

Herbicides Tolerated by Cuphea (*Cuphea viscosissima* × *lanceolata*)¹

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Abstract: Partial seed retention line #23('PSR23') cuphea is a hybrid of *Cuphea viscosissima* × *C. lanceolata*. It is a new, spring-planted, annual, potential oilseed crop that is highly susceptible to interference by weeds because of its slow growth during spring and early summer. Grass weeds are controlled easily in this broadleaf crop, but broadleaf weeds are an appreciable problem. Consequently, several broadleaf herbicides were screened for tolerance by 'PSR23' cuphea. Broadleaf herbicides to which cuphea showed tolerance in a spray cabinet and a greenhouse were tested in a field setting for 2 yr. Field tolerance was considered as absence of negative impact ($P > 0.05$) both years to any of four measured traits: overall vigor, dry weight, stand density, and time to anthesis. Cuphea showed tolerance in the field to three soil-applied herbicides (ethalfluralin, isoxaflutole, and trifluralin) and one postemergence herbicide (mesotrione). A few combinations of soil-applied and postemergence herbicides did not damage cuphea. These combinations were ethalfluralin followed by (fb) mesotrione, isoxaflutole fb imazethapyr, and isoxaflutole fb mesotrione. Availability of these herbicides for use in cuphea production may facilitate the domestication and acceptance of this new crop.

Nomenclature: 'PSR23' cuphea, *Cuphea viscosissima* Jacq. × *C. lanceolata* f. *silenooides* W. T. Aiton.

Additional index words: Capric acid, ethalfluralin, isoxaflutole, imazethapyr, lauric acid, mesotrione, oilseed, PSR23, trifluralin.

Abbreviations: fb, followed by; MCFA, medium chain fatty acid; PA, plant-applied; PPI, preplant incorporated; 'PSR23', partial seed retention line #23; SA, soil-applied.

INTRODUCTION

About 1 million tons of medium chain-length fatty acids (MCFAs), such as capric acid (C10:0) and lauric acid (C12:0), are used annually for industrial purposes, particularly in the manufacture of lubricants and detergents. Presently, all plant-derived MCFAs are produced from tropical palms. There are no temperate plant sources of MCFAs that are suitable from an agronomic perspective, except *Cuphea* (Hirsinger 1985; Knapp 1993), a genus within the Lythraceae.

The cuphea variety 'PSR23' (Partial Seed Retention line #23) was developed by Knapp and Crane (2000) and is a cross between *C. viscosissima* and *C. lanceolata*. The former species is native to the eastern United States,

and the latter species is native to Mexico. The purposeful hybridization of these two annual species and subsequent selection resulted in a genetic line with superior agronomic traits, which included reduced seed dormancy and seed shattering, and greater self-fertility. 'PSR23' is still only semidomesticated, as its varietal name implies. However, this variety grows well in temperate zones (Gesch et al. 2002, 2003), and is the anticipated forerunner of improved commercial varieties.

Slow initial growth in spring and early summer makes cuphea susceptible to interference from summer-growing weeds. Weed control tactics for cuphea are required to facilitate ongoing agronomic and breeding research, as well as eventual commercialization of this crop. These tactics must be compatible with contemporary weed management systems in the highly productive cropping regions of the northern United States. Preliminary experiments conducted in Illinois and Minnesota with soil-applied (SA) herbicides of varying modes of action in greenhouse and field trials have suggested that some classes of herbicides are potentially suitable for cuphea production. However, little was known about cuphea tol-

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Table 1. Dates and cumulative growing degree days for management and measurement events for cuphea herbicide tolerance experiments in 2003 and 2004.^a

Event	2003	2004
Seedbed preparation	June 2	June 17
Fertilizer application	July 2	June 17
PPI herbicide applications, harrow, sowing, and PRE applications	July 2	June 22
PA applications	July 26	July 23
GDD after sowing	263	316
Visual tolerance rating	August 8	August 10
GDD after PA	151	166
Dry weight and stand determination	September 5	September 2
GDD after PA	484	311
GDD after sowing	748	627

^a Abbreviations: GDD, growing degree days; PA, plant-applied; PPI, pre-plant incorporated; PRE, pre-emergence.

erance to postemergence or plant-applied (PA) herbicides. Therefore, the objectives of this study were to screen several SA and PA herbicides for tolerance by ‘PSR23’ cuphea.

MATERIALS AND METHODS

Greenhouse Screening. The sole purpose of greenhouse screening was to assess which of more than 30 herbicides merited field testing. Thus, only a cursory description of greenhouse screening methods is presented here. A cabinet sprayer was used for all herbicide applications, and each herbicide was applied at a range of rates with the highest typically being the label rate for other crops (e.g., Gunsolus et al. 2003; Zollinger et al. 2004). About half of the herbicides screened in the greenhouse were tested only once and were dismissed from further consideration, whereas the remainder was tested at least twice. Both SA and PA herbicides were examined. At 2

to 4 wk after treatment, visual inspection of vigor, height, leaf number, or dry weight was used to determine that four SA herbicides and three PA herbicides were worthy of field testing.

Field Testing. The field site was located at the U.S. Department of Agriculture Agricultural Research Service Swan Lake Research Farm near Morris, MN. In both 2003 and 2004, the soil was a Barnes loam (Calcic Hapludoll, fine-loamy, mixed, superactive, frigid; with 6% organic matter, a bulk density of about 1.0 g/cm³, and a pH of 6.8) that was chisel-plowed and field-cultivated. Fertilizer requirements of cuphea are unknown, so to ensure adequate fertility, each year the soil received the equivalent of 112, 13, 30, and 52 kg/ha of nitrogen, phosphorous, potassium, and sulfur, respectively. The previous crop was wheat both years. Dates for management and sampling events are listed in Table 1. The experiments were purposefully performed late with respect to sowing and herbicide applications so that higher soil and air temperatures would facilitate uniform seedling emergence and growth, which often is variable for early sown ‘PSR23’ cuphea.

To examine tolerance of cuphea to both SA and PA herbicides, alone and in sequence with one another, a lattice experimental design was used (Table 2). The lattice permitted easy and effective application of herbicides, but it lost some statistical power (see below). In brief, two contiguous blocks of strip plots were established in an east-west direction. In each block, one strip plot was assigned randomly to each SA herbicide as well as a nontreated check. Superimposed upon these east-west blocks were two north-south blocks, each of which also contained strip plots that were assigned randomly to each of the PA herbicides as well as a nontreated check. Consequently, each SA herbicide was tested alone in four plots and tested in combination with each PA herbicide in unique sets of four plots each. Similarly, each PA herbicide was tested alone in four plots and tested in combination with each SA herbicide in different sets of four plots each. Because each herbicide was applied in strips spanning several plots, the errors associated with continual starting and stopping of sprayers in traditional small-plot research were minimized. Finally, four plots received no herbicide and represented the nontreated checks. Each plot was 1.53 m long and 1.53 m wide.

The SA herbicides that were tested were those that appeared promising from the greenhouse screening experiments. These were ethalfluralin preplant incorporated (PPI), isoxaflutole PRE, mesotrione PRE, and trifluralin

Table 2. Example of an idealized “lattice” design for testing three soil-applied herbicides (upper case letters) and four plant-applied herbicides (lower case letters) alone and in combination.^a

X₁a₁	Z ₁ a ₁	Y ₁ a ₁	Y ₂ a ₁	X₂a₁	Z ₂ a ₁
X ₁ d ₁	Z ₁ d ₁	Y ₁ d ₁	Y ₂ d ₁	X ₂ d ₁	Z ₂ d ₁
X ₁ b ₁	Z ₁ b ₁	Y ₁ b ₁	Y ₂ b ₁	X ₂ b ₁	Z ₂ b ₁
X ₁ c ₁	Z ₁ c ₁	Y ₁ c ₁	Y ₂ c ₁	X ₂ c ₁	Z ₂ c ₁
X ₁ d ₂	Z ₁ d ₂	Y ₁ d ₂	Y ₂ d ₂	X ₂ d ₂	Z ₂ d ₂
X ₁ c ₂	Z ₁ c ₂	Y ₁ c ₂	Y ₂ c ₂	X ₂ c ₂	Z ₂ c ₂
X₁a₂	Z ₁ a ₂	Y ₁ a ₂	Y ₂ a ₂	X₂a₂	Z ₂ a ₂
X ₁ b ₂	Z ₁ b ₂	Y ₁ b ₂	Y ₂ b ₂	X ₂ b ₂	Z ₂ b ₂

^a For example, the soil-applied herbicides are sprayed first in strip plots, each one in two strips running up and down. The strip plots are assigned randomly within each of two blocks (subscripts 1 and 2). The plant-applied herbicides are sprayed next, each one in two strip plots running left to right. These strip plots are also assigned randomly within each of two blocks (subscripts 1 and 2). Thus, each plant-applied herbicide is oversprayed onto each soil-applied herbicide four times (i.e., four replicates). As an example, note herbicide combination “X a” in bold. Diagonal replicates can be averaged (e.g., [X₁ a₁ + X₂ a₂]/2 and [X₁ a₂ + X₂ a₁]/2) to meet the assumptions of parametric statistics, resulting in two replicates for analysis.

PPI applied at 840, 80, 105, and 840 g ai/ha, respectively. Treatments were applied with a CO₂-pressurized backpack sprayer equipped with a 1.53 m-boom and flat-fan nozzles calibrated to deliver 187 L/ha water at 207 kPa pressure. Clomazone PPI (1,240 g/ha) and sulfentrazone PRE (263 g/ha) also were tested in 2004 without prescreening in the greenhouse. Once the PPI herbicides were applied, the entire experimental area was rototilled lightly to a depth of 5 cm. Subsequently, the soil was packed to create a firm seedbed, and 'PSR23' cuphea seeds were sown at a rate equivalent to 1,000 seeds/m². Sowing depth was 1 cm and rows were separated by 61 cm, which allowed two rows of cuphea in each plot. PRE herbicides were applied (as above) after planting. Incorporation and activation of SA herbicides, and rapid cuphea seed germination, were facilitated by timely rains within 5 d of planting: 33 mm in 2003 and 15 mm in 2004.

The PA herbicides that were tested were those that appeared promising from the greenhouse screening experiments. These were imazethapyr, imazaquin, and mesotrione, each at 70 g/ha. Treatments were applied using the previously described equipment. Adjuvants (ammonium sulfate, crop oil concentrate, nonionic surfactant, and liquid nitrogen fertilizer) were added as per label requirements. In 2004, mesotrione also was applied post-emergence at 105 g/ha. All PA applications within a year were made on the same date (Table 1), at which time the percentage of cuphea plants in the two-leaf pair, three-leaf pair, and branching stages of growth were about 27, 14, and 54% in 2003; and 25, 21, and 51% in 2004. In 'PSR23' cuphea, axial branches begin growing after the three-leaf to four-leaf pair stage is reached. Plots were hand-weeded as needed throughout the growing season.

Cuphea tolerance to herbicides was assessed by four criteria. First, vigor was determined visually 2 wk after PA treatments. Visual assessment was performed by comparing overall plant vigor in a plot with nontreated plants within the same block. Scores ranged from 0 (dead) to 10 (most vigorous). Second, dry weight assessment was performed 8 to 11 wk after PA treatment by clipping all plants at ground level within two central 0.5-m lengths of row within each plot, and drying the plants to a constant weight at 66 C. Third, stand densities were determined by counting the clipped plants. Fourth, plots were assessed every 2 to 3 d for the presence of flowers after the first observation of cuphea flowering within the entire experiment. Days from seeding to initial flowering were recorded. Seed yield was not determined.

Daily rainfall and air temperature were recorded at a

weather station within 200 m of the experimental site. Summer temperatures were higher in 2003 than in 2004, which were reflected in differences between years in the accumulation of growing degree-days (base 10 C) from specific management to measurement events (Table 2). Consequently, attempts were made to alter dates of measurements to minimize differences in thermal time accumulation for these events between years.

Statistical Analysis. The lattice design used in the field tests allowed quick and effective application of herbicides, and every treatment was replicated in four plots. An outline of the locations of each set of four plots per treatment always formed a quadrangle. However, this lattice design also created a statistical dilemma in that the location of each plot within a treatment was not independent from two other plots in that treatment (Table 2). To overcome this lack of independence, data were aggregated from plots that opposed one another diagonally. This resulted in a completely random statistical design in which there were two replications instead of four, but each replicate was independent of the other, and the assumptions of ANOVA could be met. Tukey's honestly significant difference ($P = 0.05$) was calculated for comparisons between treatment pairs (Anonymous 1997).

Because visual ratings could vary only within a range from 0 to 10, the values were divided by 10 and arcsine-transformed prior to ANOVA. Ratings were back-transformed for presentation in Table 3. Data for dry weights, stand densities, and flowering times were not transformed because they were not constrained, and they varied homogeneously ($P > 0.05$) according to Bartlett's test of equal variances (Anonymous 1997). The effect of experimental year was tested (ANOVA) for these 20 treatments and was significant ($P < 0.05$) for each variable. Consequently, treatment means were compared separately for each year. Linear regression was used to explore relationships between visual ratings and other measurements.

RESULTS AND DISCUSSION

Greenhouse Screening. The following herbicides examined in the greenhouse were found to reduce cuphea growth or vigor significantly compared to that of nontreated plants and, consequently, were not tested in the field: acifluorfen, bentazon, bentazon + acifluorfen, bromoxynil, clopyralid, dicamba, dimethenamid, flumetsulam, flumetsulam + clopyralid, flumiclorac, fluroxypyr, fomesafen, imazapic, imazapyr, lactofen, metribuzin, MCPA, propanil, prosulfuron, pyridate, thifensulfuron,

Table 3. Visual tolerance rating, dry weight, stand density, and time of anthesis (days after planting) of cuphea 'PSR23' as influenced by herbicide treatments.^a

Herbicide treatment		Visual tolerance		Dry weight		Stand density		Anthesis time	
Soil-applied	Plant-applied	2003	2004	2003	2004	2003	2004	2003	2004
		— Scale 1–10 (arcsine) —		g/m ²		Plants/m ²		Days	
None	None	8.5 (1.13)	8.4 (1.12)	450	489	210	113	64	51
None	Imazaquin	4.4 (0.47)	3.2 (0.33)	320	53	198	62	73	>80
None	Imazethapyr	6.8 (0.81)	7.5 (0.93)	358	283	234	82	67	52
None	Mesotrione	8.5 (1.13)	8.4 (1.12)	517	417	236	137	65	53
Ethalfuralin	None	8.5 (1.13)	8.5 (1.13)	573	456	193	82	65	48
Ethalfuralin	Imazaquin	3.7 (0.38)	3.0 (0.30)	341	55	179	60	77	>80
Ethalfuralin	Imazethapyr	6.6 (0.78)	7.0 (0.85)	501	343	190	95	66	53
Ethalfuralin	Mesotrione	7.8 (0.98)	7.5 (0.93)	543	355	191	93	64	51
Isoxaflutole	None	9.3 (1.35)	8.2 (1.07)	654	426	174	78	63	51
Isoxaflutole	Imazaquin	3.0 (0.30)	2.7 (0.28)	281	44	137	42	77	>80
Isoxaflutole	Imazethapyr	7.5 (0.93)	7.1 (0.85)	541	280	151	76	64	54
Isoxaflutole	Mesotrione	8.2 (1.07)	7.7 (0.97)	548	393	172	94	64	51
Mesotrione	None	7.9 (1.02)	7.9 (1.02)	458	471	129	78	64	50
Mesotrione	Imazaquin	3.9 (0.41)	2.5 (0.25)	283	41	138	32	76	>80
Mesotrione	Imazethapyr	7.1 (0.85)	6.4 (0.74)	390	271	165	68	67	62
Mesotrione	Mesotrione	7.9 (1.02)	6.8 (0.81)	474	309	162	51	65	50
Trifluralin	None	7.9 (1.02)	8.0 (1.02)	565	512	142	98	65	49
Trifluralin	Imazaquin	3.9 (0.41)	2.7 (0.28)	316	47	141	46	75	>80
Trifluralin	Imazethapyr	6.8 (0.81)	6.2 (0.71)	511	216	164	51	67	52
Trifluralin	Mesotrione	8.2 (1.07)	6.4 (0.75)	484	259	111	55	64	52
HSD (P = 0.05) ^b		(0.46)	(0.31)	166	291	106	77	4	11

^a Values of treatments in bold do not differ from the maximum (or minimum for anthesis) within a column for any measurement or year.

^b Tukey's honestly significant difference.

and triasulfuron. Neither imazamox nor phenmedipham + desmedipham + ethofumesate seriously affected cuphea in greenhouse tests, but they were not field-tested because of insufficient availability at the time the experiments were performed.

No PA graminicide showed a deleterious effect on cuphea, a dicot, at the highest rates tested (data not shown). Graminicides and their highest rates were clethodim, fluzifop + fenoxaprop, sethoxydim, and quizalofop applied at 309, 212 + 59, 336, and 75 g/ha, respectively. Only sethoxydim was tested twice in the greenhouse.

Field Tests. Visual ratings of cuphea tolerance indicated that the SA herbicides ethalfuralin, isoxaflutole, mesotrione, and trifluralin, in the absence of PA herbicides, did not impede vigor of cuphea either year compared to the checks (Table 3). Furthermore, PA imazethapyr and mesotrione, in the absence of SA herbicides, did not reduce visual ratings of cuphea in comparison to the most vigorous plants. In contrast, PA imazaquin always reduced cuphea vigor (Table 3). Visual ratings for the high rate of PA mesotrione (105 g/ha) in 2004 were comparable to those for the low rate (70 g/ha) that same year (data not shown).

Combinations of SA and PA herbicides had varying effects on cuphea vigor. Consistently high tolerances were associated with ethalfuralin followed by (fb) imazethapyr, ethalfuralin fb mesotrione, isoxaflutole fb imazethapyr, and isoxaflutole fb mesotrione. Other combi-

nations of herbicides either were associated with poor tolerance or were variable across years (Table 3). Rate of PA mesotrione did not alter the results in 2004 (data not shown).

Dry weights of cuphea were correlated highly with visual tolerance ratings at ($R^2 = 0.90$ and $P < 0.01$, in 2003; and $R^2 = 0.85$ and $P < 0.01$ in 2004). This indicated that visual rating may be used as a surrogate for the more labor-intensive dry weight measurements. Accordingly, there were few discrepancies between ANOVA results for visual tolerance ratings (2 wk after treatment) and dry weights (8 to 11 wk after treatment). The SA herbicides that consistently allowed maximum dry weight accumulation were ethalfuralin, isoxaflutole, and trifluralin (Table 3). For PA herbicides, only mesotrione permitted high dry weight accumulation both years (and at both low and high rates for 2004). Consistently high dry weights were associated with the same SA fb PA combinations as with visual ratings of vigor (see above).

Stand densities were not affected by SA and PA herbicide treatments as greatly as were plant vigor and dry weight (Table 3). Significant reductions from maximum stand densities occurred in the presence of mesotrione only in 2003 and imazaquin, especially in 2004. The only treatment that reduced stand densities both years was trifluralin fb mesotrione.

Imazaquin negatively affected cuphea flowering by increasing time to initial anthesis (Table 3), regardless

whether it was applied alone or sequentially after an SA herbicide. All other treatments did not significantly influence time to anthesis, except mesotrione fb imazethapyr in 2004.

SA clomazone and sulfentrazone, which were tested only in 2004, significantly decreased cuphea vigor, stand, and dry weight ($P < 0.05$; data not shown). These herbicides did not merit further study.

In summary, the only treatments that never affected any of the four measured aspects of cuphea growth and development were ethalfluralin PPI, isoxaflutole PRE, trifluralin PPI, mesotrione PA, ethalfluralin PPI fb mesotrione PA, isoxaflutole PRE fb imazethapyr PA, and isoxaflutole PRE fb mesotrione PA (Table 3). Consequently, only these seven treatments can be confidently recommended for use in cuphea at present. Finally, although none of these recommended treatments can control the entire spectrum of weed species that occur in the northern United States, they represent a good start for developing weed management systems for cuphea.

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