Weed Control with Liquid Carbon Dioxide in Established Turfgrass

Denis J. Mahoney, Matthew D. Jeffries, and Travis W. Gannon*

In recent years, increasing implementation of biological, cultural, and mechanical weed-control methods is desired; however, many of these techniques are not viable in established turfgrass systems. The use of freezing or frost for weed control has previously been researched; however, is not well elucidated. Field and greenhouse experiments were conducted to evaluate liquid carbon dioxide (LCD) for weed control in established turfgrass systems. LCD was applied with handheld prototypes that were modified to reduce the amount of LCD required for weed control. Common annual and perennial turfgrass weeds included common chickweed, corn speedwell, goosegrass, large crabgrass, smooth crabgrass, Virginia buttonweed, and white clover. Turfgrass tolerance was evaluated on the following species: hybrid bermudagrass, Kentucky bluegrass, tall fescue, and zoysiagrass. The final modification allowed for lower output (0.5 kg LCD min^{-1}) when compared with the initial prototype (3 kg LCD min^{-1}). In general, weed control increased as LCD increased. When comparing weed species life cycles, annuals were controlled more than perennials ($P < 0.0001$) at 14 and 28 d after treatment (DAT). Further, exposure time affected control as white clover, Virginia buttonweed, and large crabgrass control was greater (18, 14, 15%, respectively) from the longer exposure time (30 vs. 15 s), although equivalent amounts of LCD (30 kg m^{-2}) were applied. These data also suggest that plant maturity affects control, as large crabgrass control in one- to two- and three- to four-leaf stages (> 90%) was greater than in the one- to two-tiller stage (< 70%). Turfgrass injury at 7 DAT was unacceptable (> 30%) on all species, but declined to 0% by 28 DAT. These data suggest that LCD has the potential to provide an alternative for weed control of select species where synthetic herbicides are not allowed or desired.

Nomenclature: Common chickweed, *Stellaria media* (L.) Vill.; corn speedwell, *Veronica arvensis* L.; goosegrass, *Eleusine indica* (L.) Gaertn.; large crabgrass, *Digitaria sanguinalis* (L.) Scop.; smooth crabgrass, *Digitaria ischaemum* (Schreb.) Schreb. ex Muhl.; Virginia buttonweed, *Diodia virginiana* L.; white clover, *Trifolium repens* L.; hybrid bermudagrass, *Cynodon dactylon* (L.) Pers. × *Cynodon transvaalensis* Burtt-Davey, cv. 'Tifway 419′; Kentucky bluegrass, *Poa pratensis* L. 'Unique'; tall fescue, *Lolium arundinaceum* (Schreb.) S.J. Darbyshire 'Confederate'; zoysiagrass, *Zoysia japonica* Steud. 'El Toro'.

Key words: Nonchemical weed control, turf.

En años recientes, se ha hecho deseable el aumento en la implementación de métodos de control de malezas de tipo biológico, cultural, y mecánico. Sin embargo, muchas de estas técnicas no son viables en sistemas de césped establecido. El uso de congelación para el control de malezas ha sido previamente investigado aunque no ha sido bien elucidado. Se realizaron experimentos de campo e invernadero para evaluar el carbon dioxide líquido (LCD) para el control de malezas en sistemas de césped establecido. Se aplicó LCD con prototipos manuales que fueron modificados para reducir la cantidad de LCD requerido para controlar las malezas. Las malezas anuales y perennes comunes en céspedes incluyeron *Stellaria media*, *Veronica arvensis*, *Eleusine indica*, *Digitaria sanguinalis*, *Digitaria ischaeum*, *Diodia virginiana*, y *Trifolium repens*. La tolerancia del césped fue evaluada en las siguientes especies: bermuda híbrido (*Cynodon dactylon* × *Cynodon transvaalensis*), *Poa pratensis*, *Lolium arundinaceum*, y *Zoysia japonica*. La modificación final del prototipo permitió una descarga menor (0.5 kg LCD min^{-1}) cuando se comparó con el prototipo inicial (3 kg LCD min^{-1}). En general, el control de malezas incremento al aumentar la dosis de LCD. Cuando se comparó las especies según su ciclo de vida, las anuales fueron controladas más que las perennes ($P<0.0001$) a 14 y 28 d después del tratamiento (DAT). Además, el tiempo de exposición afectó el control; así el control de *T. repens*, *D. virginiana*, y *D. sanguinalis* fue mayor (18, 14, 15%, respectivamente) bajo el tiempo de exposición más largo (30 vs. 15 s), aunque se aplicaran cantidades equivalentes de LCD (30 kg m^{-2}). Los datos también sugieren que la madurez de la planta afecta el control. Así el control de *D. sanguinalis* fue mayor en los estadios de una- a dos- y tres- a cuatro-hojas (>90%) que en los estadios de uno- a dos-hijuelos (<70%). El daño en el césped a 7 DAT fue inaceptable (>30%) en todas las especies, pero disminuyó a 0% a 28 DAT. Estos datos

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* Graduate Research Assistant, Graduate Research Technician, and Assistant Professor, respectively, Department of Crop Science, North Carolina State University, Raleigh, NC 27695-7620. Corresponding author's E-mail: djmahone@ncsu.edu.

sugieren que LCD tiene el potencial de brindar una alternativa para el control de malezas de especies selectas donde el uso de herbicidas sintéticos no está permitido o no es deseable.

From 2000 to 2007, an estimated 45 million kg of synthetic herbicides were applied to noncrop land, including residential, commercial, and governmental properties (facilities, sites, and other land) in the United States (Grobe et al. 2011). Increasing pressure from environmental groups and human health advocates has driven many laws and regulations toward reducing or prohibiting synthetic pesticide use in public and private areas (Cisar 2004). Examples include Takoma Park, MD, where the Safe Grow Act of 2013 banned cosmetic-use pesticides on both public and private property (Anonymous 2013a). Cosmetic use was defined as products used to create a "homogenous lawn." Further, the Sustainable Land Care Policy of 2011 prohibited synthetic pesticides on city-owned land in Greenbelt, MD (Anonymous 2013a). California also passed laws prohibiting the use of 28 pesticides (including dicamba, fluoxastrobin, metconazole, mesotrione, thiencarbazone, etc.) on school and childcare center grounds (Anonymous 2013b). Although weed control is known to be important in these areas, city council members believed the potential adverse effects from synthetic pesticides outweigh the benefits.

To minimize human pesticide exposure and potential adverse environmental effects, the aforementioned communities planned to educate residents on "minimum-risk pesticides" and nonchemical weed-control methods such as biological, cultural, and mechanical control techniques. Integrated weed management programs, which include biological weed control, have been successful in certain settings (Ghosheh 2005); however, when biological agents are used alone, control is inconsistent and dependent on environmental conditions (Abu-Dieyeh and Watson 2007; Johnson 1994). The authors concluded that synthetic herbicides were needed for effective control. Compared with traditional row-crop weed management, established turfgrass systems have reduced cultural weed-control options because of their perennial nature; for example, mulches and covers are not feasible in established turfgrass systems because of growth reduction from plant light interception (Bond and Grundy 2001). When properly performed, common turfgrass cultural management practices including mowing, fertilization, aerification, and verticutting have been proven to suppress weed populations (Busey 2003). Hoyle et al. (2013) noted that large crabgrass incidence decreased 64% as tall fescue mowing height increased from 2.5 to 10.2 cm. Similarly, Dernoeden et al. (1993, 1998) reported that smooth crabgrass incidence was unacceptable when tall fescue was maintained at $\leq$ 6.5 cm; however, when maintained at $\geq$ 8.8 cm, smooth crabgrass incidence was $\leq$ 2%. Mechanical weed-control practices such as discing and conventional tilling are not viable in turfgrass systems as they cause surface disruption and unacceptable turfgrass quality (Hatcher and Melander 2003). Further, hand removal and suppression practices (e.g., edging) are typically safe and efficacious; however, they are very labor intensive, cost prohibitive in most regions, and weed control is species specific (less efficacious on perennial species). For these reasons, they are not heavily adopted or researched (Busey 2003).

Previous research has shown that thermal techniques such as flaming and solarization may provide acceptable weed control (Cohen and Rubin 2007; Hatcher and Melander 2003; Hoyle et al. 2012). Flaming does not disturb the soil; however, multiple flaming events are typically needed and may unacceptably damage turfgrass (Fergedal 1993; Hoyle et al. 2012). Solarization can be used to initiate weed emergence, which can be timed with stressful climatic conditions to provide good weed control (> 80%) (Hoyle et al. 2012). However, solarization efficacy varies between plant species and is generally not feasible in established turfgrass areas because it would adversely affect the health and functionality of desirable plants (Cohen and Rubin 2007; Hoyle et al. 2012).

Although low-temperature thermal weed control is not well elucidated, the effect of low temperatures on plant growth has been examined (Fergedal 1993; Lewis et al. 2011; Malyshev and Henry 2012; Pearce 2001; Singh and Laroche 1988; Xin and Browse 2000). Cellular freezing processes are classified as extra- or intracellular. Extracellular

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and intracellular freezing can adversely affect plant cells, thereby reducing survivability. Specifically, extracellular freezing, or ice formation outside the cell, causes water potential to drop and cytoplasmic water to move into intercellular spaces, leading to dehydration and compromised cell wall integrity (Singh and Laroche 1988; Xin and Browse 2000). Intracellular freezing, or ice formation inside the cell, is the most detrimental form of plant freezing (Pearce 2001). This occurs when ambient conditions cool more rapidly than plant acclimation rates and irreversibly damage cell membranes, causing cell walls to collapse (Singh and Laroche 1988). Although plants may physiologically acclimate to freezing temperatures, these changes occur over weeks or months (Xin and Browse 2000). During summer climatic conditions, cold-hardy plants are susceptible to freezing, much like nonhardy species, because this physiological acclimation has not occurred (Warren 1998).

Jitsuyama and Ichikawa (2011) researched cryogen salt applications before snowfall for weed control. Research trends showed reduced weed establishment the following growing season; however, weed control was unacceptable (> 140 weeds m^{-2}), treatments relied on snow for activation, and the authors noted improvements for application needed to be made. Fergedal (1993) researched the efficacy of low-temperature weed-control methods using liquid nitrogen or dry ice and compared results with flaming with liquid petroleum. The researchers reported that adequate weed control with liquid nitrogen was comparable with flaming; however, > 400 times more liquid nitrogen (14,600 kg) was required for weed control compared with flaming (35 kg). Furthermore, the authors noted that many improvements could be made to the delivery system (Fergedal 1993). Lewis et al. (2011) investigated weed control with liquid carbon dioxide (LCD) and reported greater control as total LCD applied increased; further, annual species were more susceptible than perennial species and increased LCD exposure duration provided greater weed control.

Currently, there is a need for more cost-effective and efficacious weed management techniques where synthetic herbicide use is not desired. The Frostbite Weed Control System™ (Frostbite; Arctic, Inc., Clemmons, NC) discharges recaptured LCD at subfreezing temperature, creating a frost layer that may cause extra- or intracellular freezing in treated plants, providing weed control in areas where synthetic herbicides are not desired. The objective of this research was to determine the efficacy of Frostbite for weed control in established turfgrass systems.

Materials and Methods

Prototype I. LCD treatments were applied via a Frostbite handheld prototype through a 0.6-cm disc nozzle (D14-D25; TeeJet®, Spraying Systems Co. Wheaton, IL). Nozzle height was 15 cm above the plant and exposed to atmospheric conditions. Application output was 3 kg LCD min^{-1} over a 182 cm^2 treated area. Field experiments were initiated March 16, 2010 at the Lake Wheeler Turfgrass Field Laboratory (LWTFL) (Raleigh, NC) to evaluate the efficacy of LCD applications for weed control in established turfgrass systems. Experimental units were managed in utility turfgrass areas, with supplemental irrigation, weekly mowing to a 10-cm height of cut, and recommended fertilization. Soil type was a Cecil sandy loam (fine, kaolinitic, thermic Typic Kanhapludults). After treatment, mowing was discontinued for the remainder of the experiments. The trials consisted of a 4 by 4 factorial treatment arrangement of four LCD dwell times (6, 15, 30, or 60 s) and four weed species (corn speedwell, common chickweed, white clover, or Virginia buttonweed). For comparison, 2,4-D + mecoprop + dicamba (Trimec Classic®, 1.3 kg ai ha^{-1}, PBI Gordon Corp., Kansas City, MO) was used as a herbicide control standard (Johnson 1980; Kelly and Coats 2000). Two annual and two perennial weed species common to mid-Atlantic United States were included to compare efficacy between life cycles. Treatments were arranged in a randomized complete block design with three replications and a nontreated check was included. Control was visually estimated on a 0 to 100% scale (0% = no control; 100% = complete plant death) 1, 2, 3, 4, 5, 6, 7, 14, 21, and 28 d after treatment (DAT).

Prototype II. Frostbite was fit with a flat-fan nozzle (SS XR8002E or SS XR8004E) (TeeJet) and an uninsulated cone (15-cm diam) to reduce LCD required for weed control. After prototype modification, experiments were conducted to evaluate the efficacy of LCD applications for weed control in

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established turfgrass systems. Experiments were initiated on April 16 and May 1, 2010 at LWTFL. Applications were made with the cone pressed to the ground over a 182 cm^2 area with a 15-cm nozzle height. Application outputs were 2 or 1 kg LCD min^{-1} from the XR8004E or XR8002E nozzles, respectively. The trials consisted of a 3 by 2 by 5 factorial treatment arrangement of three LCD dwell times (5, 15, or 30 s), two nozzles (flat-fan SS XR8002E or SS XR8004E), and five weed species (goosegrass, large crabgrass, smooth crabgrass, Virginia buttonweed, or white clover). All grass species were one- to three-tiller, whereas white clover was one to two trifoliate and Virginia buttonweed was two- to 14leaf at experiment initiation. For comparison, quinclorac (Drive 75 DF®, 0.8 kg ai ha^{-1}, BASF Corp., Research Triangle Park, NC) + methylated seed oil (MES-100, 2.1 L ha^{-1}, Drexel Chemical Company, Memphis, TN) was included as a herbicide standard for white clover, Virginia buttonweed, smooth crabgrass, and large crabgrass control (Hart et al. 2004), whereas fenoxaprop (Acclaim Extra®, 0.1 kg ai ha^{-1}, Bayer Environmental Science, Research Triangle Park, NC) + nonionic surfactant (Induce®, 0.3% v v^{-1}, Helena Chemical Co., Collierville, TN) was included for goosegrass control (Zabihollahi 2009). Treatments were arranged in a randomized complete block design with three replications and a nontreated check was included. Control was visually estimated as previously described.

Prototype III. *Weed Control.* Final Frostbite modifications were made to enhance system efficiency. Frostbite modifications included a full cone nozzle (1/4T D1-33) (Spraying Systems Co.) held at a 25-cm height above the surface inside an insulated cone (30-cm height by 15-cm diam with 1.3 cm of foam insulation). Nozzle height differed from prototypes I and II because of cone design. Application output was 0.5 kg LCD min^{-1} over a 103 cm^2 treated area. Greenhouse experiments were initiated November 16 and 30, 2010 at the Method Road Greenhouse Complex (Raleigh, NC) to determine the influence of LCD exposure times at varying plant growth stages on large crabgrass control. Large crabgrass (Lorenz's OK Seeds, LLC, Okeene, OK) was seeded in pots (182 cm^2 surface area, 1,670 cm^3 volume) and thinned 14 and 21 d after emergence to six uniform plants per pot. Growing medium consisted of 60% Norfolk clay loam (fine-loamy, kaolinitic, thermic Typic Kandiudults) and 40% river bottom sand. Greenhouse day/night temperatures were 31/20 C and supplemental light was provided at 350 µmol m^{-2} s^{-1} for a 16-h d. Pots were irrigated three times per day with overhead irrigation and fertilized weekly with a 20–20–20 soluble fertilizer at a rate of 12.2 kg (N–P–K) ha^{-1} (Peters Professional 20–20–20 water soluble fertilizer, Scotts-Sierra Horticultural Products Co., Marysville, OH). Pots were arranged under a wooden structure to create a flush surface surrounding the top of the pots, creating a semi enclosed treatment area but allowing room for pressure release during LCD application. A 4 by 3 factorial treatment arrangement of four LCD dwell-time treatments (0.5, 1.5, 3, or 5 s) and three large crabgrass growth stages (one- to two-leaf, three- to four-leaf, or one- to two-tiller) were evaluated in a randomized complete block design with three replications and a nontreated check. Experimental units were re-randomized biweekly to minimize the effect of variation and control was visually estimated as previously described.

Turfgrass Tolerance. Field experiments were initiated July 12 and 19, 2012 at LWTFL to determine turfgrass tolerance to LCD applications. The trial consisted of a 5 by 4 factorial treatment arrangement of five LCD dwell times (1, 2, 3, 4, or 5 s) and four turfgrass species (hybrid bermudagrass, Kentucky bluegrass, tall fescue, or zoysiagrass). Treatments were arranged in a randomized complete block design with three replications and a nontreated check was included. Visual injury was estimated on a 0 to 100% scale (0% = no injury; 100% = complete plant death) with cover also being visually estimated on a 0 to 100% scale (0% = bare ground; 100% = complete plant cover) 1, 2, 3, 4, 5, 6, 7, 14, 21, and 28 DAT.

Data Analysis. Although only visual parameter estimations are presented, they have been strongly correlated with nonsubjective data collection methods in turfgrass research (Hoyle et al. 2013; Jeffries et al. 2013, Lewis et al. 2010). Data were subject to ANOVA using general linear models with SAS (SAS® for Windows, v 9.3, Statistical Analysis Systems Institute, Cary, NC) to determine the effect of LCD for weed control and turfgrass tolerance. Significant main effects and interactions are presented accordingly with precedent given to interac-

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Table 1. Broadleaf weed control 14 and 28 d after liquid carbon dioxide treatments with disc nozzle (prototype I).[a–c]

	14 DAT				28 DAT			
	Annual		Perennial		Annual		Perennial	
Treatment[d]	VERAR	STEME	TRFRE	DIQVI	VERAR	STEME	TRFRE	DIQVI
kg LCD m^{-2}	% control[e]							
16	73	92	40	27	73	98	23	15
40	80	94	57	27	95	98	33	12
80	77	94	73	47	98	100	47	32
160	78	96	77	45	100	100	72	47
Herbicide[f]	28	73	27	33	97	98	82	73
LSD[g]	7				6			
Annual vs. perennial[h]	$P < 0.0001$				$P < 0.0001$			

[a] Research conducted at the Lake Wheeler Turfgrass Field Laboratory (Raleigh, NC).

[b] Abbreviations: DAT, days after treatment; LCD, liquid carbon dioxide; VERAR, corn speedwell; STEME, common chickweed; TRFRE, white clover; DIQVI, Virginia buttonweed.

[c] Disc nozzle: D14-D25.

[d] LCD exposure times: 6, 15, 30, or 60 s (16, 40, 80, or 160 kg LCD m^{-2}, respectively).

[e] Control was visually estimated on 0 to 100% scale (0% = no plant injury; 100% = complete plant death).

[f] 2,4-D + mecoprop + dicamba (1.3 kg ha^{-1}).

[g] LSD values for comparison within DAT.

[h] P-value obtained from orthogonal contrast.

tions of increasing magnitude (Steele et al. 1997). Means were separated according to Fisher's Protected LSD at $P = 0.05$. Orthogonal contrasts ($P < 0.05$) were performed to compare weed species life cycles.

Results and Discussion

Maximum weed control was observed 14 DAT, whereas regrowth had occurred by 28 DAT; therefore, data from both evaluation dates are presented. Maximum turfgrass injury was observed 7 DAT, with reduction of turfgrass coverage noted 28 DAT. In general, injury symptoms on susceptible plants progressed from a water-soaked appearance to tissue chlorosis, followed by necrosis on all treated plant tissue, and eventual plant death. Further, injury was greatest on plant tissue closest to point of application and decreased closer to the soil surface. The discussion will focus on: (1) effect of LCD on weed control and turfgrass injury and (2) effect of prototype modifications on weed control.

Prototype I. A treatment-by-species interaction was detected 14 and 28 DAT. At 14 DAT, annual and perennial broadleaf control ranged from 73 to 96% and 27 to 77%, respectively, with LCD (Table 1). Excellent common chickweed control (> 90%) was observed regardless of LCD rate 14 DAT, whereas control of other species including corn speedwell, white clover, and Virginia buttonweed was unacceptable (< 80%) (excluding corn speedwell at 40 kg LCD m^{-2}). However, for all species and treatments, control from LCD was greater than the herbicide standard (excluding Virginia buttonweed at 16 and 40 kg LCD m^{-2}) by 14 DAT. Excellent common chickweed control was observed (≥ 98%) with all LCD application rates and the herbicide standard 28 DAT. White clover and Virginia buttonweed control from LCD applications was unacceptable (< 80% and < 50%, respectively) at either rating date. Excellent corn speedwell control (> 94%) was observed at the three highest LCD application rates (40, 80, and 160 kg m^{-2}) and was similar to the herbicide standard. Annual broadleaf weed control was greater than perennial broadleaf weed control 14 and 28 DAT ($P < 0.0001$), which may be due in part to morphological differences. Because of the nonsystemic effect of LCD, plant organs (e.g., thick stems or underground structures) may allow perennial species such as white clover and Virginia buttonweed to recover after freezing damage to aboveground biomass. In contrast, 2,4-D + mecoprop +

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Table 2. Weed control 14 and 28 d after liquid carbon dioxide treatments with uninsulated cone apparatus and flat-fan nozzle (prototype II).[a,b]

		14 DAT					28 DAT				
		Perennial		Annual			Perennial		Annual		
Nozzle	Treatment[c]	TRFRE	DIQVI	DIGIS	DIGSA	ELEIN	TRFRE	DIQVI	DIGIS	DIGSA	ELEIN
	kg LCD m^{-2}	% control[d]									
XR8002E	5	33	0	15	27	24	65	0	8	23	20
	15	68	12	78	37	43	75	0	35	28	32
	30	94	42	98	58	58	91	0	86	48	56
XR8004E	10	38	0	68	25	30	74	0	30	23	20
	30	76	28	94	43	57	78	0	73	42	60
	60	97	76	100	78	71	91	0	94	78	70
	Herbicide[e]	21	31	12	53	49	83	44	100	77	84
	LSD[f]	5					5				
Annual vs. perennial[g]		$P < 0.0001$					$P < 0.0001$				

[a] Research conducted at the Lake Wheeler Turfgrass Field Laboratory (Raleigh, NC).

[b] Abbreviations: DAT, days after treatment; LCD, liquid carbon dioxide; TRFRE, white clover; DIQVI, Virginia buttonweed; DIGIS, smooth crabgrass; DIGSA, large crabgrass; ELEIN, goosegrass.

[c] LCD exposure times: XR8002E: 5, 15, or 30 s (5, 15, or 30 kg LCD m^{-2}, respectively); XR8004E: 5, 15, or 30 s (10, 30, or 60 kg LCD m^{-2}, respectively).

[d] Control was visually estimated on 0 to 100% scale (0% = no plant injury; 100% = complete plant death).

[e] Quinclorac (0.8 kg ha^{-1}) + methylated seed oil (2.1 L ha^{-1}) for TRFRE, DIQVI, DIGIS, and DIGSA; fenoxaprop (0.1 kg ha^{-1}) + nonionic surfactant (0.25% v v^{-1}) for ELEIN.

[f] LSD values for comparison within DAT.

[g] P-value obtained from orthogonal contrast.

dicamba are systemic herbicides that generally provide better perennial weed control presumably due to their systemic activity.

Prototype II. The LCD output was reduced with flat-fan nozzles XR8004E (2 kg LCD min^{-1}) or XR8002E (1 kg LCD min^{-1}) compared with prototype I (3 kg LCD min^{-1}). A treatment-by-species interaction was detected at both evaluation dates (Table 2). At 14 and 28 DAT, excellent white clover control (> 90%) was obtained at the highest application rate with the XR8004E (60 kg m^{-2}) or XR8002E (30 kg m^{-2}) nozzle, whereas poor control (< 80%) was observed with all other rate and nozzle combinations. Poor Virginia buttonweed control was observed (≤ 76%) regardless of LCD rate or nozzle 14 DAT, whereas no injury symptoms were observed 28 DAT. The aforementioned results may be explained by species morphology; white clover has stolon death and little resurgence during the summer months after aboveground biomass destruction (Sanderson et al. 2003). In contrast, Virginia buttonweed has better resurgence during summer months because of high adventitious bud capacity, which allows greater plant emergence from root systems in hot climatic conditions (Baird et al. 1992).

At 14 DAT, excellent smooth crabgrass control (> 90%) was observed at two rates with the XR8004E nozzle (30 and 60 kg LCD m^{-2}) and one (30 kg m^{-2}) with the XR8002E nozzle; however, unacceptable control (< 80%) of other annual grass species was observed, with control ranging from 24 to 78% (Table 2). Similar trends were observed 28 DAT, with 94 and 86% smooth crabgrass control at 60 kg LCD m^{-2} (XR8004E) and 30 kg LCD m^{-2} (XR8002E), respectively. As with prototype I, annual weeds were more susceptible than perennial weeds ($P < 0.0001$).

Although comparisons were not made among prototypes, trends indicated that greater weed control could be achieved with less LCD when using prototype II; this may indicate increased efficiency with incorporated modifications (flat-fan nozzle and cone). For example, at 28 DAT, using prototype II white clover was controlled 74% with 10 kg LCD m^{-2} compared with only 23% when 16 kg LCD m^{-2} was applied via prototype I. It should

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Table 3. Large crabgrass control at various growth stages 14 and 28 d after liquid carbon dioxide treatments with insulated cone and full cone nozzle (prototype III).[a–c]

	14 DAT			28 DAT		
($kg\ LCD\ m^{-2}$)[d]	One- to two-leaf	Three- to four-leaf	One- to two-tiller	One- to two-leaf	Three- to four-leaf	One- to two-tiller
	% DIGSA control[e]					
0.4	24	23	24	26	24	28
1	31	37	35	64	37	34
2	79	63	56	79	68	55
5	93	92	66	96	92	64
LSD[f]	8			10		

[a] Research conducted at the Method Road Greenhouse Complex (Raleigh, NC).

[b] Full cone nozzle: 1/4T D1-33.

[c] Abbreviations: DAT, days after treatment; LCD, liquid carbon dioxide; DIGSA, large crabgrass.

[d] LCD exposure times: 0.5, 1.5, 3, or 5 s (0.4, 1, 2, or 5 kg LCD m^{-2}, respectively).

[e] Control was visually estimated on 0 to 100% scale (0% = no plant injury; 100% = complete plant death).

[f] LSD values for comparison within DAT.

also be noted that at 14 DAT white clover, Virginia buttonweed, and large crabgrass control was greater (18, 14, and 15%, respectively) with the flat-fan XR8002E nozzle as opposed to the XR8004E at the same LCD application rate (30 kg LCD m^{-2}). This trend was also observed in white clover and smooth crabgrass at 28 DAT (13% greater control). This may be attributed to the application duration of the flat-fan XR8002E nozzle (30 s) being double that of the XR8004E nozzle (15 s). Increasing freezing exposure duration may have increased plant mortality. Pearce (2001) detected rapid ice growth (4 to 40 mm s^{-1}) through plant cells and noted that this initial rapid growth is of great importance as it may cause lethal freezing at any site it reaches.

Prototype III. *Weed Control*. The final modification further reduced output (0.5 kg LCD min^{-1}) compared with prototype I (3 kg min^{-1}) and prototype II (1–2 kg min^{-1}). A treatment-by-growth stage interaction was detected at both evaluation dates (Table 3). In general, control increased as dwell time increased on both evaluation dates. Across evaluation dates, excellent one- to two- and three- to four-leaf large crabgrass control (> 90%) was observed at 5 kg LCD m^{-2}; however, control of one- to two-tiller plants was unacceptable (< 70%).

At 14 DAT, no differences in large crabgrass control were observed with 0.4 and 1 kg LCD m^{-2} between growth stages; however, with 2 kg LCD m^{-2}, control was greater at the one- to two-leaf stage compared with the three- to four-leaf or one- to two-tiller stage (Table 3). At the highest application rate (5 kg m^{-2}), no differences were detected 14 and 28 DAT between the one- to two-leaf (93 and 96%, respectively) and three- to four-leaf (92%) stages; however, less control was observed at the one- to two-tiller stage (66 and 64%, respectively). At 1 kg m^{-2}, one- to two-leaf stage plants were more susceptible than those at the three- to four-leaf and one- to two-tiller stage (64, 37, and 34%, respectively) 28 DAT. Further, with 2 kg LCD m^{-2}, control decreased as plant maturity increased, with one- to two-leaf, three- to four-leaf, and one- to two-tiller large crabgrass control measuring 79, 68, and 55%, respectively. These results are similar to those by Reed et al. (2013) who observed that smooth crabgrass control with the synthetic herbicides aminocyclopyrachlor and fenoxaprop was higher in the multileaf stage (59 and 94%, respectively) as compared with the multitiller stage (31 and 66%) 9 wk after treatment (WAT). Similarly, Brosnan et al. (2010) reported reduced control as plant maturity increased ,with dithiopyr providing 93, 85, and 45% control of one-leaf, one-tiller, and three-tiller smooth crabgrass, respectively, 10 WAT.

Turfgrass Tolerance. A treatment-by-species interaction was detected 7 and 14 DAT. In general, injury increased as LCD rate increased. Injury was similar across all species at the lowest LCD rate (0.7 kg LCD m^{-2}) at 7 (7 to 18%) and 14 DAT (2 to 10%) (Table 4). Trends in tall fescue and zoysiagrass injury were similar, as no differences were detected

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Table 4. Turfgrass injury 7 and 14 d after liquid carbon dioxide treatments with insulated cone and full cone nozzle (prototype III).[a–c]

	7 DAT				14 DAT			
	FESAR	POAPR	CYNDA	ZOYJA	FESAR	POAPR	CYNDA	ZOYJA
kg LCD m^{-2}[d]	% injury[e]							
0.7	17	7	12	18	10	5	5	2
1.5	30	33	40	33	10	23	8	17
2.1	47	53	50	28	30	20	18	17
3.0	50	53	63	37	23	33	25	22
3.7	43	80	83	37	20	53	40	30
LSD[f]	19				14			

[a] Research conducted at the Lake Wheeler Turfgrass Field Laboratory (Raleigh, NC).

[b] Full cone nozzle: 1/4T D1-33.

[c] Abbreviations: DAT, days after treatment; LCD, liquid carbon dioxide; FESAR, tall fescue 'Confederate'; POAPR, Kentucky bluegrass 'Unique'; CYNDA, hybrid bermudagrass 'Tifway 419′'; ZOYJA, zoysiagrass 'El Toro'.

[d] LCD exposure times: 1, 2, 3, 4, or 5 s (0.7, 1.5, 2.1, 3, or 3.7 kg LCD m^{-2}, respectively).

[e] Injury was visually estimated on 0 to 100% scale (0% = no plant injury; 100% = complete plant death).

[f] LSD values for comparison within DAT.

between species (excluding 2.1 kg LCD m^{-2} at 7 DAT). At 7 DAT bermudagrass and Kentucky bluegrass injury was $>$ 36% more than tall fescue or zoysiagrass at the highest evaluated rate (3.7 kg m^{-2}). This may be explained by comparing temperature tolerances between the species. Of the evaluated species, bermudagrass and Kentucky bluegrass have the poorest cold and heat tolerances, respectively (Turgeon 1999). Consequently, bermudagrass may have been most adversely affected by the LCD treatment, whereas high summer temperatures (high temperature ranges 30 to 39 C) during experimentation may have limited the recovery of Kentucky bluegrass.

Although tall fescue and zoysiagrass injury was observed at 14 DAT, it was $\leq$ 30% regardless of LCD rate (Table 4). However, LCD rates evaluated did not provide adequate large crabgrass control (Table 3). Kentucky bluegrass (53%) and bermudagrass injury (40%) at the highest LCD rate (3.7 kg LCD m^{-2}) was considered unacceptable. Kentucky bluegrass was injured $\geq$ 23% more than tall fescue and zoysiagrass at the highest LCD rate. Injury was not observed after 14 DAT for any species; however, turfgrass thinning was observed at 28 DAT, with Kentucky bluegrass cover measuring significantly less than bermudagrass, zoysiagrass, and tall fescue, which may be attributed to Kentucky bluegrass' heat tolerance (data not shown) (Turgeon 1999).

In conclusion, adequate weed control of some species, primarily annual weeds, was obtained after LCD application via Frostbite. Modifications to this technology allowed for similar control to be achieved with less LCD. However, further improvements to Frostbite are needed to reduce economic and environmental impacts. On the basis of this research, a 5 kg LCD m^{-2} rate delivered with prototype III (lowest application rate providing excellent control of leaf-stage large crabgrass) would require 250 kg of LCD per 50 m^2. Additionally, 5 s was required to apply this rate, which will increase time per application by 10 (assuming 0.5 s for spot application with herbicide). This amount of LCD per application may not be economically sound in many settings. Further, the addition of this amount of a greenhouse gas per application may pose environmental concerns as it has been indicted for a rise in global temperatures (Montzka et al. 2011). These data suggest that LCD has the potential to provide control of select weed species where synthetic herbicides are not allowed or desired. Additional research is needed to improve Frostbite system efficiency (materials, setup, nozzles, etc.) and evaluate various techniques before or after treatment to reduce LCD required for acceptable weed control.

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