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Liverwort (*Marchantia polymorpha*) Response to Quinoclamine in a Pine Bark Substrate

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Quinoclamine is an herbicide under development for control of liverwort, a weed common in nursery crops. With respect to liverwort control, quinoclamine has been considered to primarily have POST activity. However, some PRE activity has been reported. Growth media sorption studies with ^{14}C-quinoclamine indicate that only 0.64% of the quinoclamine amount that enters the media remains unadsorbed and thus available to be taken up by established plants or propagules. Computer modeling revealed that a large portion of the surface of the quinoclamine molecule is positively charged, which likely is the reason for its high adsorptivity. In a simulation of PRE activity, hydroponically grown liverwort and germinating gemmae were exposed to increasing quinoclamine concentrations. Phytotoxicity to both plants and gemmae was obtained with a minimal concentration of 4 to 6 mg L^{-1}. Based upon the projected use rate, and assuming minimal vertical infiltration depth, the theoretical concentration of quinoclamine within the aqueous phase of a pine bark substrate would be approximately 8 mg L^{-1}. In toto, results indicate that the projected use rate will result in sufficient quinoclamine in the aqueous phase of a pine bark substrate to provide PRE control of gemmae propagules as well as to contribute to the efficacy of POST applications to established liverwort.

Nomenclature: Quinoclamine; liverwort, *Marchantia polymorpha* L.
Key words: Herbicide adsorption, hydroponic solution, nursery crops, gemmae, thalli.

Liverwort is a common weed in nursery containers throughout the United States. It is a primitive spore-bearing plant in the class Hepaticae. Liverwort spreads via both sexual and asexual means. Ross and Puritch (1981) identified liverwort and thread moss (*Bryum argenteum* L.) as the most abundant cryptogams in greenhouse crops and attributed their predominance to their ability to propagate both asexually and sexually. Asexual reproduction in liverwort is via dispersal of clonal diaspores called gemmae. Gemmae disperse via splashing, in which water drops from irrigation or rainfall splash gemmae up to 1.6 m from the mother plant (England and Jeger 2006). Vegetative reproduction can also occur via fragmentation, in which small sections of the liverwort thalli regenerate entire plants. Fragmentation could be an important dispersal mechanism if hand-weeding efforts do not remove the entire liverwort plant.

Efficacy of quinoclamine applied PRE has been variable. Suggesting only limited residual activity, Senesac (2005) reported a slight, but significant, level of residual control of new liverwort infestations over a 5-wk period when quinoclamine was applied to weed-free containers. Newby et al. (2007) observed a longer period of residual activity with quinoclamine. They documented effective PRE liverwort control up to 14 wk after application; however, these authors also noted that control with quinoclamine was much less than that provided by other commonly used granular PRE herbicides. With the strongest suggestion of residual activity, Svenson et al. (1997) stated that quinoclamine is most effective when used for PRE liverwort control.

To better understand the efficacy of quinoclamine applied for PRE liverwort control, experiments were conducted to determine the amount of quinoclamine in solution on the surface of a typical nursery substrate. Then, the concentration of quinoclamine required in solution to control liverwort gemmae and thalli was determined.

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Materials and Methods

Quinoclamine Sorption by a Pine Bark Substrate. Quinoclamine[1] rates used in this laboratory experiment represented the range of application rates on the proposed Gentry label and included 3.8, 5.7, and 7.6 kg ai ha^{-1} (38.1, 57.1, and 76.2 μg cm^{-2}). The substrate used was 5 : 1 loblolly pine (*Pinus taeda* L.) bark : sand (v/v) with a cation-exchange capacity (CEC) of 30 mEq 100 cm^{-3}, 64% organic carbon (loss on ignition), a water-holding capacity of 46%, and a bulk density of 0.27 g cm^{-3}. Assuming 0.5-cm infiltration of the substrate surface, quinoclamine concentration in the substrate surface would range from 282 to 564 mg kg^{-1}.

Quinoclamine sorption on the pine bark substrate was evaluated using radiotracer methodology and a soil solution technique previously described (Adams et al. 1982; Goetz et al. 1986, 1989). Briefly, appropriate amounts of formulated quinoclamine and ^{14}C-quinoclamine were added to the substrate (1.0 kg samples) to achieve the desired substrate concentration of 282 to 564 mg kg^{-1} on a dry weight basis. The formulated quinoclamine and ^{14}C-quinoclamine were first combined with 460 ml of tap water, added to the substrate, and the substrate sample was brought to field capacity. Field capacity had been previously determined to be 46% by the method described by Adams et al. (1982). This solution, which had a radioactivity concentration of 460 Bq ml^{-1}, was applied to the dry substrate sample in a stainless steel pan, mixed thoroughly, covered with both plastic film and aluminum foil to prevent evaporation, and allowed to equilibrate for 3, 6, 12, 24, 36, 48, or 72 h at room temperature (approximately 20 C). After equilibration, substrate samples were divided into four subsamples and placed into individual substrate solution-extraction cups. An extraction cup consisted of Plexiglas cylinder (8 cm inner diameter by 20 cm deep) with a perforated bottom. This design allowed the aqueous phase to be extracted from the substrate and collected in a catch cup that was attached below the substrate-containing column. Filter paper was placed between the substrate sample and the perforated bottom to prevent substrate particles from clogging the perforations. Samples were centrifuged at 1,960 × *g* for 1 h, and 1-ml subsamples of

the extracted aqueous phase were assayed for ^{14}C using a liquid-scintillation spectrometry. Radioactivity in the 1-ml samples typically ranged from 3 to 6 Bq ml^{-1}, and minimal counting efficiency, based on an automatic external standard quench curve, was at least 94%. The difference between the radioactivity in solution before adding the substrate and the radioactivity in solution recovered from the soil was assumed to represent the quinoclamine that had been sorbed by the bark substrate. An experimental unit consisted of an individual substrate-containing pan, and each equilibration time was assigned to three experimental units. Sorption for an individual experimental unit was determined by the average of four subsamples. A completely randomized design was used, and the experiment was repeated once.

Computer Modeling of Quinoclamine Molecule. The geometry of the quinoclamine molecule was first optimized in its most likely configuration using the Gaussian 03 Program.[2] Then, the electrostatic-potential map of the molecular surface was constructed using the Visual Molecular Dynamics (VMD) visualization program (Humphrey et al. 1996). A warm color (i.e., red) indicates net accumulation of a positive charge; conversely, a cool color (i.e., green) indicates net accumulation of a negative charge.

Gemmae Control as Influenced by Quinoclamine Concentration. Stock liverwort plants were grown and all subsequent hydroponic studies were conducted in a greenhouse in Auburn, AL, during March and April 2007. Liverwort stock plants used in all trials were grown with the following procedure. A black plastic bedding plant flat with cells 2 cm wide by 3 cm deep was inserted into a 0.47-L Gladware[3] plastic container. Containers were filled with 0.3 L of a half-strength Hoagland solution (Hoagland and Arnon 1950). Pieces of fiberglass 1 cm wide by 5 cm long were folded length-wise and inserted into individual cells of the bedding plant flat such that the middle section of the fiberglass rose above the solution level. Capillarity caused the section of fiberglass above the water level to remain consistently moist. Liverwort fragments approximately 3 mm square were excised from liverwort stock plants and placed on the section of fiberglass strips elevated above the nutrient solution. Thalli fragments were maintained until they regenerated and grew to more than 10 times their original size. At the time of experimentation, liverwort fragments were 3 to 5 cm wide, and rhizoids had grown through the fiberglass such that they could not be removed from the thalli. Greenhouse temperatures were maintained between 16 and 24 C.

Eleven separate batches of half-strength Hoagland solution were spiked with quinoclamine to achieve concentrations of 0 to 10 mg L^{-1} quinoclamine, in 1-mg L^{-1} increments. Containers were partially filled with quinoclamine-spiked nutrient solutions. Glass petri dishes were placed upside down in the containers such that the nutrient solution just covered the bottom of the inverted dish. Fiberglass strips were placed on the bottom of the dish; the intent was to allow the solution to wick completely through the fiberglass but not submerge the gemmae. An experimental unit consisted of a single container with a single sheet of fiberglass on which was placed at least 10 gemmae. There were three replications per quinoclamine concentration treatment. Gemmae were collected from gemmae cups on the stock liverwort, using a moistened needle, and transferred to the clean strip of fiberglass in each of the aforementioned containers. Gemmae size were measured using an ocular scale on a stereomicroscope 5 d after treatment (DAT), and control was calculated as the percentage of reduction in growth compared with the nontreated controls. The experiment was repeated once.

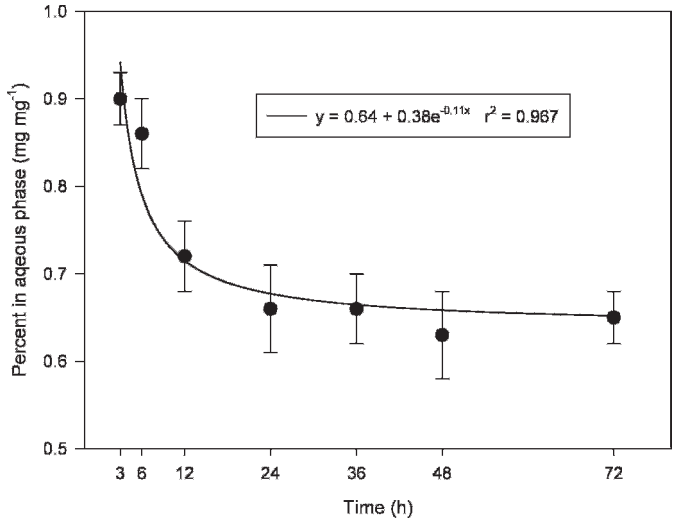

Figure 1. Percentage of applied quinoclamine remaining in the aqueous phase of a pine bark substrate over time.

Thalli Control as Influenced by Quinoclamine Concentration. To evaluate the response of liverwort plants (thalli in particular) to rhizoid-available quinoclamine, procedures similar to that described above with gemmae were followed. Liverwort stock plants, and the fiberglass strips to which they were attached (by rhizoids), were removed from the stock containers and blotted free of nutrient solution. Plants were placed into the quinoclamine-spiked, nutrient solution–filled containers as described above, such that the solution could wick through the fiberglass but never contact tissue of the liverwort plant other than the rhizoids. An experimental unit consisted of a single container with three liverwort plants, with their attached fiberglass strips arranged in a triangle across the bottom of the inverted petri dish. Each of the 11 quinoclamine concentrations treatments were replicated three times. Thalli were rated visually 4 DAT on a scale from 0 to 100 where 0 was no injury, and 100 was complete thalli death. The experiment was repeated once.

Data in all experiments were analyzed with nonlinear regression using the NLIN procedure in SAS (SAS Institute 2001). Curves were compared, and the most appropriate model was selected using the lack-of-fit test as described by Seefeldt et al. (1995).

Results and Discussion

Quinoclamine Sorption by a Pine Bark Substrate. Quinoclamine remaining in solution decreased exponentially with time (Figure 1). After 3 h, less than 1% of quinoclamine remained in solution, and by 24 h, the amount leveled off to approximately 0.64%. With an application rate of 3.81 kg ai ha^{-1} and an infiltration depth of 0.5 cm, the concentration of quinoclamine in the substrate would be 282 mg kg^{-1} on a dry-weight basis. Field capacity of the pine bark substrate used in this study was 46%, thus the maximum

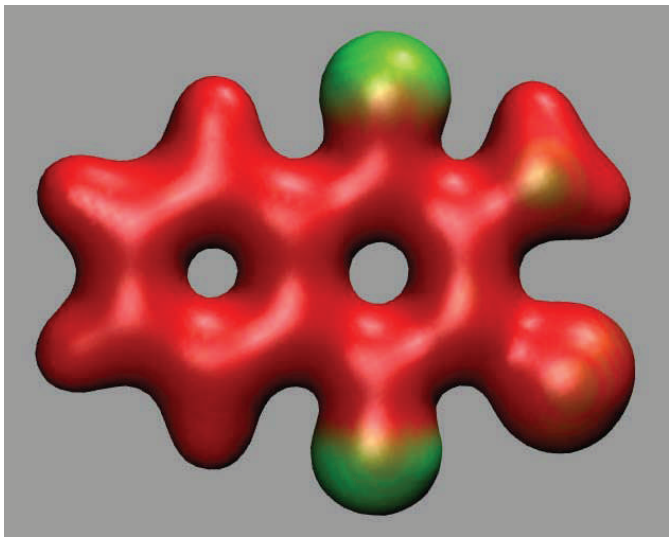

Figure 2. Electrostatic potential map of the quinoclamine molecular surface, where warm colors (red) represent areas of positive charge, and cool colors (green) represent regions of negative charge.

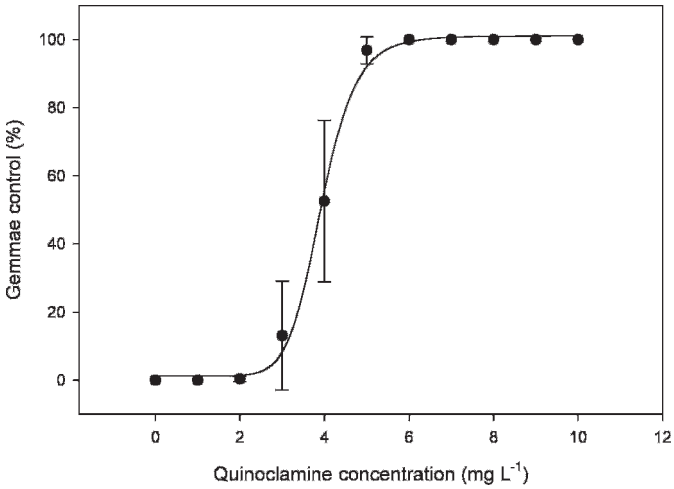

Figure 3. Liverwort gemmae response to quinoclamine concentration in hydroponic solution. Control is expressed as the percentage of reduction in growth of gemmae, after 5 d, compared with the nontreated controls.

potential quinoclamine in solution would be 613 mg L^{-1}. After 72 h, only 0.64% of the applied quinoclamine remained in solution (Figure 1), thus one would expect 3.9 mg L^{-1} of quinoclamine to remain in aqueous phase and be available for absorption by liverwort. By doubling the application rate to the maximum proposed 7.62 kg ai ha^{-1}, the amount of quinoclamine projected to be in solution of a pine bark substrate is 8.0 mg L^{-1}. Solubility of quinoclamine is 20.7 mg L^{-1} at 20 C, thus the calculated quantities of quinoclamine in solution are within its solubility limits.

Following Weber et al. (2000), an herbicide partition coefficient (K_d) of 70.11 ml g^{-1} was determined. The substrate in this study was 64% organic carbon (OC), thus K_{doc} is 109.5 ml g^{-1}. The K_d value calculated in our study is much higher than most herbicides, as summarized by Weber et al. (2000), which range from 0.01 to 10 ml g^{-1} at OC levels between 0 and 3% (with the exception of oxyfluorfen with K_d values of 8.5 to 228.61 ml g^{-1}). When normalized for OC, K_{doc} levels fell within the range of most other herbicides (30 to 400 ml g^{-1}) (Weber et al. 2000).

Computer Modeling of Quinoclamine Molecule. Although the quinoclamine molecule has no overall net charge, the computer modeling predicts most of the surface is positively charged (Figure 2). This would render the molecule very strongly attracted to the negative charges associated with organic matter of pine bark substrates, and thus, removed from the aqueous phase. This is in agreement with our media sorption study, where only a relatively small proportion of the quinoclamine (0.64%) remained available in the aqueous phase after 72 h. The authors have used the procedure described herein to evaluate the media sorption of other PRE-active herbicides commonly used in nursery production, including oxadiazon (Wehtje et al. 1993), isoxaben (Wehtje et al. 2006), and oryzalin (Wehtje et al. 1994). None of those herbicides were adsorbed to the extent that quinoclamine was adsorbed in our study.

Gemmae Control as Influenced by Quinoclamine Concentration. Gemmae response to quinoclamine in the two repetitions of the experiment were fit with four-parameter logistic curves (Seefeldt et al. 1995). Dose–response curves to quinoclamine concentration were similar in the two repetitions of the study according to the lack-of-fit test (P = 0.990); thus, both repetitions were pooled for curve fitting (Figure 3). In nontreated controls, spherical gemmae were initially 0.6 mm in diameter and grew to 2.2 mm over the 5-d experimental period. Gemmae maintained their green color and grew normally in hydroponic solution spiked with either 1 or 2 mg L^{-1} quinoclamine. Control increased rapidly from 2 to 6 mg L^{-1}, with those gemmae in greater than 6 mg L^{-1}, quickly turning brown within 1 d (observed, not measured). Based on fitted curves, the quinoclamine concentration required to provide 90% control (LC_{90}) is calculated to be 4.9 mg L^{-1} (Table 1). Although quinoclamine sorption to pine bark is very high or nearly complete, the relatively small amount that remains available in the water phase, by calculation, is sufficient to provide PRE control of gemmae.

Thalli Control as Influenced by Quinoclamine Concentration. Thalli dose–response curves to quinoclamine concentration differed from the two dates according to the lack-of-fit test (P = 0.0001). Parameter estimates, along with their standard errors, suggest that LC_{50} for each date differed (Table 1), although even these differences were minor. As quinoclamine concentration increased from 0 to 3 mg L^{-1}, there was little or no observed response in thalli (Figure 4). Control improved rapidly over the range of 3 to 7.6 mg L^{-1}, with 7.6 and 6.4 mg L^{-1} providing 90% control in repetitions 1 and 2, respectively (Table 1). At the highest labeled rate, we calculated 8.0 mg L^{-1} quinoclamine to be in solution in a pine bark substrate. This concentration would be high enough to provide liverwort control. Furthermore, because thalli never came in contact with the hydroponic solution, it demonstrates that rhizoids, either passively or actively, absorbed and translocated quinoclamine.

Cation-exchange capacity of pine bark ranges from 6 to 10 mEq 100 cm^{-3} (Tucker 1995), which is higher than sandy soils but less than most silt and clay soils (Krewer and Ruter 2005). Media sorption data, coupled with electrostatic-potential rendering, suggest that quinoclamine is quickly sorbed to negatively charged particles within the substrate. In sandy loam to loam soils, dissipation time for loss of 50% and

Table 1. Parameter estimates and their standard errors (in parentheses) for sigmoid dose–response curves[a] that predict the relationship between thalli and gemmae response to quinoclamine concentration.

Tissue	Repetition	D	C	LC_{50}	LC_{90}[b]	b	r^2
		%		mg L^{-1}			
Thalli	1	102.6 (3.6)	−2.1 (2.5)	5.2 (0.1)	7.6	−5.8 (0.8)	0.984
	2	101.2 (2.8)	−3.5 (2.9)	4.2 (0.1)	6.4	−5.3 (0.7)	0.977
Gemmae	pooled	101.0 (1.7)	1.9 (2.5)	3.9 (0.1)	4.9	−9.8 (1.7)	0.996

[a] Curve model is $y = C + \{[D - C]/[1 + (x/LC_{50})^b]\}$, where C, maximum value in the curve; D, minimum in the curve: and LC_{50}, dose required for 50% control.
[b] LC_{90} is calculated from the fitted-regression model and represents the dose required for 90% control.

90% (DT_{50} and DT_{90}) was 31 and 103 d, respectively (Anonymous 2005). It is difficult to correlate dissipation times in soils to dissipation in a pine bark substrate. However, DT_{50} of 31 d suggests that quinoclamine is in sufficient concentration for at least several weeks. This corroborates research by Newby et al. (2007) showing quinoclamine provides some PRE liverwort control but less than other granular PRE herbicides.

Gemmae response to quinoclamine concentration is most indicative of the potential for quinoclamine to provide PRE liverwort control in nursery and greenhouse containers. Although liverwort can reproduce sexually via spores and asexually via splashing of gemmae, Ross and Puritch (1981) attributed the greater numbers of liverwort and silvery thread moss, compared with other cryptogams, to their ability to propagate asexually. Data from Ross and Puritch (1981), coupled with our observations, suggest that gemmae dispersal is most responsible for liverwort spread within a nursery or greenhouse system. Calculated LC_{90} for gemmae was 4.9 mg L^{-1} of quinoclamine, thus assuming DT_{50} of just 31 d, there would still be sufficient quinoclamine in the aqueous phase to provide control of gemmae for several weeks following application of the maximum proposed label rate.

Thalli response to quinoclamine concentration in hydroponic solution was surprising. Previous research (Altland et al. 2007) documented rapid uptake and translocation of quinoclamine in liverwort when applied in a spray formulation to the dorsal surface. Rapid uptake was attributed to the large number of pores on the thalli surface, which lack guard cells present in higher plants (Doyle 1970). The dorsal surface of the thalli used in the present study did not come in direct contact with the hydroponic solution. Because the site of action for quinoclamine is photosystem I and because the only photosynthetic tissue in liverwort occurs in a thin layer along the dorsal surface, quinoclamine must have been moved along the rhizoids, absorbed by the parenchymatous tissue on the ventral surface of the thalli, and translocated to the photosynthetic layer. McConaha (1941) described how scales and rhizoids on the ventral surface of liverwort can rapidly distribute water via external capillarity to absorptive areas throughout the liverwort thallus. These data further corroborate conclusions by Altland et al. (2007) that quinoclamine is translocated within liverwort thalli, despite its lack of a vascular system.

Cumulatively, data herein suggest that quinoclamine has a largely positive electrostatic surface and is quickly and nearly completely sorbed to pine bark substrates but remains in sufficiently high concentrations within the aqueous phase to provide control of both gemmae and liverwort thalli. The respective LC_{50} and LC_{90} values for gemmae and for thalli of established plants differed only slightly.

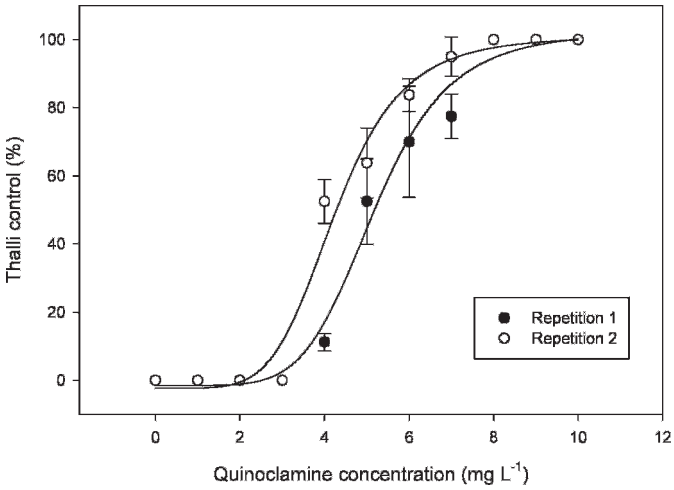

Figure 4. Liverwort thalli response to quinoclamine concentration in hydroponic solution. Liverwort were rated 5 d after exposure to spiked hydroponic solution and evaluated on a scale from 0 to 100 where 0 is no injury, and 100 is complete death.

Sources of Materials

[1] Gentry, 25% wettable powder, Chemtura Corp., Middlebury, CT 06762.

[2] Gaussian 03 Program. Revision C.02 (released 2004), available from Gaussian Inc., Wallingford, CT 06492.

[3] Glad Products Co., Oakland, CA 94612.

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