ABSTRACT
Velvetleaf (Abutilon theophrasti Medik.) was used as a bioindicator to examine factors (temperature, soil moisture, soil incorporation) which might influence clomazone (2-[(2-chlorophenyl)methyl]-4,4-dimethyl-3-isoxazolidinone) volatilization. Young plants were periodically exposed to atmosphere inside boxes containing clomazone-treated soil. Exposed plants were returned to the greenhouse for 5 to 7 d, and new leaves were assayed for chlorophyll. Maximum chlorophyll inhibition occurred in velvetleaf exposed during the first 2 wk after clomazone application. Reductions in degree of bleaching were first observed in plants exposed in the treatments with lower soil moisture (2% moisture at 19 days after clomazone treatment, DAT; 9% at 42 DAT). The least amount of clomazone was measured in soils incubated in the 35 °C treatment, indicating elevated temperatures enhanced volatilization, and may have contributed to the increased chlorophyll content observed observed earlier than other temperatures. The effect of herbicide incorporation was not conclusive.

KEYWORDS
Clomazone, 2-[(2-chlorophenyl)methyl]-4,4-dimethyl-3-isoxazolidinone, volatilization, temperature, moisture.

INTRODUCTION
Clomazone is used as a pre-emergence herbicide in cotton (Gossypium hirsutum L.) and soybean [Glycine max. (L.) Merr.] to control several annual grass and broadleaf weeds. Clomazone inhibits carotenoid and chlorophyll biosynthesis in susceptible species, resulting in tissue bleaching(1). Bleaching observed in non-target plants, including numerous shrubs and trees, has been
attributed largely to clomazone drift following application. Volatilization subsequent to application also has the potential to injure sensitive plants in proximity to the site of application. Clomazone injury from volatilization was measured in velvetleaf plants up to 2 wk after herbicide application\(^2\).

The dispersal of herbicides by volatilization is dependent on a variety of environmental factors\(^3\). High ambient temperature may destabilize the affinity of a chemical for the soil/plant matrix resulting in volatilization. Mervosh et al.\(^6\) observed greater clomazone volatilization with increasing temperature, while effects of soil moisture on volatilization were minimal. Effects of tillage on clomazone persistence were described by Mills et al.\(^5\). Clomazone concentration in no-tillage soil on the day of application was approximately 43\% less than in conventional tillage soil, and the authors speculated that the herbicide in no-tillage was intercepted by wheat straw residue and/or was volatilized.

The vapor pressure of clomazone at 25 °C is 18.9 mPa and is similar to that of trifluralin (2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl)benzenamine) (14.5 - 15.0 mPa) \(^3,8\). Volatilization is a major dissipation mechanism for trifluralin, and soil incorporation following application is recommended. Clomazone has a relatively high water solubility (1100 mg L\(^{-1}\)) \(^6\). However, it is relative immobile in the soil\(^5\). Loux et al.\(^7\) suggested that hydrophobic bonding to organic components is a mechanism of clomazone sorption in soil. In another study, Loux et al.\(^8\) showed that clomazone was less available for dissipation in a soil with higher organic matter content. Greater clomazone movement was observed in soil with coarser texture and low organic carbon content\(^9\). Approximately one month after application, soil-incorporated clomazone was detected (between 1.6 and 2.3 ppb) in leachate from lysimeters placed at 0.3 and 0.6 m depths\(^10\).

Off-site herbicide movement into the atmosphere, either from application drift or from volatilization, may be minimized by management practices such as soil incorporation or by using various formulations or additives when applying clomazone. Thelen et al.\(^5\) measured less clomazone volatilization damage when the herbicide was incorporated. Halstead and Harvey\(^11\) observed that applying clomazone impregnated onto dry fertilizer significantly reduced off-site herbicide movement. Starch encapsulation reduced clomazone volatilization when compared to clomazone applied as an emulsified concentrate\(^6\).

Presently, there are frequent reports of off-target damage from clomazone during the spring in the southern United States. To develop methods to minimize off-target injury, it is important to identify the relative contribution of application drift and post-application volatilization to plant damage.
The objective of this study was to evaluate factors which might contribute to clomazone volatilization by assaying a sensitive plant species after foliar exposure to minute concentrations of clomazone vapor.

**MATERIALS AND METHODS**

**Moisture effects**

Six plastic trays (34 cm x 46 cm x 7 cm) were filled with 5 kg air-dried (2% w/w) Dundee silty clay loam (fine-silty, mixed, thermic, Aeric Ochraqualf). No water was added to two of the trays (2%, w/w), and sufficient deionized water was added to the remaining trays to adjust gravimetric moisture to 9% or 18% (w/w) (two trays for each moisture). Clomazone (Command 4EC™, FMC Corp.) was applied to the soil surface at a maximum labeled rate of 1.4 kg ha\(^{-1}\) to three of the trays (one for each moisture level), and the remaining trays were not treated with herbicide. No additional water was added to the soil during the study period (50 d). Each tray was sealed inside a plexiglass box (0.38 m width, 0.48 m length, 0.38 m height; 0.069 m\(^3\)) for the duration of the study except for short periods of time when plants were transferred into or out of the box (see below). The boxes were stored in a controlled environment growth chamber (24 h light, 25 °C).

Velvetleaf was chosen as an indicator species because it is extremely sensitive to clomazone and has been successfully used in other studies\(^{[2]}\). One h after clomazone application, four plants (4- to 7-leaf stage), each in 10-cm diameter pots, were placed above the soil surface of each tray on a wire mesh platform to ensure no direct contact with the soil surface. The trays/plants were sealed in the plexiglass boxes described previously. The boxes containing the soil trays and plants were then placed in a controlled environment growth chamber for 24 h (24 h light, 25 °C). After 24 h, the plants were removed from the boxes and placed in a greenhouse (32 °C, 12 h light). After 6 d in the greenhouse, the most recent fully expanded leaf at the time of exposure to clomazone and the fully expanded leaves above this leaf were harvested. Leaf pigments were extracted with dimethyl sulfoxide (DMSO) and assayed for chlorophyll content with a spectrophotometer set at 645 and 663 nm\(^{[12]}\). At various times during the study, new groups of four plants were placed in each box for 24-h periods and then removed and assayed for chlorophyll content as described above. At corresponding times, groups of four unexposed plants were assayed for chlorophyll as controls.

Prior to sealing the velvetleaf plants in the boxes with the trays, the boxes were vented outside to remove clomazone-contaminated air. During the venting, the plants were kept in a separate area to prevent exposure to clomazone. By this method, the plants were exposed only to clomazone volatilized during the 24-h period of exposure.
**Temperature effects**

Using general procedures described above, temperature (15, 25, and 35 °C) effects on clomazone volatilization were also measured. Air-dried soil (2% w/w) was used for all three temperature regimes to minimize effects of moisture fluctuation caused by the addition of water. Soils were incubated at the above temperatures, but during plant exposure (24 h), the trays were moved to a 25 °C controlled environment chamber to avoid detrimental effects on the plants at the extreme temperatures. New groups of four plants were placed in each plexiglass box for all sampling periods.

Soil from each tray was sampled periodically and extracted with 1:1 Soil:ACN for 24 h. Clomazone analysis was by liquid chromatography (Reverse phase ODS2 Column, 50:50 acetonitrile:water, 1 ml min⁻¹, UV 230 nm), with technical grade clomazone used as a standard.

**Incorporation effects**

General procedures as described previously were used to evaluate effects of incorporating clomazone in soil. Soil incorporation was performed by disturbing the top 2 cm of soil immediately after clomazone application. Soil moisture was maintained at 12% (w/w) as measured on an oven-dry basis.

**Postemergence applications**

Velvetleaf was germinated in 10-cm diameter pots with 3 to 5 plants per pot. When plants reached the three-leaf stage, clomazone was applied to the foliage using a moving-track sprayer equipped with Teejet 8001E nozzles (Spraying Systems Co., Wheaton, IL) at 380 kPa in a spray volume of 173 L ha⁻¹. Spray droplet volume median diameter was measured at 119.5 μm under those conditions. This droplet size corresponds to a fine spray, which is approximately the size of droplet expected from spray drift following application of herbicides. Clomazone was applied at concentrations ranging from 0 to 5% of a recommended rate of 1.4 kg ha⁻¹. Off-target plants exposed to spray drift typically receive up to 0.1% of the applied herbicide rate. Following treatment, plants which received <0.5% of the rate were placed in a separate greenhouse from those which received 0.5% or more. Greenhouse conditions were set at 14-h photoperiod and day/night temperatures of 29/25 °C. The 5th and 6th leaf of three plants from each pot were removed 10 DAT and assayed for chlorophyll content as described above. These particular leaves were assayed because they exhibited the most significant and consistent injury symptoms.
Field observations

Clomazone was applied in early May, 1994 and 1995, at 1.4 kg ha$^{-1}$ on field plots (Dundee silt loam soil) containing rye (*Secale cereale* L.) and hairy vetch (*Vicia villosa*) as cover crops, or fallow (no cover crop). At various times after clomazone application (1 h, and 1, 3, and 10 DAT in 1994; 1, 5, 7, and 10 DAT in 1995), four potted velvetleaf plants were transferred from the greenhouse to each plot, where they remained for 24 h. The pots containing velvetleaf were in placed on trays to prevent contact with the soil surface. After exposure, the plants were returned to the greenhouse for 5 d and then leaves were harvested and extracted with DMSO as described above.

Statistical analyses

The moisture, temperature, and incorporation experiments were conducted in a completely randomized design. In each experiment, plants were grown in the greenhouse under the same conditions to the 4- to 7-leaf stage. At each exposure time, four plants (replicates) were randomly selected to be placed in each plexiglass box (treatment) for a designated period of time. After exposure, the plants were returned to the greenhouse for further growth under the same conditions. Similarly, for the field study, plants were randomly selected from the greenhouse (four replicates), placed grown in the field plots at each exposure time, and then returned to the greenhouse for further growth. The postemergence application experiment was conducted twice in a randomized complete block design with four replications. Statistical analyses included analysis of variance procedures with mean separation by Fishers LSD test. Means were also delineated by standard error.

RESULTS AND DISCUSSION

Moisture effects

Visual symptoms of clomazone damage (lack of chlorophyll) in velvetleaf were generally observed in young and newly-formed leaves within 2 to 3 d after exposure to the herbicide for all moisture treatments. Differential effects with varying exposure times were obvious when comparing plants exposed for only 1 h on the day of clomazone application (data not shown) with those exposed for 24 h at the same time. The 24-h exposed plants exhibited a higher degree of bleaching in new leaves developed after exposure, while most of the bleaching in the 1-h exposed plants was in the leaf margins. Chlorophyll content relative to the control in the air-dried treatment was 37.2% for the one-h versus 1% for the 24-h exposure. The higher chlorophyll content observed after the one-h exposure period versus the 24-h exposure period is indicative of less clomazone injury on plant leaves.
Maximum chlorophyll inhibition occurred in plants exposed to treated soil during the first two wk after clomazone application for all moisture treatments (Figure 1). Among soil moisture treatments, no differences in chlorophyll content were observed during this period, but after three wk, significantly greater chlorophyll levels were measured in the air-dried (2% moisture) treatment (Figure 1). It was somewhat unexpected that volatilization would be sustained for 20 to 30 d in air-dried soil. Symptoms of decreased volatilization in the air-dried soil were characterized by bleaching primarily along the outer edge of the new leaves, while the entire area of new leaves was bleached in the 9% and 18% moisture treatments. Bleaching began to decrease in the 9% moisture treatment 30 to 40 DAT, and by 50 DAT, volatilization was minimal in soils from the air-dried and 9% moisture treatments but was still occurring in soil from the 18% moisture treatment (Figure 1). When the study was terminated (50 DAT), effects of clomazone in the 18% treatment were beginning to diminish.

![Relative chlorophyll content in velvetleaf leaves developed after 24-h exposure in plexiglass chambers containing clomazone-treated soil at 2, 9, and 18% initial soil moisture content. LSD (α = 0.05) for comparing means among moisture treatments within each exposure event is indicated by vertical bars.](image)

**Figure 1.** Relative chlorophyll content in velvetleaf leaves developed after 24-h exposure in plexiglass chambers containing clomazone-treated soil at 2, 9, and 18% initial soil moisture content. LSD (α = 0.05) for comparing means among moisture treatments within each exposure event is indicated by vertical bars.

**Temperature**

Increases in chlorophyll content in leaves were first observed in the 35 °C treatment 40 DAT and in the 25 °C treatment 49 DAT (Figure 2). Although plant exposure to soil from the three temperature regimes continued on a weekly basis, leaves were not always assayed for chlorophyll. However,
visual observations indicated that clomazone damage was occurring in the 15 °C treatment until the last chlorophyll assay time (75 DAT) when there was no difference among temperature treatments (Figure 2).

![Figure 2](attachment:image)

**Figure 2.** Relative chlorophyll content in velvetleaf leaves developed after 24-h exposure in plexiglass chambers containing clomazone-treated soil which was incubated at 15, 25, or 35 °C. LSD (α = 0.05) for comparing means among temperature treatments within each exposure event is indicated by vertical bars.

Less extractable soil clomazone was measured in the soil of the 35 °C treatment (Table 1), indicating that elevated temperatures enhanced volatilization. Similarly, in a study where clomazone-treated soils were incubated at various temperatures for up to 84 days, less clomazone was desorbed from soils incubated at higher temperatures (14). In the present study, reduced levels of labile clomazone available for volatilization may have contributed to the increased chlorophyll observed in the leaves at the highest temperature (Figure 2). Even though this reduction in soil clomazone level was observed, it was not closely correlated with clomazone damage in new leaves of velvetleaf plants (Figure 2). Because developing tissue in velvetleaf is so sensitive to clomazone, only a small proportion of the total clomazone present in the soil would have to volatilize to have an effect. The mature leaves, which are less sensitive, were affected when exposed during the first 6 d. However, after the initial flush of clomazone volatilization (after 6 d), the effects on mature leaves diminished. This scenario was observed for a longer period of time with the developing leaves.
Table 1. Effect of temperature (15, 25, and 35 °C) on clomazone extracted from soil during a 28-day incubation.

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<thead>
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<th>% Relative Concentration</th>
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<td>Days After Application</td>
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<td>Standard Error</td>
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Incorporation

Incorporation of clomazone into soil should reduce volatilization loss\(^2\), but the effect of herbicide incorporation in this study was not conclusive. Averaged over sampling time, there was a slightly higher chlorophyll content in plants from the no-incorporation treatment (no incorp. 29.2%, incorp. 22.5%; LSD\(_{0.05}\) = 4.9). However, most of this occurred toward the end of the incubation period (Figure 3). More chlorophyll in plants from the no-incorporation treatment relative to those from the incorporated treatment at later incubation times may indicate that the bulk of clomazone volatilization occurred earlier in the incubation period for the no-incorporation treatment. Volatility from the incorporated treatment may have been in smaller increments throughout the incubation period. The result was a reduced availability of clomazone for later volatilization in the no-incorporation treatment. The volatilization data did not corroborate with soil extraction data, which was variable and inconsistent (data not shown). These results might have been influenced by a combination of clomazone concentration and physical positioning in the soil.
Figure 3. Relative chlorophyll content in leaves developed after 24-h exposure in plexiglass chambers containing clomazone-treated soil, where clomazone was either incorporated or not incorporated. Vertical bars represent standard error for each exposure event.

One factor relating to volatilization is herbicide concentration in soil (9). The affinity of the herbicide for soil is reduced as herbicide concentration increases, as indicated in other studies (7,16) where clomazone sorption was evaluated. Clomazone in the incorporated treatment was diluted in the soil, resulting in lower concentrations at the soil surface. Physical position in the soil can also influence herbicide volatilization. Placement below the soil surface is more desirable for inhibiting volatilization. For the study duration, volatilization was occurring in the incorporated treatment at levels sufficient to induce chlorophyll damage. However, although initial volatilization losses may have been less in the no-incorporation treatment, the effect was masked because of the extreme sensitivity of velvetleaf. The volatilization effect might have been observed over a shorter period of time if a less sensitive species was used.

Postemergence applications

Velvetleaf tissue was sensitive to postemergence applications of clomazone (Figure 4). Application of clomazone at a rate of 7 g ha$^{-1}$ (0.5% of the 1.40 kg ha$^{-1}$ rate) reduced chlorophyll content in developing leaves by approximately 50%.

The injury to velvetleaf from both direct applications and exposure to treated soil demonstrated that off-target movement of clomazone can occur in spray droplets or in vapor form. Typically, no
more than 0.5% of an applied pesticide moves off-target under normal conditions\textsuperscript{(13)}. Therefore, the relatively high degree of injury to velvetleaf from volatilized clomazone compared to direct applications at 0.5% suggests that volatilized clomazone could be the major contributor to clomazone injury of off-target plants. Velvetleaf is highly sensitive to clomazone, and the degree of injury from clomazone may vary with plant species.

![Figure 4](image)

**Figure 4.** Chlorophyll content of velvetleaf as affected by low rates of postemergence applications of clomazone. Bars represent standard error for each herbicide dose.

**Field observations**

From 1 to 10 DAT, some damage was observed in velvetleaf plants placed in plots receiving no clomazone in both 1994 and 1995, indicating contamination by clomazone volatilization from treated plots (data not shown). The chlorophyll content in velvetleaf placed in the fallow, vetch, and rye plots was similar, and injury occurred up to the 10-d measurement in 1994 (Figure 5a). Clomazone damage was observed through 5-d in 1995 (Figure 5b). In a Michigan study, Thelen et al.\textsuperscript{(2)} observed clomazone damage in velvetleaf placed in plots 2 wk after application. However, lower mean temperatures compared to Mississippi conditions may have prolonged volatilization effects. Also, in the present study, plants were exposed for only 24 h, whereas Thelen et al.\textsuperscript{(2)} exposed plants for 5 to 11 d.
Figure 5. Relative chlorophyll content in velvetleaf leaves exposed to clomazone-treated field plots in (a) 1994; and (b) 1995 at various times after herbicide application. Vertical bars represent standard error for each exposure event.

SUMMARY AND CONCLUSIONS
In greenhouse studies, exposure of velvetleaf plants to clomazone vapors up to 10 d following soil application caused severe (>80% relative to control) damage under all moisture and temperature conditions, with no significant differences among treatments. After three to four weeks, differences in chlorophyll content of exposed plants were apparent among moisture and temperature
treatments, with a trend toward less clomazone effect with higher temperatures and lower soil moisture. The early (up to 10 DAT) clomazone effect regardless of moisture/temperature treatment suggests that, aside from applying clomazone when wind speeds are low, restricting applications to optimal environmental conditions may not be very effective for reducing off-site movement. Incorporation was not an effective method to reduce clomazone movement up to 3 wk after clomazone application, as velvetleaf injury was similar with or without incorporation.

In field studies, clomazone damage was observed in velvetleaf plants exposed to clomazone-treated plots up to 5 to 10 days after application. Clomazone damage to velvetleaf plants exposed in fallow, vetch, and rye plots was similar. Field research evaluating reduction in clomazone movement from target sites by manipulating formulations, application techniques, or soil incorporation merits further study.

REFERENCES


