J Plant Growth Regul (1991) 10:73–78

Journal of
Plant
Growth
Regulation

Gas Chromatographic Determination of Flurprimidol in a Submersed Aquatic Plant (*Myriophyllum spicatum*), Soil, and Water

Tara Chand and Carole A. Lembi

Department of Botany and Plant Pathology, Purdue University, West Lafayette, Indiana 47907, USA

Received July 24, 1990; accepted October 10, 1990

Abstract. Methods for the extraction and quantification of flurprimidol residues in Eurasian watermilfoil (*Myriophyllum spicatum*), soil, and water are described. The compound was detected and quantified by gas chromatography (GC) with a thermionic specific detector. Its identity was confirmed by gas chromatography–mass spectrometry (GC-MS) with detection at m/e 40–320. Recoveries from samples spiked with flurprimidol at 10–10,000 ng ml^{-1} or g^{-1} averaged 86.8% for Eurasian watermilfoil shoots, 85.2% for roots, 79.3% for loam soil, and 93.3% for water. In a small-scale experiment under field conditions, approximately 88% of the applied flurprimidol dissipated in 4 weeks. The majority of recovered flurprimidol was found in the water and upper 5 cm soil layer. The half-life of the compound in water was 6.8–8 days during June/July 1989.

Flurprimidol [α-(1-methylethyl)-α-[4-(trifluoromethoxy)phenyl]-5-pyrimidine-methanol] is one of several gibberellin synthesis inhibitors, including ancymidol, paclobutrazol, and uniconazole, that have been shown to reduce stem elongation in many terrestrial plants (Barrett 1982, Dernoeden 1984, Hare 1984, Steffens 1988, Sterrett and Tworkoski 1987). Flurprimidol is also effective in reducing the elongation of the submersed aquatic weeds Eurasian watermilfoil (*Myriophyllum spicatum* L.) and hydrilla (*Hydrilla verticillata* Royle) when applied to water at very low concentrations (0.75 and 75 μg L^{-1}, respectively) and for exposure times as short as 2 h (Lembi et al. 1989, Netherland 1989). Reduction of stem length in these aquatic plants would minimize weediness but allow the plants to remain viable and retain beneficial characteristics, such as oxygen production, fish habitat, and sediment stabilization.

An ideal plant growth regulator or herbicide, whether applied directly to aquatic weeds or to terrestrial sites with potential for residue runoff into an aquatic environment, should have low persistence. However, no information is available on persistence of any of the gibberellin synthesis inhibitors in the aquatic environment. The purpose of this study was to develop methods of extracting flurprimidol residues from Eurasian watermilfoil (roots, shoots, and buds), and to determine residues in water, soil, and plant parts.

Materials and Methods

Extraction from Plant Tissues

Eurasian watermilfoil tissue was freeze dried, macerated into a fine powder, and stored. All solvents used for extractions were of high-performance liquid chromatographic (HPLC) grade. Two different methods of extraction (Reed 1988, Stahly and Buchanan 1986) were tried. The most satisfactory method, resulting in less contamination from plant pigments, was the extraction method of Stahly and Buchanan (1986) but with the following modifications. (1) Plant samples were extracted in 80% methanol at 55°C for 30 min rather than at room temperature for 5 min with a blender. (2) Samples were first purified through 1 g LC-florisil SPE (Suppelco, Inc.) tubes; the flurprimidol was eluted with a 5-ml mixture of anhydrous ether and methanol (97:3 vol/vol) and evaporated in vacuo. Residues were dissolved in 1 ml of 100% methanol, and 24 ml of water was added to dilute the solution to 4% methanol. In the final step of purification, 0.5-g Sep-Pak C_{18} cartridges (Water Associates, MA, USA) were conditioned with 5 ml of 100% methanol and then 10 ml of 4% methanol. The samples were loaded on the cartridges with a 10-ml rinse of 4% methanol. Flurprimidol was eluted with 5 ml of 80% methanol which was collected, vacuum evaporated, and dissolved in 100% anhydrous methanol for gas chromatography (GC) and/or GC-mass spectrometry (GC-MS) analysis.

Extraction from Soil

Free water was removed from each soil sample by vacuum-

filtering the soil through Whatman No. 1 filter paper in a Buchner funnel. The extraction method for the wet soil was the same as that used for plant tissue.

Extraction from Water

Flurprimidol was extracted from water samples by the method described by West (unpublished, available from Eli Lilly Research Laboratories, Indianapolis, IN, USA). The major modification was eliminating the hexane addition prior to eluting the sample through the Sep-Pak C_{18}. Elution was conducted with 80% instead of 100% methanol. Fewer impurities were obtained when this sequence was used.

Recovery of Known Amounts of Flurprimidol

Watermilfoil plants were grown under controlled environmental conditions (25 ± 1°C, 400 μE m^{-2} s^{-1}, 16:8 h light:dark) in 3-L flasks (Selim et al. 1989, Smith et al. 1989). The plants were harvested after 6–8 weeks of growth and were washed twice with distilled water prior to freeze-drying. Flurprimidol (99.8% technical grade, Eli Lilly) dissolved in 100% methanol was added to 1–2 g of the freeze-dried, macerated plant tissue, 25–200 ml of well water, and 20 g wet weight of soil.

Small-Scale Field Experiments

Analysis of flurprimidol residues in plant parts, soil, and water in a small-scale outdoor experiment was conducted during June/July 1989. Metal barrels (67-L capacity) were lined with plastic liners. Loam soil (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 suspended soil was allowed to settle. Ten healthy milfoil stem apices (10 cm length) without roots were planted in each barrel and allowed to acclimate for 1 week prior to flurprimidol treatment on June 4, 1989. Flurprimidol (50% WP, Elanco Products Company, Indianapolis, IN, USA) was applied at a concentration of 0.0 (control) and 500 μg active ingredient (a.i.) L^{-1} with three replicates per treatment. Water samples were taken immediately after treatment and 28 days after treatment when plants were harvested and soil was sampled for flurprimidol analysis. The plants were washed twice with distilled water and segregated into shoots, buds, and roots. The plant parts were blot-dried, and their wet weight was recorded before freeze drying within 24 h of collection. Soil cores were taken using a hollow plastic cylinder (5 cm inner diameter by 15 cm in length). Water and soil samples were frozen for storage. For analysis, thawed soil samples were divided into upper and lower 5 cm layers before removing the free water.

To study the dissipation of flurprimidol in water over time, another small-scale barrel experiment, similar to the experiment described above, was conducted in the field. In this experiment only two milfoil apices were planted in each barrel. On June 4, 1989 flurprimidol was applied at concentrations of 0.0 (control), 7.5, and 75.0 μg a.i. L^{-1}. There were two replicates per treatment. One liter water samples were taken from each barrel prior to treatment, immediately after treatment, and 2 h, 1, 3, 7, 14, and 28 days after treatment.

GC and GC-MS

GC was conducted using a Varian 3400 GC equipped with a model 8035 autosampler; 1075 split/splitless capillary injector set at a split ratio of 1:6 with a 2 μl injection volume; thermionic specific detector (TSD) (Varian Associates, Inc., Walnut Creek, CA, USA); and a DB-17 (30 m × 0.32 mm) fused silica capillary column (J & W Scientific, Folson, CA, USA). Gas flow velocity for the hydrogen carrier gas was 45 cm min^{-1}; for make-up gas N_2, 30 ml min^{-1}; H_2, 4.95 ml min^{-1}; and air, 175 ml min^{-1}. The TSD bead current was 2.950 A, and bias voltage was −4.0 V. The temperature for chromatography was 250°C for the injector and detector. The initial column temperature was 150°C for 1 min followed by a 3°C min^{-1} increase to a final temperature of 230°C with a 5-min hold time. Under these conditions, flurprimidol retention time was 11.74 min.

GC-MS was conducted using a Hewlett-Packard GC 5890A with a HP mass selective detector (MSD) 5970 and a HP7673A autosampler. The same column, injection volume (splitless mode), and temperature program as in the GC analysis were used, except that the initial column temperature was 170°C. Helium gas flow was 32 ml min^{-1}. Electron ionization was at 70 eV with a scan range of m/e 40–320. The retention time of flurprimidol was 11.89 min.

Standard curves for GC were developed by injecting 2 μl volumes of standard solutions (0.1, 0.25, 0.5, 1.0, 2.5, 5.0, 10.0, 25.0, 50.0, and 100 μg ml^{-1}) of technical grade flurprimidol (99.8% pure) in 100% methanol. While running samples on the GC or GC-MS every third or fourth sample was a flurprimidol standard to detect variability in the sensitivity of the instruments.

Results and Discussion

The peak area response of the TSD was linear over a concentration range of 0.1–100 μg ml^{-1}. The average correlation coefficient for peak area linearity of the standard solutions was 0.995–0.998 on 7 different days. As noted by West and Rutherford (1986) we also found that the peak area response of the GC-MS was highly variable on different days and was dependent on the autotune of the instrument. Consequently, GC-MS was only used for the confirmation of flurprimidol residues. The mass spectrum of technical grade flurprimidol at 5 μg ml^{-1} and an injection volume of 1 μl consists of four major ions at m/e values of 79, 107, 189, and 269 (Fig. 1) with relative intensities (%) of 20, 100, 7, and 69, respectively; retention time was 11.89 min. The molecular ion (MW 312.3) was not recorded until the flurprimidol concentration was at least 25 μg ml^{-1}. Even at this concentration, the relative intensity of the molecular ion was less than 1%. In all treated samples (fortified and field) the retention time of flurprimidol was the same. The relative intensities (%) of ions 79, 107, 189, and 269 were 17–23, 100, 4–8, and 59–65, respectively, which confirms the identity of flurprimidol.

The extraction efficiency at different levels of for-

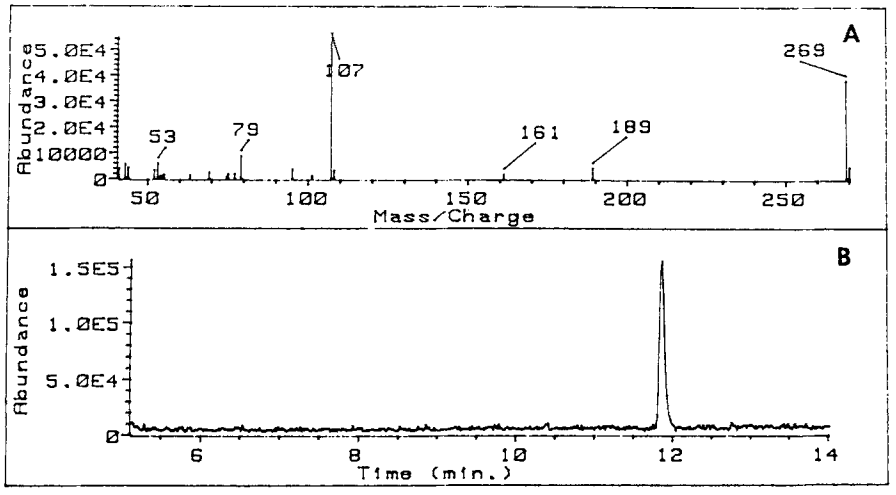

Fig. 1. GC-MS characteristics of flurprimidol at 5 ng injection. **(A)** Mass spectrum; the major ions of flurprimidol are 79, 107, 189, and 269. **(B)** Total ion chromatogram.

Table 1. Recovery of flurprimidol from Eurasian watermilfoil shoots, roots, soil, and water samples spiked with different flurprimidol concentrations.

Sample type	Added flurprimidol[a]	Recovery level[a,b]	% recovery
Plant shoots	100	83 ± 2	82.9
	400	360 ± 52	89.6
	2000	1847 ± 91	90.6
	5000	4225 ± 24	83.9
	10,000	8725 ± 34	87.2
Plant roots	100	78 ± 4	78.5
	400	332 ± 23	83.0
	2000	1744 ± 35	87.2
	5000	4505 ± 40	90.1
	10,000	8710 ± 38	87.1
Loam soil	25	17 ± 4	68.4
	100	79 ± 3	79.3
	200	182 ± 6	91.0
	500	388 ± 17	77.6
	1000	801 ± 49	80.1
Water	10	9 ± 2	88.5
	25	23 ± 2	91.0
	125	117 ± 19	93.4
	250	236 ± 18	94.3
	500	476 ± 15	95.3
	1000	972 ± 11	97.2

[a] ng g^{-1} dry weight basis in plant shoots and roots, ng g^{-1} fresh weight basis in soil, ng ml^{-1} in water.
[b] Each value is the mean ± SD of two experiments with two replicates each.

tification was tested by spiking plant, water, and soil samples with known concentrations of flurprimidol (Table 1). Mean recoveries were 86.8% from watermilfoil shoots, 85.2% from roots, 79.3% from loam soil, and 93.3% from water. These recoveries were similar to those obtained for a soil–grass mixture (78%), soil (80%) (West and Rutherford 1986), and peach leaves (83.6%) (Reed 1988), which were also analyzed by GC, and considerably better than those obtained from plant tissue (40%), which was analyzed with HPLC (Booth et al. 1989).

Flurprimidol concentrations in field-grown plants after 28-day exposures were highest in the buds (Table 2). Flurprimidol was also found in the stems and roots. The milfoil plants at the time of treatment did not have roots but produced them during the exposure period. This suggests that the flurprimidol either moved basipetally in the plant or entered newly forming roots via the water–soil solution. The former seems unlikely since most of the literature on terrestrial plants suggests that flurprimidol and other gibberellin synthesis inhibitors are translo-

Table 2. Recovery of flurprimidol from Eurasian watermilfoil plant parts, water, and soil collected from barrels 28 days after treatment with 500 μg L^{-1} flurprimidol.

Sample[a]		Flurprimidol		
Type	Wet wt or vol. $barrel^{-1}$	Concentration[b]	Total (ng) $barrel^{-1}$	Distribution (%) of recovered flurprimidol
Stems	12.8 ± 0.8	67 ± 11	870 ± 20	0.021
Buds	3.6 ± 1.8	93 ± 21	332 ± 20	0.008
Roots	8.1 ± 1.8	51 ± 1	410 ± 10	0.010
Soil				
Upper 5 cm	7065 ± 432	220 ± 51	$1.56 \times 10^6 \pm 3.94 \times 10^5$	37.9
Lower 5 cm	6664 ± 368	10 ± 2	$6.80 \times 10^4 \pm 1.10 \times 10^4$	1.7
Water	55,000	45 ± 12	$2.47 \times 10^6 \pm 2.12 \times 10^5$	60.4
Total recovery (ng) after 4 weeks			4.10×10^6	
Total applied (ng)			3.49×10^7	
Dissipation (%)in 4 weeks			88.3	

[a] Wet weight (g) of Eurasian watermilfoil and soil; volume (ml) of water.
[b] ng g^{-1} fresh weight basis in Eurasian watermilfoil and soil; ng ml^{-1} in water.

cated primarily in the xylem (e.g., Sterrett 1988). The compound probably entered all of the plant parts through the aqueous medium; however, a slightly higher accumulation of flurprimidol in buds may indicate some upward movement in the plant. Sterrett and Tworkoski (1987) found that 10% of the flurprimidol applied to woody terrestrial plants by stem injection had moved into new shoots by 35 days after treatment. The majority of the compound remained near the application site, and none was detected in the roots.

The amount of flurprimidol applied to each barrel at a dose of 500 μg a.i. L^{-1} was approximately 3.49×10^7 ng. At the end of 28 days, a total of approximately 4.1×10^6 ng of flurprimidol was recovered in the plant, soil, and water components (Table 2). Residues in the combined plant parts accounted for only 0.039% of the total flurprimidol recovered (Table 2). Soil accounted for 39.6% of the total recovered, although the highest concentration and recovery was found in the upper 5 cm soil layer. Of the total recovered in the wet soil, approximately 4.2% had moved into the lower 5 cm of soil. The flurprimidol concentration in the free water from the upper layer was approximately the same as the concentration detected in the water from the barrel; however, no flurprimidol was detected in the free water from the lower 5 cm of soil. Flurprimidol is weakly adsorbed and easily desorbed from soils (Lilly Research Laboratories 1983) and therefore appears to be readily available for plant uptake and leaching. In leaching columns, 7.3% of applied flurprimidol had moved through 30 cm of terrestrial soils after 45 days (Lilly Research Laboratories 1983).

Approximately 60% of the recovered flurprimidol was present in the water fraction (Table 2). However, this only represented approximately 7% of that initially applied (3.49×10^7 ng); the estimated half-life was 8 days. The actual analysis of flurprimidol residues with time showed that its half-life in water at 7.5 and 75.0 μg a.i. L^{-1} was 6.8 days (Fig.

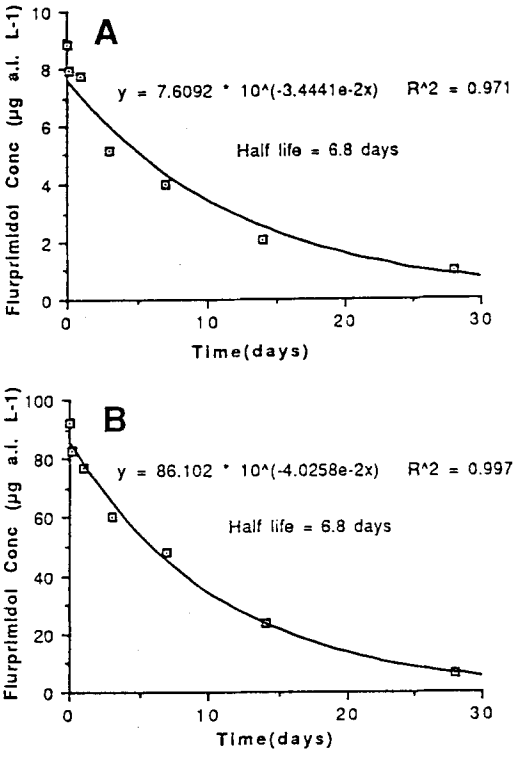

Fig. 2. Recovery of flurprimidol in barrel water: **(A)** 7.5 μg a.i. L^{-1} treatment and **(B)** 75 μg a.i. L^{-1} treatment. Half-life of flurprimidol at both concentrations is 6.8 days.

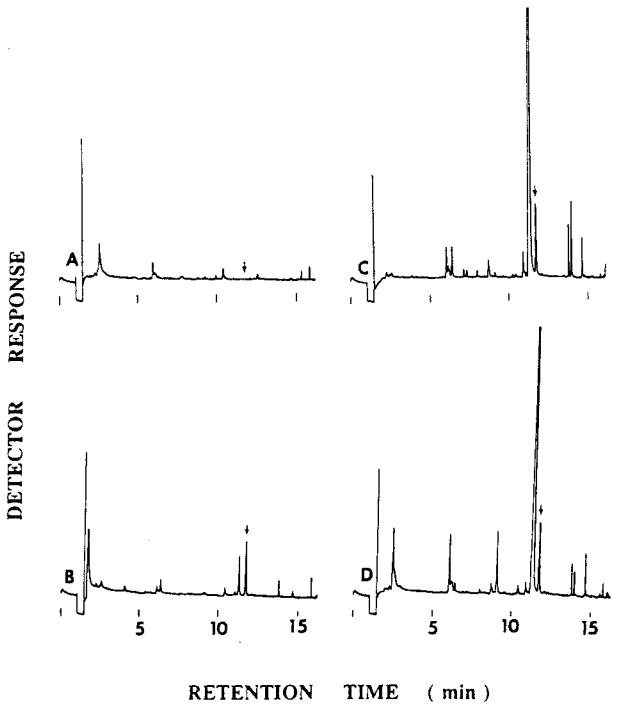

Fig. 3. Capillary GC analysis of flurprimidol: **(A)** shoots of untreated Eurasian watermilfoil (no flurprimidol detected), **(B)** roots, **(C)** shoots, and **(D)** buds of flurprimidol-treated Eurasian watermilfoil after preparation through florisil and C_{18}. Peak identification, RT = 11.74 min (arrows).

2). In addition to loss to the soil and plant components, flurprimidol is highly susceptible to photolysis with a half-life of 3 h in pure water under high light intensities (Lilly Research Laboratories 1983).

Approximately 88.3% of the flurprimidol initially applied had disappeared within the 28-day period (Table 2). However, even at low concentrations, flurprimidol may retain its activity in reducing plant elongation. At least 2 or 3 years of stem elongation reduction have been monitored on woody species using foliar or soil drench applications of flurprimidol and paclobutrazol (Williams 1984, Wood 1986). In a separate study with hydrilla, we found stem reduction at 4 weeks after only a 2-h exposure to the compound at concentrations as low as 75 $\mu g\ L^{-1}$ (unpublished results).

With our extraction procedures no peak was found in the flurprimidol area of the chromatogram when untreated watermilfoil shoots (Fig. 3A), soil, or water samples were analyzed. However, a large peak was recorded in front of the flurprimidol peak in every chromatogram of plant shoots, buds, or roots from flurprimidol-treated plants (Fig. 3B–D). This peak did not appear in flurprimidol-treated water or soil (data not shown). To get a good resolution of the flurprimidol peaks, the column was programmed at 3°C min^{-1} as described in Materials and Methods. In the published literature, high temperature programs for the column have been used to get shorter retention times of flurprimidol and other plant growth retardants (Reed 1988, Stahly and Buchanan 1986). This saves time and is acceptable if there is no interfering peak. Initially, we ran our samples at the higher temperature program for the column (initial 170°C for 1 min followed by 20°C min^{-1} increase to a final of 250°C with a 10 min hold). With this temperature program these two peaks were recorded as one, and we subsequently modified the column temperature to get good resolution of peaks.

The results of these small-scale field experiments showed that approximately 88% of the applied flurprimidol dissipated in 28 days and that its half-life in water is short (6.8–8 days). Due to these desirable characteristics flurprimidol may have a potential use in the management of watermilfoil and other aquatic weeds. Further study of the residues in a natural pond or lake system is the next step in assessing the acceptability of this compound for the aquatic market.

Acknowledgments. Flurprimidol was provided by Lilly Research Laboratories. The assistance of A. Ekanayake, J. Hazebroek, and T. Clark Reed is gratefully acknowledged. We also thank R. Coolbaugh and P. Nelson (Purdue University) for the use of their analytical equipment and S. West (Eli Lilly Research Laboratories) for technical advice. The tests described and the resulting data presented herein, unless otherwise noted, were obtained from research conducted under the Aquatic Plant Control Research Program of the United States Army Corps of Engineers by the US Army Engineer Waterways Experiment Station. Permission was granted by the Chief of Engineers to publish this information.

References

Barrett JE (1982) Chrysanthemum height control by ancymidol, PP333, and EL-500 dependent on medium composition. Hortic Sci 17:896–897

Booth MC, Campidonica MJ, Fujino DW, Sachs RM (1989) HPLC methods for detection of uniconazole-P in soils and plant tissues. J Plant Growth Regul 8:293–300

Dernoeden PH (1984) Four-year response of Kentucky bluegrass–red fescue turf to plant growth retardants. Agron J 76:807–813

Hare RC (1984) EL-500: An effective growth retardant for dwarfing southern pine seedlings. Can J For Res 14:123–127

Lembi CA, Chand T, Reed TC (1989) Plant growth regulator effects on submersed aquatic plants. Proceedings, 24th Annual meeting, Aquatic Plant Control Research Program, Army Corps of Engineers, Huntsville, Alabama (in press)

Lilly Research Laboratories (1983) Technical report on EL-500. Eli Lilly and Company, Indianapolis, IN 46285

Netherland MD (1989) A laboratory bioassay used to test the effect of three related plant growth regulators on the growth of two submersed aquatic plant species. M.S. Thesis, Purdue University, W. Lafayette, IN 47907

Reed AN (1988) Quantitation of triazole and pyrimidine plant growth retardants. J Chromatogr 438:393–400

Selim SA, O'Neal SW, Ross MA, Lembi CA (1989) Bioassay of photosynthetic inhibitors in water and aqueous soil extracts with Eurasian watermilfoil (*Myriophyllum spicatum*). Weed Sci 37:810–814

Smith CS, Chand T, Harris RF, Andrews JH (1989) Colonization of a submersed aquatic plant, Eurasian water milfoil (*Myriophyllum spicatum*), by fungi under controlled conditions. Appl Environ Microbiol 55:2326–2332

Stahly EA, Buchanan DA (1986) Extraction, purification, and quantitation of paclobutrazol from fruit tissues. Hortic Sci 21:534–535

Steffens GL (1988) Gibberellin biosynthesis inhibitors: Comparing growth-retarding effectiveness on apple. J Plant Growth Regul 7:27–36

Sterrett JP (1988) XE-1019: Plant response, translocation, and metabolism. J Plant Growth Regul 7:19–26

Sterrett JP, Tworkoski TJ (1987) Flurprimidol: Plant response, translocation, and metabolism. J Am Soc Hortic Sci 112:341–345

West SD, Rutherford BS (1986) Gas chromatographic determination of the plant growth regulator flurprimidol in soil and soil–grass samples. J Assoc Off Anal Chem 69:572–575

Williams MW (1984) Use of bioregulators to control vegetative growth of fruit trees and improve fruiting efficiency. Acta Hortic 146:97–104

Wood BW (1986) Influence of paclobutrazol (PP-333), flurprimidol and Ortho XE-1019 (Chevron) growth retardants on growth and selected chemical and yield characteristics of *Carya illinoensis*. Acta Hortic 179:287–288