J. Aquat. Plant Manage. 32: 15-20

Dissipation of Gibberellin Synthesis Inhibitors in Small-scale Aquatic Systems[1]

TARA CHAND AND C. A. LEMBI[2]

ABSTRACT

Water, plant and soil samples from 67-L barrels planted with Eurasian watermilfoil (*Myriophyllum spicatum* L.) and exposed to the gibberellin synthesis inhibitor flurprimidol ([α-(1-methylethyl)-α-(4-trifluoromethoxy) phenyl]-5 pyrimidinemethanol) were analyzed for flurprimidol using gas chromatography. Half lives of flurprimidol in water and plant tissues were 8.4 and 9.1 days, respectively, at an initial treatment concentration of 75 μg a.i. L^{-1} and 9.8 and 8.8 days, respectively, at an initial treatment concentration of 200 μg a.i. L^{-1}. Half life of the compound in sediment at an initial treatment concentration of 1000 μg a.i. L^{-1} was 178 days. Approximately 14.5% of the flurprimidol initially applied was recovered after 28 days of exposure. An internal threshhold level of 20-30 ng flurprimidol per gram dry weight milfoil tissue appeared to be necessary for maintaining adequate stem height reduction. Dissipation characteristics of two other gibberellin synthesis inhibitors, paclobutrazol ([2RS,3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl) pentan-3-ol]) and uniconazole ((E)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1- yl)-1 penten-3-ol), differed from those of flurprimidol. Half lives of paclobutrazol and uniconazole in water were 24.4 and 5.2 days, respectively. The half life of uniconazole in soil was 102 days whereas paclobutrazol did not dissipate over the 168 day sampling period.

Key words: persistence, flurprimidol, *Myriophyllum spicatum*, paclobutrazol, plant growth regulation, PGRs, uniconazole.

INTRODUCTION

Gibberellin synthesis inhibitors have been shown to reduce the shoot height of hydrilla [*Hydrilla verticillata* (L.f.) Royle] and Eurasian watermilfoil (*Myriophyllum spicatum* L.) in laboratory (Netherland and Lembi 1992) and small-scale outdoor trials (Lembi and Chand 1992) without adversely affecting plant viability. These compounds have the potential of maintaining submersed aquatic weeds at reduced heights while allowing them to retain useful functions such as oxygen production and fish habitat.

Very little is known about the dissipation characteristics of these compounds in the aquatic environment. Chand and Lembi (1991) described extraction and quantification procedures for the gibberellin synthesis inhibitor flurprimidol from Eurasian watermilfoil tissue and soil using gas chromatography. In barrels set out-of-doors with water, Eurasian watermilfoil, and soil, approximately 88% of the applied flurprimidol had dissipated from the system within 4 weeks. The half-life of the compound in water was 6.8 to 8 days during June/July 1989.

The major goal of the present study was to determine the dissipation characteristics of flurprimidol in Eurasian watermilfoil tissue and soil. In addition, a relationship between percent reduction in shoot height of watermilfoil and the concentration of flurprimidol in plant tissue was estimated. We also compared the dissipation of flurprimidol in water and soil with that of two other gibberellin synthesis inhibitors, paclobutrazol and uniconazole.

MATERIALS AND METHODS

Metal barrels (67-L capacity) with plastic liners were set in an unshaded outdoor area. Loam soil (46.5% sand, 41.0% silt, 12.4% clay; 1.6% organic matter, pH 6.2) free from plant growth regulators, herbicides and other pesticides was added to a 10-cm depth in each barrel. Approximately 55 L of well water was added, and the soil was allowed to settle for 2 to 3 days. Two stem apices (10 cm length) of healthy Eurasian watermilfoil from Martel pond (Tippecanoe Co, Indiana) were planted per barrel and allowed to establish for 7 days prior to flurprimidol treatment. Flurprimidol (50% WP, DowElanco Products Company, Indianapolis, IN) was applied by diluting the compound in 10 ml of water, then gently stirring the solution into the barrels, without disturbing the soil, to insure even dispersal.

Flurprimidol treatments at 75 and 200 μg a.i. L^{-1} were made on June 1, 1990. The milfoil plants were exposed to flurprimidol for 2 hours, 1, 3, 7, 14, and 28 days. Treatments were arranged in a randomized complete block, and each exposure time/concentration combination consisted of two replications. Two replicate untreated barrels were established for each of four groups of exposure times: 1) 2 hours, 1 and 3 days, 2) 7 days, 3) 14 days, and 4) 28 days. Water, milfoil and soil samples were taken from the treated and appropriate control barrels at the end of the exposure time. Water samples were also taken immediately before and after treatment. One-liter water samples were frozen for storage. One of the two milfoil plants was removed, washed twice with distilled water, and blot-dried. The plant wet weight (roots and shoots were combined) was recorded prior to freeze drying, which was done within 24 hr of collection. Soil cores were taken using a hollow plastic cylinder (5 cm inner diameter by 15 cm in length) and were

[1]Part of these data were previously published in the US Army Corps of Engineers Aquatic Plant Control Research Program Annual Proceedings, Misc. Papers A-92-2.

[2]Postdoctoral Associate and Professor, respectively, Department of Botany and Plant Pathology, Purdue University, West Lafayette, Indiana 47907. Received for publication June 20 1992 and in revised form June 6, 1993.

frozen for storage. For analysis, thawed soil samples were divided into upper and lower 5 cm layers before removing the free water.

After removal of samples for flurprimidol analysis, the water was removed from the barrels (including untreated controls) by siphoning, and new untreated water was carefully added in a manner to minimize soil disturbance. After 4 weeks in untreated water, the remaining milfoil plant from each barrel was harvested and prepared as described above for flurprimidol analysis.

The dissipation pattern of flurprimidol in water and soil was compared with that of paclobutrazol (21.8% liquid, ICI Americas, Inc., Goldsboro, NC) and uniconazole (50% WP, Chevron Chemical Company, Richmond, CA) in barrels set up as described above except that no plants were present. Two barrels for each compound were treated on June 19, 1990 to achieve a final concentration of 1000 μg a.i. L-1. Water samples (100 ml) were taken before treatment, immediately after treatment, and at 2 hours, 1, 3, 7, 14, 28, 56, and 112 days after treatment. The soil was sampled after 28, 56, 112, and 168 days of treatment.

Extraction of the gibberellin synthesis inhibitors and analytical procedures were the same as those described for water, plant tissue and soil in Chand and Lembi (1991). Standards were technical grade flurprimidol (99.85%, Eli Lilly and Company, Indianapolis, IN), paclobutrazol (97.5%, ICI Americas, Richmond, CA) and uniconazole (78.5%, Valent U.S.A. Corporation, Walnut Creek, CA). Since all three gibberellin synthesis inhibitors behave similarly during extraction (Reed 1988) we used flurprimidol as an internal standard to quantify the residues of uniconazole and paclobutrazol and used paclobutrazol for the quantification of flurprimidol. Residues were quantified using a Varian 3400 GC equipped with a model 8035 autosampler, thermionic specific detector (TSD), and a DB-17 (30 m X 0.32 mm) fused silica capillary column. To confirm the identity of the compounds, mass spectra of technical grade flurprimidol, paclobutrazol and uniconazole (dissolved in 100% methanol) were obtained using a Hewlett Packard GC 5890A gas chromatography-mass spectrometer with HP mass selective detector (MSD) 5970 and a HP7673A autosampler.

RESULTS AND DISCUSSION

Flurprimidol was not detected in untreated barrels or in barrels sampled before treatment. Water samples collected immediately after treatment showed flurprimidol residues were slightly less, but within 10%, of the target amounts of 75 and 200 μg L^{-1}.

Flurprimidol was present in water, plant tissue and soil in the treated barrels throughout the 28 day sampling period (Table 1). The amounts in water and plant tissue decreased over the 28 day period at both treatment concentrations but generally increased in the upper and lower soil layers. At 28 days, the amounts of flurprimidol remaining in the barrels were 14.6 and 14.4% of that initially applied at 75 and 200 μg L^{-1}, respectively. These percent recoveries matched reasonably well with an 11.7% recovery in an earlier study (Chand and Lembi 1991) conducted in 1989 in which barrels were treated with 500 μg L^{-1} of flurprimidol.

Residue data calculated as ng ml^{-1} of water and ng g^{-1} fresh weight of plants and soil were used to determine dissipation curves and half lives of flurprimidol (Figure 1). Best fit regression equations showed that the half life of flurprimidol in water was 8.4 and 9.8 days at 75 and 200 μg L^{-1}, respectively. The compound was present at its maximum value in water within 1 day of treatment and then decreased through the 28 day period.

Flurprimidol in water is known to be highly susceptible to photolysis, with a half life of 3 h in pure water under high light intensities (Lilly Research Laboratories 1983). Photolysis of flurprimidol in our system seems likely since light could readily penetrate through the short water column (46 cm) in the barrels. Flurprimidol half lives of 6.8 days (Chand and Lembi 1991) to 9.8 days in this work indicate that the compound is indeed short-lived in water that is subject to good light penetration. Further work is needed to determine the fate of flurprimidol in water under lower light conditions.

Maximal concentrations of flurprimidol in milfoil tissue appeared within 1 to 3 days after treatment and decreased (Fig. 1). The half life of the compound in milfoil tissue (on per g fresh weight basis) was similar to that in water: 9.1 and 8.8 days at 75 and 200 μg L^{-1}, respectively. Although plants treated at 75 μg L^{-1} did not appear to lose flurprimidol on a per plant basis (Table 1), the dry weight per plant increased more than 5-fold over the 28 day period resulting in a decrease from 1200 ng flurprimidol g^{-1} dry weight at 2 h posttreatment to 190 ng flurprimidol g^{-1} dry weight 28 days after treatment. In contrast, at a treatment of 200 μg L^{-1}, the amount of flurprimidol did decrease per plant over the 28 day period. This treatment was more effective at reducing shoot growth than the 75 μg L^{-1} treatment (data in Lembi and Chand 1992), and dry weight per plant increased only 2-fold. Again, the amount of flurprimidol on a dry weight basis decreased from 3500 ng g^{-1} dry weight at 2 h to 510 ng g^{-1} dry weight at 28 days.

Gibberellin synthesis inhibitors are relatively persistent in terrestrial plant tissues and are effective in reducing stem length in tree species for at least 3 years (Williams 1984, Tukey 1986, Mauk et al. 1990) and in herbaceous species for several months (Dernoeden 1984). As much as 22% of the paclobutrazol detected at one week was still present in apple tissues 13 weeks after a soil drench application (Reed et al. 1989), and as much as 81% of ^{14}C-flurprimidol was recovered in apple tissue 35 days after stem injection (Sterrett and Tworkoski 1987). The compounds can be applied effectively to foliage (Lehman and Unrath 1988), suggesting that terrestrial plants can take up these compounds through their leaves as well as from the roots and via stem injection. The detection of nearly maximum amounts of flurprimidol in milfoil tissues 2 hours after treatment suggests that submersed plants may, at least initially, take up the compound from the water, presumably through the shoots. However, the relatively short half life of flurprimidol in milfoil tissue suggests that persistence of the compound in submersed aquatic plant tissues will more likely mirror that of terrestrial herbaceous species than woody species.

In contrast to water and plant tissue, flurprimidol concentration in the upper 5 cm of soil increased over the first

TABLE 1. MASS BALANCE OF TOTAL FLURPRIMIDOL RESIDUES IN WATER, SOIL AND PLANT TISSUES [% = PERCENT OF APPLIED (4072.8±16 μg PER BARREL FOR 75 μg L^{-1} AND 11584.2±470 μg PER BARREL FOR 200 μg L^{-1} FLURPRIMIDOL). % = PERCENT OF MEAN VALUES

Days after application	Flurprimidol residues (μg) Water	%	Soil(U)[a]	%	Soil(L)[b]	%	Plant	%	Total	%
75 μg L^{-1} Treatment										
0.08	3969	92.9	22.6	0.51	1.97	0.04	0.18	0.01	3991.8	93.5
	3600		18.8		1.74		0.45		3619.3	
1.0	3528	88.9	69.8	1.78	2.64	0.05	0.50	0.01	3598.9	90.8
	3718		74.9		1.71		0.57		3794.4	
3.0	3156	77.9	83.5	1.98	2.11	0.06	0.73	0.02	3240.3	79.9
	3194		77.4		2.67		0.58		3272.0	
7.0	2230	60.6	117.7	2.90	3.18	0.07	0.53	0.02	2348.4	63.6
	2707		118.4		2.88		0.92		2827.2	
14.0	1764	34.4	144.1	3.72	4.30	0.13	0.31	0.01	1910.1	38.3
	1039		159.1		6.18		0.17		1201.5	
28.0	382	9.3	293.9	5.06	6.27	0.16	0.36	0.01	680.1	14.6
	377		118.6		6.47		0.18		504.5	
200 μg L^{-1} Treatment										
0.08	11384	95.7	58.6	0.51	1.99	0.02	0.74	0.01	11443.7	96.3
	10795		58.3		2.08		1.21		10854.7	
1.0	11690	97.8	318.4	2.52	4.01	0.03	0.86	0.01	12009.6	100.5
	11011		286.2		1.71		1.40		11278.8	
3.0	9836	82.4	292.9	2.22	4.36	0.03	1.94	0.02	10131.3	84.7
	9313		222.5		3.21		2.08		9538.4	
7.0	7267	61.2	352.2	3.17	3.95	0.03	1.21	0.01	7621.2	64.3
	6891		381.3		3.47		1.31		7274.2	
14.0	3945	33.7	239.9	2.84	7.90	0.08	0.77	0.01	4187.8	36.6
	3856		419.1		8.78		0.53		4278.4	
28.0	1440	11.2	379.7	3.07	10.35	0.08	0.32	0.01	1834.6	14.4
	1163		331.8		9.50		0.31		1501.2	

[a] U = upper 5 cm.
[b] L = lower 5 cm.

7 to 14 days after treatment; after that point, the rate of increase appeared to level off (Fig. 1).

Of the flurprimidol still detectable in the barrels at the end of the 28 day period (14.6 and 14.4% of the initial amount applied), the highest percentage (64.1 and 78.1% of the total at 75 and 200 μg L^{-1}, respectively) was found in the water (Table 2). The upper 5 cm soil layer contained the next highest percentage (34.8 and 21.3% of the total at 75 and 200 μg L^{-1}, respectively). Very little of the compound moved into the lower soil layers. Only 2.7-3.1% of that present in the upper soil layer was present in the lower layer after 28 days (Table 2).

In terrestrial systems, the gibberellin synthesis inhibitors are considered to be susceptible to leaching under severe leaching conditions. After 45 days of leaching in a 30-cm soil column, 7.3% of the flurprimidol was found in the leachate; the remainder was evenly distributed in the column (Lilly Research Laboratories 1983). Between 27% and 53% of the paclobutrazol found in the first 5 cm of a treated soil was found in the 5 to 10-cm layer (Mauk et al. 1990). After 3 months, 28% of the uniconazole found in the 0 to 13-cm soil layer was present in the 25 to 38-cm layer although after 7 months that percentage dropped to 2% (Booth et al. 1989). In contrast to being exposed to a unidirectional mass flow of water as in terrestrial systems, the soil in our barrels was thoroughly water-saturated throughout the experimental period. The minimal downward movement of flurprimidol may have been due to diffusional processes, which tend to be slow, rather than through mass flow.

It is difficult to extrapolate our soil movement data directly to an aquatic system since we used a terrestrial loam soil with a low OM percentage rather than aquatic sediments which typically have a high OM content with potential binding properties. However, the chemistry of the gibberellin synthesis inhibitors does not indicate the

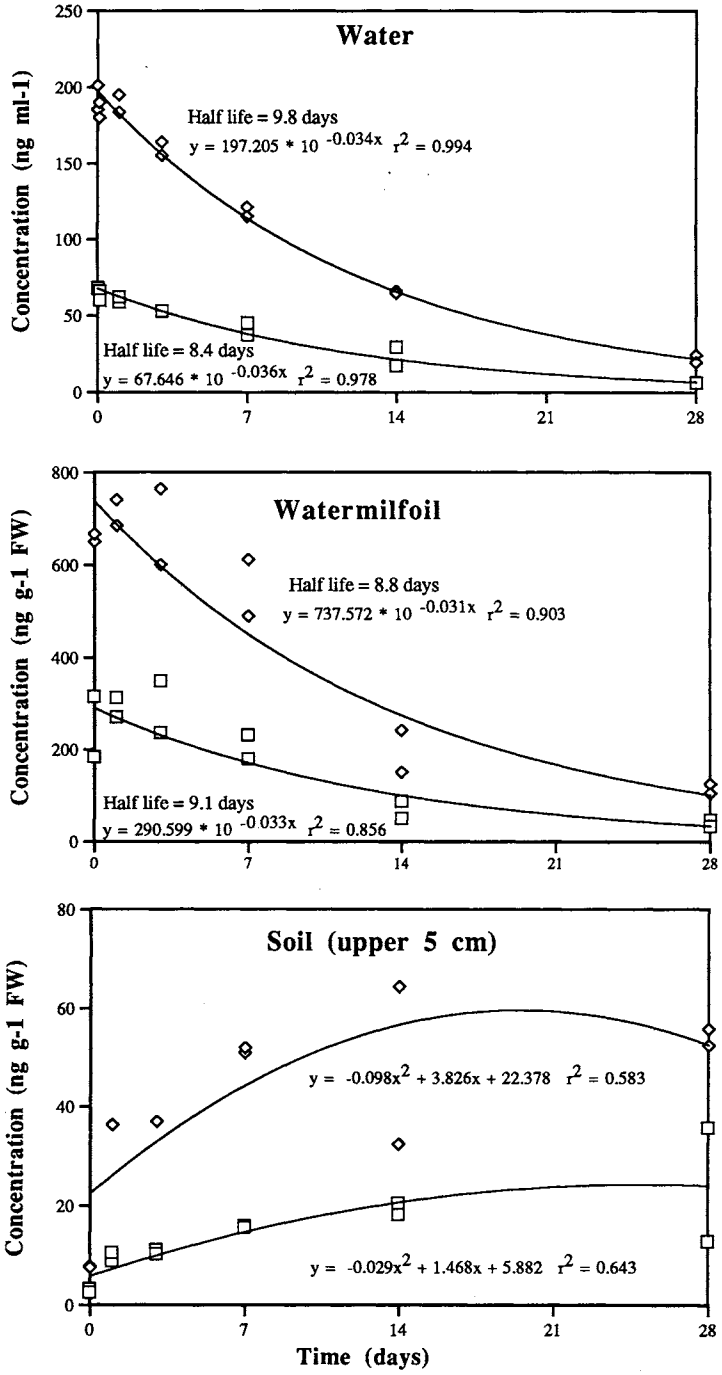

Figure 1. Dissipation curves of flurprimidol in water, watermilfoil tissue and the upper 5 cm soil layer. Treatments were 75 μg L^{-1} (diamonds) and 200 μg L^{-1} (squares).

presence of cationic groups that might bind to anionic components (clay or OM) in sediments. The addition of peat moss to greenhouse mixes did not change efficacy or leachability of the compounds when compared to their performance in mineral soils (Larson et al. 1974, Bonamino and Larson 1978, Barrett 1982). This suggests that the compounds are not tightly bound to organic matter, a factor that could influence both leachability and persistence. Interestingly, the addition of pine bark to greenhouse mixes did decrease efficacy and leachability; Barrett (1982) suggested that a hydrophobic attraction between nonpolar portions of the compounds and the bark accounted for increased binding.

TABLE 2. DISTRIBUTION (%) OF RECOVERED FLURPRIMIDOL 28 DAYS AFTER APPLICATION.

Sample	Treatment concentration (μg L^{-1}) 75	200
Plant	0.05	0.02
Soil (upper 5 cm)	34.79	21.33
(lower 5 cm)	1.07	0.59
Water	64.09	78.06

Milfoil tissue at 28 days contained less than 0.1% of the remaining flurprimidol in the barrels (Table 2), a result of the small amount of plant tissue present (0.06% of the total weight) in relation to the other components. However, when the concentrations of residues in plants, soils, and water were compared (on a per unit wet weight basis) during the treatment period, the plant tissue contained as much as 87 times more flurprimidol than the soil and between 2.93 and 6.39 times that in water (Table 3). The plant to soil ratios of flurprimidol decreased over the 28 day period, reflecting the gradual loss of detectable flurprimidol from the plant tissue and its increase in the soil. The plant/water ratios seem relatively stable over the 28 day period, suggesting that as the compound dissipates from the water, it is also dissipating from the plant tissue. Thus, there may be an equilibrium between tissue-held flurprimidol and that present in the water, so that flurprimidol concentration in water may be an important factor in dictating internal tissue concentration.

Another potential route of flurprimidol absorption by submersed plants is from the soil. The fact that the plant/soil ratios decreased over the 28 day period does not negate the possibility of uptake from the soil. Even though flurprimidol concentration in the plant does not seem to respond in a positive way to changes in flurprimidol concentrations in the soil, the concentrations in both the plant and the soil may be so high that they mask potential uptake and/or equilibrium relationships. Whether the plant is obtaining flurprimidol from the water column or from the soil cannot be determined using these data. Further studies following the fate of ^{14}C-labelled flurprimidol applied to the soil versus the water are needed to determine the major route by which the plants take up the compound.

Milfoil plants that had been exposed to flurprimidol for varying periods and then allowed to grow in untreated water for 28 days were harvested, measured for main stem length (data in Lembi and Chand 1992), and analyzed for flurprimidol residues. In this way we obtained plants with different % reductions in main stem lengths and internal flurprimidol concentrations. The relationship between % length reduction and internal concentration was linear and positive: % main stem reduction increased with increasing internal flurprimidol concentration (Figure 2). Plants that were reduced in length by at least 60% contained approx-

TABLE 3. RATIOS OF FLURPRIMIDOL IN PLANT TISSUE IN RELATION TO WATER AND SOIL. BASED ON FLURPRIMIDOL CONCENTRATION IN EURASIAN WATERMILFOIL (ng g^{-1} FRESH WEIGHT), SOIL (ng g^{-1} FRESH WEIGHT) AND WATER (ng ml^{-1}).

Days after Treatment	Plant/water 75	Plant/water 200	Plant/soil 75	Plant/soil 200
	$\mu g\ L^{-1}$		$\mu g\ L^{-1}$	
0.08	3.95	3.56	86.79	85.81
1.0	4.83	3.77	29.90	19.62
3.0	5.53	4.29	27.37	18.43
7.0	4.99	4.67	13.03	10.71
14.0	2.93	3.02	3.53	4.05
28.0	6.39	5.31	1.67	2.13

imately 55 to 85 ng flurprimidol per gram dry weight. At plant heights where no significant reduction in length had been obtained when compared to untreated controls (less than 20% reduction; Lembi and Chand 1992), internal flurprimidol concentrations were between 13 to 22 ng per gram dry weight. The data suggest that there may be an internal threshhold level of approximately 20 to 30 ng per gram dry weight, above which substantial length reduction can be expected and below which stem length reduction will be minimal. A similar relationship and threshhold value was obtained in separate experiments on hydrilla (data not shown). Although additional testing is required to confirm this particular relationship, such information would be valuable in screening treated plants in the field to determine whether uptake of the compound is sufficient to give reduced plant heights.

The dissipation patterns of paclobutrazol and uniconazole in water were similar to that of flurprimidol (Figure 3); however, half lives differed: 9.3, 24.4 and 5.2 days

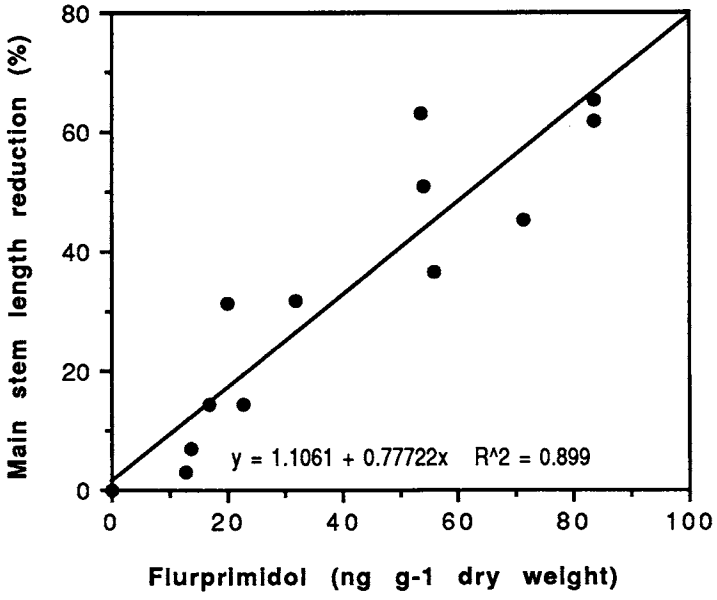

Figure 2. Relationship of internal flurprimidol content in milfoil tissue and % main stem length reduction. Main stem length reductions of less than 20% were not statistically different from untreated controls (data in Lembi and Chand 1992).

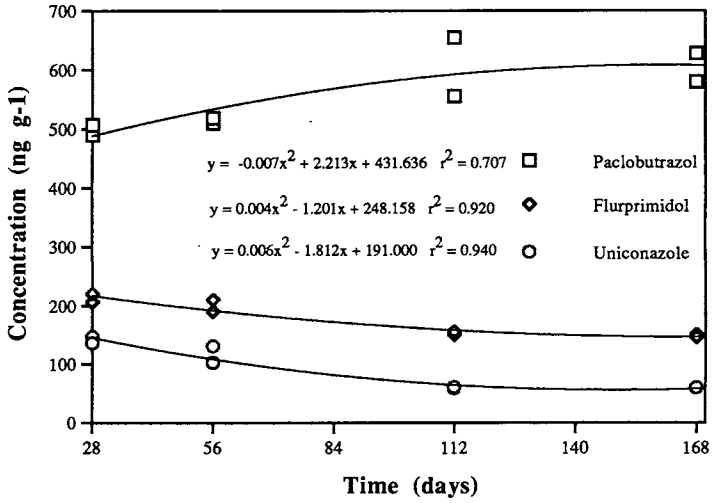

Figure 3. Dissipation of paclobutrazol, flurprimidol and uniconazole in water. Treatment was at 1000 $\mu g\ L^{-1}$.

for flurprimidol, paclobutrazol and uniconazole, respectively. When the compounds were measured in soil over a period of 28 to 168 days, flurprimidol and uniconazole gradually decreased (half-lives of 178 and 102 days, respectively) whereas mean values of paclobutrazol remained at concentrations of 500-700 ng g^{-1} fresh weight soil (Figure 4). The longer persistence of paclobutrazol (and shorter persistence of uniconazole) in these barrel systems was also indicated by the percent recovery at 168 days of the initial amount applied: 3.9, 0.9 and 27.4% of the flurprimidol, uniconazole, and paclobutrazol, respectively.

Clearly, the persistence of different gibberellin synthesis inhibitors in water varies. The only obvious structural difference between the shortest-lived compound, uniconazole, and the longest-lived compound, paclobutrazol, is the presence of a covalent double bond in uniconazole. In paclobutrazol, the carbons are saturated with hydrogen. However, the sterioisomers of the biologically-active forms of these two compounds also differ, a point that Steffens

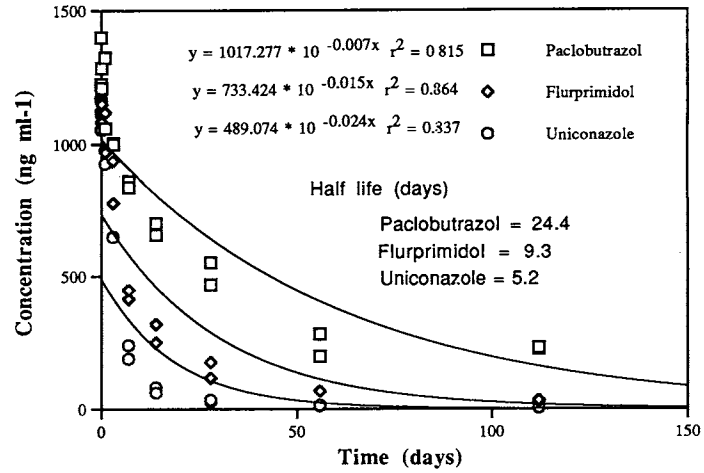

Figure 4. Dissipation of paclobutrazol, flurprimidol and uniconazole in soil. Treatment was at 1000 $\mu\ L^{-1}$.

(1988) suggested might partially account for the fact that uniconazole was the more effective of the two compounds in reducing stem length in greenhouse-grown apple trees, even at 115 days after treatment. Whether uniconazole in apple stem tissue is simply more biologically active than paclobutrazol or whether it persists longer, thereby providing long-term stem reduction, is unknown. In laboratory studies, uniconazole also was more effective than either flurprimidol or paclobutrazol in reducing main stem length in hydrilla (Netherland and Lembi 1992). This finding along with our data showing a shorter persistence time in water and soil suggests that the effectiveness of uniconazole may be due to biological activity rather than to persistence.

There is some indication from terrestrial systems that paclobutrazol may be more persistent in soil than flurprimidol, as it also appeared to be in our system. The half life of flurprimidol is estimated to be less than 6 months under conditions of adequate rainfall or irrigation (Lilly Research Laboratories 1983); however, paclobutrazol only decreased at a rate of 50% per year over a three year period in an apple orchard in North Carolina (Mauk et al. 1990). Since our long term soil persistence study was initiated in mid-June and the last sampling occurred in early December, when lake water temperatures in central Indiana are typically between 5-10 C (unpubl. data), it is possible that under warmer climatic conditions in other parts of the country the half lives of all three compounds will be shorter than those projected here. In addition, the ability of natural microbial populations in lake sediments to degrade these compounds still needs to be investigated. However, it is interesting to note that the soil half life of flurprimidol in our barrels was 178 days (5.9 months), similar to that projected for terrestrial systems by the Lilly Research Laboratories (1983). This may have been due to the fact that we used a typical terrestrial loam soil with low organic matter (OM).

In summary, flurprimidol shows rapid dissipation in water. Although flurprimidol dissipates from plant tissue, apparently low dosages (25-30 ng g^{-1} dry weight of plant tissue) are sufficient to achieve significant main stem length reduction. The compound dissipates slowly from soil, but further studies using lake sediments are required before a complete picture of sediment persistence is obtained. A half life of 6 months in sediment may be advantageous in providing a source of compound for long term plant uptake. The dissipation characteristics of the three gibberellin synthesis inhibitors in water and soil appear to differ; these differences may turn out to be important factors in determining which of these compounds is eventually developed for the aquatic market. Further information will also be needed on the breakdown products of these compounds and their dissipation characteristics.

ACKNOWLEDGMENTS

This research was funded by the U.S. Army Engineer Waterways Experiment Station under Contract DACW39-89-K-0022. We thank Drs. Thomas Jordan, Ronald Coolbaugh, and three anonymous reviewers for their critical reading of this manuscript. Purdue University Agricultural Experiment Station Journal No. 13439.

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