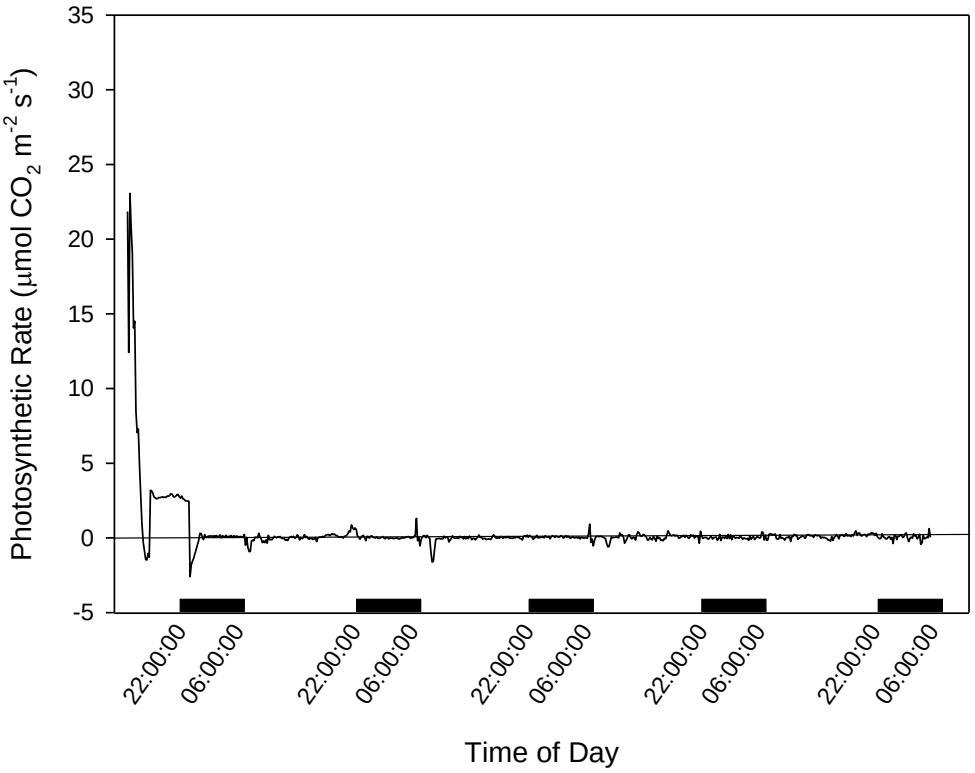

Figure 3.3. Photosynthetic rate for watered-stressed purslane measured every 10 min over 5 d on a single attached leaf. Data were recorded simultaneously for well-watered purslane. Data was collected with a closed chamber infrared gas analyzer photosynthesis system using ambient light, temperature, and humidity. Leaf area within the chamber was measured with a leaf area meter. Black bars on horizontal axis indicate the dark photoperiod.

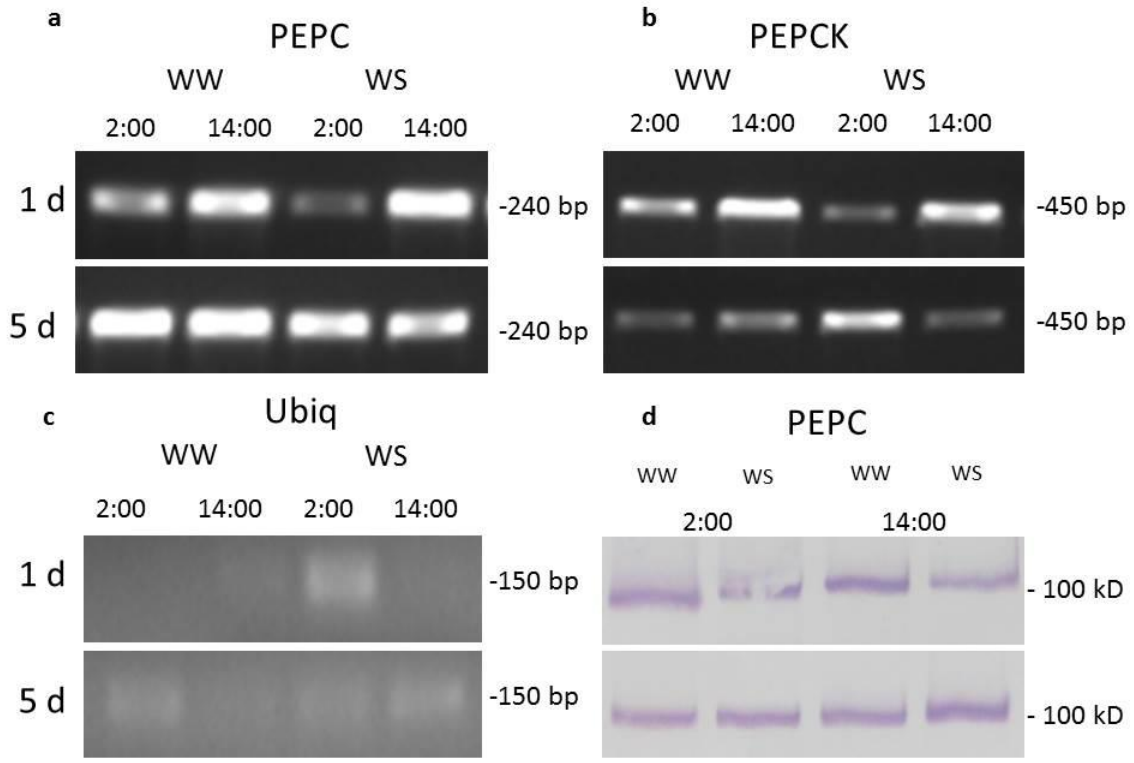

Figure 3.4 PEPC (**a**) and PEPCK (**b**) transcripts in the leaves of purslane collected at 02:00 and 14:00 h from well-watered (WW) and water-stressed (WS) plants 1 d and 5 d after drought initiation. Ubiq (**c**) is the internal control showing equal cDNA input and RT-PCR conditions. Western blots(**d**) showing PEPC abundance from well-watered and water-stressed plants from leaves collected at 2:00 and 14:00 h, 1 and 5 d following drought initiation.

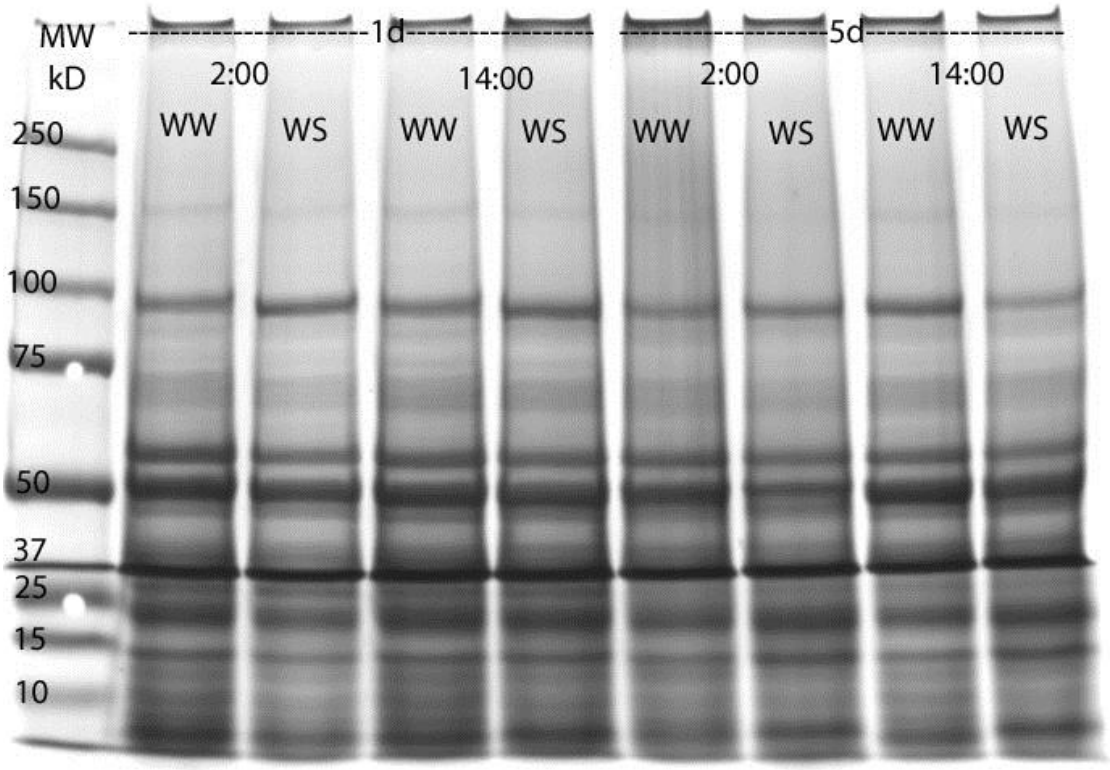

Figure 3.5 30 µg protein extracted from purslane leaves 2:00 and 14:00 h for well-watered (WW) and water-stressed (WS) samples 1 d and 5 d after drought initiation. SDS polyacrylamide gel is a 4% stacking and 10% resolving gel. Figure shown as a loading control for Western blot.