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Interspecific Differences in Weed Susceptibility to Steam Injury

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Thermal weed control methods have been incorporated into weed control programs in organic and conventional production systems. Flaming is commonly used, but steaming has been proposed to increase efficiency of heat transfer to weeds and reduce the risk of fire. The objective of this research was to measure injury to leaves of plant species that differ in leaf morphology and to measure injury to plants at different stages of plant development. The study was conducted in a glasshouse and plants were exposed to steaming at 400 C for 0.36 s—equivalent to a steaming speed of 2 km/h. Overall, leaf thickness was the best morphological characteristic to predict injury (r^2 = 0.51), with greater thickness resulting in less injury. For broadleaf species only, species with wider leaves were injured more than species with narrower leaves (r^2 = 0.64). Injury was greatest when plants had fewer than six true leaves and when their shoots were less than 10 cm long. There was a wide range of injury across species, and the grass species bermudagrass and perennial ryegrass were injured (68 to 81%) more than other species such as common purslane and English daisy (23 to 34%). Biomass of all species tested was reduced by approximately 40%, indicating that leaf injury was not the sole effect of steaming on plant growth. These results indicated that considering both visual estimates of injury and morphological characteristics is important to properly assess thermal weed control effectiveness.

Nomenclature: Bermudagrass, *Cynodon dactylon* (L.) Pers.; common purslane, *Portulaca oleracea* L.; English daisy, *Bellis perennis* L.; perennial ryegrass, *Lolium perenne* L.

Key words: Alternative weed management, steaming, heat, flaming, organic, physical, thermal, weed control.

Thermal approaches are being reconsidered for commercial weed control due to increasing organic production acreage, concerns about pesticide toxicity and public health, and the pervasive appearance of herbicide-resistant weeds (Ascard 1994; Bond and Grundy 2001; Hansen et al. 2004; Melander et al. 2005; Timmons 2005).

The key for efficacious results from thermal weed control is to transfer energy effectively from the heating device to the plant cell in order to raise the temperature to at least 58 C and cause irreversible damage to cell and organelle membranes (Daniell et al. 1969; Sirvydas et al. 2006). The challenge of transferring heat to plant tissue in a cost-effective manner has promoted the design and development of different equipment with various heat transfer mechanisms (e.g., air, steam, water, radiation, etc.) and heat generation methods while using the minimum amount of fuel.

Most of the information available for thermal weed control refers to flaming, but also to other tactics such as hot water; steaming; and microwave, infrared, and laser radiation (Ascard 1998a; Bond and Grundy 2001; Sartorato et al. 2006). Among these tactics, the use of steaming promises to be useful because it efficiently transfers heat (Rask and Kristoffersen 2007) and reduces fire risk, especially in areas where dry plant residue is present (Hansson and Ascard 2002). However, there is limited information about the factors that determine the effectiveness of steaming as a POST weed control tactic. Ascard (1994, 1995) conducted a series of detailed studies to determine the importance of plant size, developmental stage, and density on the effectiveness of flaming for POST weed control. This research clearly showed that smaller and younger plants were more susceptible to heat damage, and that weed density played a minor role on flaming effectiveness. In addition, Ascard (1995) explained that for effective weed killing, the meristems of the plant must be exposed to the heat directly. Otherwise, only the leaf tissue is injured, and the plant can recover by producing new growth from unharmed meristems. For this reason, he used the criterion of meristem exposure based on plant morphology to predict the susceptibility of a species to thermal weed control.

Because the efficiency of thermal weed control relies on heat transfer, it is important to evaluate how the morphology and physiology of the plant can influence this process. Therefore, identifying morphological characteristics that are related to heat injury could be useful to predicting the likelihood of thermal weed control success. However, species differ in their morphology, so weed community composition must be considered in conjunction with other predictors (Hanson and Ascard 2002). Several plant morphological characteristics including pubescence, high levels of lignification, and wax and water content have been reported as important for thermal weed control tolerance (Ascard 1995; Hansson and Ascard 2002), but it is not clear how they relate to tissue injury and its resulting effect on plant growth. Furthermore, factors such as leaf morphology (e.g., shape, width, length, area, etc.) have not been considered in detail although they might directly affect heat interception and transfer, thus potentially influencing heat injury susceptibility.

The objective of this study was to determine how plant developmental stage and interspecific differences in leaf morphology affect susceptibility to steam injury.

Materials and Methods

Experiments were conducted in a glasshouse in San Luis Obispo, CA from October 2006 to May 2007. The

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Table 1. Leaf morphological characteristics of seven weed species.[a]

Weed species	Length	Width	Area	Thickness
	mm		mm^2	μm × 10
Bermudagrass	33 ± 12[b]	3 ± 2	72 ± 62	19 ± 15
Bristly oxtongue	67 ± 16	16 ± 7	750 ± 213	51 ± 30
Common mallow	26 ± 8	30 ± 8	602 ± 145	38 ± 30
Common purslane	18 ± 7	11 ± 6	146 ± 87	93 ± 44
English daisy	20 ± 7	13 ± 6	170 ± 81	32 ± 25
Perennial ryegrass	73 ± 11	3 ± 2	151 ± 60	24 ± 25
White clover	16 ± 7	22 ± 7	202 ± 95	18 ± 16

[a] Measurements were conducted on the first three fully developed leaves of untreated plants.
[b] Average of 20 plants ± SE.

temperature varied between 20 and 30 C, and the sunlight intensity reached 620 $\mu mol/m^2/s$ at zenith.

Seven weed species locally considered as problematic in agricultural and urban areas were chosen for their different leaf characteristics (Table 1). These species were bermudagrass, bristly oxtongue (*Picris echioides* L.), common mallow (*Malva neglecta* Wallr.), common purslane, English daisy, perennial ryegrass, and white clover (*Trifolium repens* L.). The seeds of these species were collected during the summer of 2006 from local populations, air dried at 25 C and stored until used.

The treatments tested were steaming vs. no steaming at three different developmental stages: seedling, three to five leaves (L3–5) and six or more leaves (L6). Plants with cotyledons and/or two true leaves and a shoot length of less than 5 cm were included within the seedling stage. The L3–5 stage was comprised of plants with a shoot length between 5 and 10 cm. Finally, plants in the L6 stage showed a shoot length greater than 10 cm. In order to achieve these developmental stages the seeds of the different species were planted in 400-ml pots filled with sterilized soil at intervals of 2 to 3 wk. The pots were watered daily to maintain field capacity. When the plants reached the desired size and developmental stage, they were steamed at 400 C for 0.36 s with a steaming unit[1] pulled by a tractor at 2 km/h, releasing steam at 6 cm above the plants. This temperature was chosen because it was the highest temperature achieved, maintaining a small variation of ± 10 C. Next, the plants were returned to the greenhouse to the growing conditions described above.

Shoot length and visual injury caused by the steaming (i.e., stunting, chlorosis, and necrosis) were determined 1, 3, 7, and 14 d after treatment (DAT). Immediately after this last evaluation, the plants were harvested by clipping their aboveground structures, dried in an oven at 65 C for 2 d, and then weighed. In order to determine leaf thickness, the first three fully developed leaves of an untreated plant were clipped, and a transversal cut was made in the widest point of the leaf. Leaf blade thickness was measured between the middle vein and the leaf blade edge using a microscope equipped with an ocular micrometer.

The experiment was arranged as a completely randomized design with four replications and was conducted twice. The data met the assumptions of normality and homogeneity of variances. Data were analyzed using the GLM model to conduct ANOVA and regression analysis (SAS 1998). Treatment mean separation was done with Tukey's Studentized test ($\alpha = 0.05$).

Results and Discussion

No significant interactions between species and developmental stage or between experiment repetition and any other factor were observed. Therefore, the experiments were combined for the analyses, and the data are presented without interactive effects.

Injury caused by the steam was observed in some plants as early as 3 h after treatment, when the plants showed signs of stunting and necrosis (data not shown), and at 1 DAT all species were clearly injured (Table 2). Maximum injury was observed at 3 DAT, and after this point, most plants started to recover, although none of the species had recovered completely by 14 DAT.

No significant interaction between species and developmental stage was observed in response to steaming (Table 2 and 3). However, most of the P-values for these interactions for the different variables were close to the level of significance ($0.12 \geq P > 0.05$) because in the case of the grass species, the differences in injury and growth among developmental stages were smaller than in the rest of the weed species. In general, plants treated at the seedling and L3–5 stages were injured and reduced in size more than plants at the L6 stage (Tables 2 and 3). Larger plants had less tissue injury due to a "shading" effect in which the leaves at the top of the plant were directly exposed to the steam and showed significant injury, but also acted as shields protecting leaves located underneath them. In addition, older plants with lateral growth (i.e., tillers in grasses and branches in broadleaf species), could produce new growth from lateral buds when the main growing axis was damaged. It must be mentioned that this new growth was not considered for the visual injury estimate, so the reduction in injury by 14 DAT was due to the recovery of steamed leaves. Based on our results, weeds should be steamed before they have six true leaves, but because we studied tissue injury and growth, and not mortality, these results may not apply to field conditions. Other studies that considered mortality found that weeds should be controlled between the cotyledon and the two–true leaves stage. Otherwise, the effectiveness of the thermal treatment is reduced, and an increase in fuel consumption is required (Ascard 1994, 1995).

Table 2. Visual steam injury at 1, 3, 7, and 14 d after treatment (DAT) for seven weed species at different developmental stages: seedling, three to five leaves (L3–5), and six or more leaves (L6).

Treatment	1 DAT	3 DAT	7 DAT	14 DAT
	Injury (%)			
Bermudagrass	72 ± 6[a] a[b]	81 ± 4 a	71 ± 6 a	65 ± 6 a
Bristly oxtongue	39 ± 7 bcd	37 ± 7 bcd	31 ± 7 cd	26 ± 6 bcd
Common mallow	60 ± 13 ab	62 ± 11 ab	59 ± 10 ab	31 ± 10 bcd
Common purslane	25 ± 7 d	23 ± 6 d	24 ± 6 d	9 ± 4 d
English daisy	31 ± 5 cd	34 ± 6 cd	27 ± 5 d	21 ± 4 cd
Perennial ryegrass	63 ± 6 ab	68 ± 6 a	56 ± 5 abc	48 ± 5 ab
White clover	53 ± 7 abc	56 ± 6 abc	43 ± 6 bcd	37 ± 6 bc
Seedling	54 ± 5 a	59 ± 5 a	49 ± 5	54 ± 5
L3–5	49 ± 5 ab	48 ± 5 ab	43 ± 5	49 ± 5
L6	40 ± 4 b	45 ± 5 b	38 ± 4	40 ± 4
	P-value			
Species	< 0.001	< 0.001	< 0.001	< 0.001
Developmental stage	0.04	0.05	0.13	0.16
Species by developmental stage	0.12	0.06	0.07	0.07

[a] Average of eight replications ± SE.

[b] Species or developmental stages with the same letter within columns are not statistically different based on Tukeys Studentized test ($\alpha = 0.05$).

The injury caused by steaming varied across species ranging from 23 to 81% at 3 DAT (Table 2). It must be noted that the temperature used in this experiment was lower than temperatures reported as necessary to cause high levels of weed mortality and leaf tissue injury across several species (i.e., > 800 C), so the level of control of the studied species can be improved by increasing steam temperature or decreasing steaming speed (Ascard 1997, 1998a, 1998b). Bermudagrass, perennial ryegrass, and common mallow were the species that showed the highest injury levels, while common purslane and English daisy showed limited signs of injury. Previous reports indicated that grasses were more tolerant to flaming than broadleaf species, but this tolerance was attributed to the ability to produce new growth from creeping vegetative reproductive structures or protected meristems, rather than the capacity of the leaf tissue to withstand heat damage (Ascard 1995).

Table 3. Effect of steaming on shoot length and biomass reduction of seven weed species at different developmental stages: seedling, three to five leaves (L3–5) and six or more leaves (L6), determined 14 d after treatment (DAT).

Treatment	Shoot length	Biomass
	% Based on untreated control	
Bermudagrass	66[a] ± 4 b[b]	54 ± 7
Bristly oxtongue	88 ± 5 ab	69 ± 9
Common mallow	80 ± 11 ab	71 ± 13
Common purslane	97 ± 6 a	64 ± 8
English daisy	79 ± 4 ab	61 ± 6
Perennial ryegrass	69 ± 3 b	71 ± 4
White clover	69 ± 4 b	59 ± 7
Seedling	75 ± 3 b	60 ± 5 ab
L3–5	72 ± 4 b	54 ± 5 b
L6	86 ± 3 a	73 ± 4 a
	P-value	
Steaming	< 0.001	< 0.001
Species	0.04	0.48
Developmental stage	0.01	0.01
Species by developmental stage	0.54	0.07

[a] Average of eight replications ± SE.

[b] Species or developmental stages with the same letter within columns are not statistically different based on Tukeys Studentized test ($\alpha = 0.05$).

Although there was a clear difference in the susceptibility of leaf tissue to steaming, differences across species in reductions in shoot length and biomass were minimal, having a more evident impact on biomass for which the reduction was approximately 40% in comparison with untreated plants (Table 3). These results suggested that the reductions in growth were not exclusively a consequence of a decreased leaf area due to necrosis or desiccation (Ascard 1995), and that the plants suffered other injuries not visually evident. Daniell and coworkers (1969) studied the effect of lethal and sublethal temperatures on plant cells, and they found that although plasmolysis occurred as a clear consequence of high temperatures (56 to 57 C), this was not the main cause of cell death, which was attributed to the loss of cell membrane integrity and function. In addition, at nonlethal temperatures (51 to 53 C), they observed a series of changes such as modifications in cell and organelle membrane permeability, "coagulated" cytoplasm, reduction in protoplasmic streaming and swelling, and bleaching of chloroplasts. This type of transient disruption of cellular functioning in the absence of external signs of injury could explain why common purslane, which had very limited visual injury, had biomass reductions similar to bermudagrass, which had more than 80% injury.

The fact that thermal injury can significantly reduce weed growth even in the absence of visual injury has important practical implications. For example, in agricultural systems that do not require complete weed elimination (e.g., perennial and semiperennial crops, or crops with dense tall canopies), a thermal treatment to delay weed growth integrated with other tactics could be enough to provide the needed level of weed control and even reduce the need for high fuel consumption (Bond and Grundy 2001). However, using thermal control tactics might have unintended negative effects on beneficial arthropods (Hatcher and Melander 2003). Thus, complete

Table 4. Linear relationship between leaf morphological characteristics and visual injury caused by steaming determined at 3 d after treatment (DAT).[a]

Leaf characteristic	Slope	Intercept	r^2	P-value
Width				
All species only	−3.5	56.4	0.03	0.035
Broadleaf species only	14.0	16.5	0.64	< 0.001
Length	1.5	46.2	0.04	0.022
Area	−0.9	54.4	0.02	0.075
Thickness	−53.0	72.3	0.51	< 0.001

[a] The linear regression analysis was conducted with $n = 140$ ($n = 20$ per weed species). Different regression models were tested, but the linear model best described the data.

weed removal using thermal approaches would not be energetically or ecologically justified in several agricultural systems, especially where reductions in crop yield due to weed competition would not threaten the profitability of the system (Norris and Kogan 2000).

The large range in visual injury observed across weed species prompts the question about what it is that determines the susceptibility of a species to steam injury. The answer to this question is critical to predicting the effectiveness of steaming for POST control. Using linear regression analysis to explore the relationship between steam injury and different leaf morphological characteristics, it was determined that leaf thickness was the trait that best explained the variation (51%) in visual injury (Table 4). Thus, above 500 µm thickness, injury was 35% or lower, and when leaf thickness was below 300 µm, injury was at least 56% (Ascard 1994, 1995). Another characteristic that explained steaming injury variation was leaf width. However, this was only the case when considering broadleaf species alone, because unlike the grass species that had only narrow leaves (3 mm) and were highly injured (60 to 85%), for broadleaf species, wider leaves were injured more than narrow leaves (Table 1 and 2). When excluding the grass species from the regression analysis, leaf width explained more than 60% of the steaming injury variation (Table 4). This result suggests that wider leaves increase the surface area for steam interception, facilitating heat transfer. Nevertheless, there was no relationship between leaf area and injury. This apparent contradiction is easily explained by the fact that while the leaves of bristly oxtongue, English daisy, and common mallow have a large area, they are also relatively long, and their blades and petioles tend to bend. Because of this bending, the effective area of steaming interception was reduced. We also looked at the relationship between leaf thickness and width, but this was very low (less than 15%; data not shown). Therefore, it seems that leaf thickness and width affect the susceptibility to steaming in different ways.

The importance of leaf thickness was evident not only across species but also in individual leaves. In the case of the species with thicker leaves such as bristly oxtongue, common purslane, and English daisy, most of the injury (total necrosis) occurred on the edges where leaf blades were thinner. In addition, it was noticed that towards the middle vein where the blades are thicker, there was some tissue darkening on the adaxial side of the leaf, but no injury on the abaxial side. Studies using infrared radiometry to measure leaf temperature during thermal treatments showed that thicker parts of the leaves—and especially veins—tended to increase their temperature at lower rates than the thinner parts of the leaf blade, and differences of up to 50 C were observed between those tissues in the same leaf (Rahkonen and Jokela 2003). Thus, leaf blades with more protected dorso-ventral cell layers will be more capable of maintaining tissue structure because only external cell layers will collapse after the thermal treatment. This type of heat injury protection is more effective when the external cell layers are under a thick protective cuticle (Ascard 1995). All these characteristics might explain why species with thicker leaves showed less visual tissue injury.

The results of this research indicate that across all species, leaf thickness had a strong inverse relationship on steaming injury susceptibility. In addition, for broadleaf species, wider leaves had injury levels after steaming that were almost double those observed in narrower leaves. Regardless of visually apparent injury, all species suffered a 40% reduction in growth, even at temperatures that were lower than recommended in the literature for proper thermal control (Ascard 1997). Our discussions with farmers in California suggest fuel costs and low speed of treatment may limit the use of steam for weed control. Thus, it is important for future research to determine if sublethal steaming will reduce weed interference, and result in a cost-effective integrated weed management practice.

Sources of Materials

[1] Stinger, D. J. Batchen, Pty. Ltd., New South Wales, Australia.

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