JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Application and Fate of Cyromazine in a Closed-Cycle Hydroponic Cultivation of Bean (*Phaseolus vulgaris* L.)

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The fate of cyromazine applied via the nutrient solution (20, 40, and 60 mg of active ingredient per plant) in a closed-cycle soilless cultivation of bean with zero discharge of effluents was traced in both the recycled drainage solution and the plant tissues for 99 days. The insecticide was applied once, 15 days after planting (16 days prior to the first harvest). In addition to cyromazine, the residues of melamine, its metabolite, in the drainage solution and plant tissues were also regularly determined during the 99 days. The two higher application doses induced toxicity symptoms on the leaves of the bean plant. The maximum cyromazine levels were measured 8 days after application in the drainage solution (17–46 mg l^{-1}), 16 days in the roots (1.1–2.4 mg kg^{-1} fresh weight [f. wt.]) and the vegetative shoot (4.5–9.5 mg kg^{-1} f. wt.), and 24 days after application in the pods (2.6–4.1 mg kg^{-1} f. wt.). However, the cyromazine residues in pods were clearly below the maximum acceptable levels for bean. The half-life of cyromazine in the drainage solution ranged from 16 to 19 days for the three doses. The melamine residues in the drainage solution and in the roots reached a concentration peak 16 days after cyromazine application, whereas in the vegetative shoot and the pods they were constantly increasing over the 99 days after application. Nevertheless, the melamine residues were constantly much lower than those of cyromazine, although on the last sampling day (99) they tended toward convergence. Cyromazine proved to be highly persistent, as indicated by the remarkably high residues measured in both the drainage solution and the plant tissues, even 99 days after application. Nevertheless, the application of cyromazine via the nutrient solution to beans grown in closed-cycle hydroponic systems at doses not exceeding 20 mg per plant seems to be safe with respect to both phytotoxicity and residue levels in the edible pods.

KEYWORDS: Pesticide; cyromazine; melamine; root application; hydroponics; soilless culture; recycling; bean; *Phaseolus vulgaris*

INTRODUCTION

Soilless culture provides not only a pathogen-free root environment at planting, thus circumventing the need for soil disinfection, but also promotes earlier and higher yields (*1*, *2*). Another advantage of soilless cultivation is the reduced environmental pollution originating from leaching of fertilizer residues, provided that the drainage water is recycled (*3*–*5*). Furthermore, if the fertilization effluents are recycled by growing the crop plants in closed-loop soilless systems, it is possible to apply suitable systemic pesticides via the nutrient solution, thereby minimizing or even completely eliminating the release of residues into the environment (*6*, *7*). Furthermore, the application of pesticides via the root system eliminates both the exposure of labor to residues originating from spraying and the spotting of leaves and harvested products through dried spray drops. However, previous research has indicated that some pesticides may persist for several weeks in the root zone and concomitantly in the plant tissues, especially in roots and leaves, when applied in the recycled nutrient solution (*6*, *7*). Residues of pesticides in plant tissues of ornamentals do not constitute a threat for human health, because these plants are not edible. In contrast, the persistence of pesticide residues at levels exceeding the safe limits in the edible parts of vegetables is unacceptable. Hence, to assess the possibility of applying plant protective agents to vegetables crops via the root system in closed-cycle

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10.1021/jf071726i CCC: $37.00
Published on Web 11/03/2007

Table 1. Physicochemical Properties of Cyromazine and Melamine

characteristic	cyromazine value	cyromazine source	melamine value	melamine source
solubility in water, 20 °C, pH 7.1 (g L^{-1})	13	*16*	3.1	*21*
log K_{ow} (pH 7.0)	−0.061	*17*	−1.14	*21*
log K_{oc}	0.8	*18*	No data	
pK_a	5.22	*17*	No data	
Henry's law constant (Pa m^3 mol^{-1}, 20 °C, pH 7.5)	2.72 × 10^{-6}	*19*	No data	
aerobic soil half-life (d)	150	*20*	730–1,100	*21*
acute oral toxicity, rat (LD_{50}, mg kg^{-1})	3,387	*17*	3,161	*21*
acute dermal toxicity, rat (LD_{50}, mg kg^{-1})	>3,100	*17*	>1,000	*21*
CAS number	66215–27–8	*17*	108–78–1	*21*

cultivation systems, it is essential to test different doses and to study their fate in both the recycled nutrient solution and the plant tissues.

Cyromazine (N-cyclopropyl-1,3,5-triazine-2,4,6-triamine) is an atypically substituted *s*-triazine acting as an insect growth regulator (*8*). Cyromazine proved to be highly effective as a foliar spray of horticultural crops against leafminers (*Liriomyza* sp.) and various other insects, including fleas, thrips, and coleoptera (*9, 10*). When released into the environment, cyromazine is initially metabolized into melamine (*11, 12*). Residues of cyromazine and its metabolites have been detected after spray applications in tomato (*13*), and various *Brassica* sp. vegetables (*14, 15*). Some physical and chemical properties of cyromazine and melamine, which are relevant to this study, are listed in **Table 1** (based on *16–21*). The structural formulas of cyromazine and melamine are shown in **Figure 1**.

Cyromazine is suitable for application via the nutrient solution in closed hydroponic systems because of its ability to move acropetally (*13, 22*), in combination with its high water solubility (*16, 19*). The application of cyromazine via the nutrient solution in closed-cycle cultivation systems is an efficient strategy to reduce residual leaching to the groundwater, which is a serious problem in soil-grown crops, as indicated by the appreciably low soil adsorption coefficient (log K_{oc}) of this pesticide (*18, 20*). However, the available information in the literature regarding the fate of cyromazine after application to crops via a nutrient solution is limited. A previous study with soilless-grown gerbera under Mediterranean climatic conditions revealed a persistence of cyromazine residues in the leaves for more than four months in the winter and more than seven weeks in the summer, following application via the recycled nutrient solution (*7*). Nevertheless, the residues of cyromazine in gerbera flowers were considerably lower, appeared later, and disappeared earlier than in the leaves, presumably because of lower transpiration rates in combination with shorter exposure times. The transpiration rates of fruits are much lower than those of leaves. Indeed, the transpiration rates of bean pods were 15% of the values measured in leaves under equivalent conditions (*23*). Obviously, the differences in transpiration rates between leaves and fruits would be much larger if they were expressed on a weight and not on an area basis. Hence, the residue concentrations of pesticides are expected to be considerably lower in fruits than in leaves when applied to fruit vegetables via the nutrient solution in soilless culture. Consequently, if an appropriately low dose of cyromazine is supplied to fruit-bearing vegetables at an early vegetative growth stage, then a sufficient protection level against leaf insects might be attained, while maintaining acceptably low residue concentrations in the edible fruits.

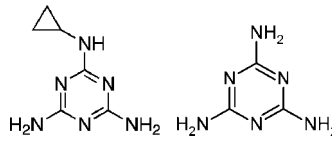

Figure 1. Structural formulas of cyromazine (left) and its derivative, melamine (right).

To test the above hypothesis, three different doses of cyromazine were applied via the recycled nutrient solution to a bean crop grown in a closed-cycle soilless system to track the fate of this insecticide and its metabolite melamine in both the plants and the environment. The data obtained may be used to assess the risks and possibilities of applying cyromazine by this alternative method to greenhouse vegetables grown in closed-cycle hydroponic systems.

MATERIALS AND METHODS

Experimental Design. The experiment was conducted in a glasshouse located in Arta (lat. 39° 7′ N, long. 20° 56′ E), Greece. Bean seedlings (*Phaseolus vulgaris* L. cv. 'Helda') propagated in peat plugs were transferred at the stage of the second true leaf to 24 channels (5 × 0.25 m) filled with pumice as growing medium. The channels were connected to 12 independent, fully automated hydroponic installations, which enabled complete recycling of the nutrient solution effluents. Each closed hydroponic system, or circuit, constituted an experimental unit comprising two channels, and each channel accommodated 80 plants (160 plants per experimental unit). Plant spacing along each channel was 12 cm, with two plants at each position, corresponding to a crop density of 12.8 plants per m^2. The plants were supported by plastic twine attached 2.2 m above the plant row on a horizontal wire. Trickle irrigation was automatically applied by a computer program at intervals depending on solar radiation intensity, which was monitored using a pyranometer. All channels were covered with black–white polyethylene sheets, and thus, water evaporation was negligible. In all experimental units, the entire quantity of drainage water was collected and recycled, regardless of its volume, by mixing it with water and concentrated fertilizer solutions. The pH of the nutrient solution effluents, which was automatically registered prior to recycling, ranged from 5.3 to 7.4 during the experiment. The amounts of fertilizers and water blended with the drainage solution to prepare the nutrient solution supplied to the crop were roughly equal to those absorbed by the plants to maintain a constant nutrient regime in the root zone. The amounts of nutrients and water absorbed by the plants were estimated by means of a previously developed model (CDW), which is based on frequent chemical analysis of the drainage water (*4*). The drainage fraction was maintained within the range of 0.35–0.45 by properly adjusting the cumulative solar energy at which irrigation events were triggered. No drainage water was deliberately discharged, and any losses due to technical failures were negligible.

The bean seedlings were planted on 10 October 2004. Recycling of the drainage solution was initiated on 15 October 2004. The first harvest of commercially ripe pods took place on 10 November 2004, and the experiment was terminated on 1 February 2005. Three experimental treatments were established by adding 40, 80, and 120 mg L^{-1} of cyromazine active ingredient into fresh nutrient solution, or 20, 40, and 60 mg of cyromazine active ingredient per plant, respectively. Each dose was applied in four circuits, and thus, there were four replications for each treatment, corresponding to four blocks. In each block, the NaCl concentration in the nutrient solution supplied to the plants was different, but this was considered to be block variation. The application of cyromazine (Trigard 75% WP, Syngenta), took place on 25 October 2004, early in the morning (8:00 a.m.). In each circuit, the required amount of cyromazine was mixed into 80 L of fresh nutrient solution, which was subsequently supplied to the plants in six irrigation cycles throughout the whole day.

The experiment was considered as a single factor (cyromazine application dose) randomized complete blocks design, with three treatments and four blocks per treatment. The PlotIT3.2 work package was used for statistical analysis and plotting graphs.

Sampling. During the experiment, samples of drainage solution were collected from each circuit on days 1, 4, 8, 16, 32, 65, and 99 after application of the insecticide and were used to determine the cyromazine residue concentrations. Immediately after the collection of each drainage solution sample, one whole plant was randomly sampled from each circuit, separated into shoot and roots, and used to determine cyromazine residue concentrations. Furthermore, 16, 24, 32, 40, 65, and 99 days after application of the insecticide, samples of commercially ripe pods were also selected and used to determine the residue concentrations of cyromazine. Thus, for each sampled material, there were four samples from each treatment at each time. After separation of each plant sample into shoot and roots, the entire mass of each sample was chopped and homogenized. Furthermore, all commercially ripe pods selected from each experimental unit on each particular sampling date were also chopped and homogenized. Thereafter, an aliquot of 20 g was obtained from each homogenized sample, packed in a bag, and placed in an ice chest. As soon as sampling was complete, the samples were transported to the laboratory and stored at −4 °C. The nutrient solution samples were filtered and analyzed directly within 48 h of collection. On all sampling dates, nutrient solution not used in the experiment, which was free from cyromazine, and bean plants irrigated with this nutrient solution, were sampled. These samples were analyzed for cyromazine residues too.

Chemicals and Solvents. Cyromazine (99.5%) and melamine (99.5%) reference standards were purchased from Riedel-de Haen (Germany). Potassium dihydrogen phosphate (99.5%), ortho-phosphoric acid (85%) and HCl were supplied by Merck (Darmstadt, Germany). Methanol and water were HPLC-grade from Merck (Darmstadt, Germany). The cleanup cartridges of carbon black (Superclean ENCI-Carb 120/400) were purchased from Supelco (USA), and the 0.45 μm PTFE filters were from Millipore (Millex DG). Standard stock solutions of cyromazine and melamine were prepared in methanol and a mixture of methanol:water (1:1, v/v), respectively. Phosphate buffer of pH 2 was prepared by dissolving 3.4 g of potassium dihydrogen phosphate powder in 2.7 mL of ortho-phosphoric acid and diluting with water to a final volume of 1000 mL.

Determination of Cyromazine and Melamine in Drainage Solution Samples. Because of the high concentrations of both cyromazine and melamine residues in the drainage solution throughout the experiment, no extraction was required. Initially, an aliquot of each drainage solution sample (100 mL) was prefiltered through a 0.45 μm PTFE filter. Subsequently, 20 μL of filtered sample were injected into the HPLC - DAD system for analysis.

Extraction of Cyromazine and Melamine from Plant Tissue Samples. The plant tissue samples were initially blended using a high-speed electric mixer (IKA-A11, Germany). Afterward, 50 mL of methanol at pH 2 (adjusted by injecting 0.5 M HCl) were added, and the samples were extracted using a Fisher–Kahn shaker for 30 min. The extracts were centrifuged at 4000 rpm for 15 min and filtered through a 0.45 μm PTFE filter to remove solid matter. The above extraction procedure was repeated. The combined organic extracts were transferred into a 250-mL Erlenmeyer flask and evaporated to 5 mL using a vacuum rotary evaporator at 35 °C. The final solution was passed through a cleaned-up cartridge filled with 1 g of carbon black, which had been previously preconditioned with 5 mL of acidified methanol (pH 2) while taking care to avoid drying up. The compounds were eluted from each cartridge using 50 mL of methanol (pH 2). The final extract was evaporated in a rotary evaporator at 35 °C to approximately 5 mL. Subsequently, the samples were evaporated under a nitrogen stream to 1 mL, and finally, 20 μL of this solution were injected into the HPLC–DAD system for analysis.

Recovery studies were performed by fortifying subsamples of each substrate with cyromazine and melamine of appropriate concentrations. For the recovery studies, the samples were processed 1 h after fortification. The average recovery of cyromazine and melamine ranged from 75% to 89% and from 70% to 81%, respectively, for the tested substrates. The limit of detection (LOD) for each compound was calculated as the lowest fortification level at which the compound was detected with a signal-to-noise (S/N) ratio above three and was found to be 3 ppb (ng g^{-1}) for both compounds according to the above method.

Chromatographic Analysis. The LC system was comprised of a Shimadzu online DGU-14A degassing system coupled to an FCV-10AL controller unit, an LC-10AD high-pressure solvent-delivery pump, with a 20 mL sample loop injector, and a Shimadzu SPD-M10A UV/diode array detector. The column material was a Discovery C18 (Supelco), with 5 mm particles (25 cm × 4.6 mm i.d.) with a guard column of the same material (8 mm × 3 mm). The mobile phase used was a methanol:water (containing phosphate buffer, pH 2) gradient, where the percentage of methanol was changed linearly as follows: 0 min, 25%; 2 min, 25%; 12 min, 90%; 22 min, 5%; 26 min, 25%). The injection volume of standards and samples was 20 μL, the flow rate was 1 mL min^{-1}, and the UV detector was operated at 215 nm for cyromazine and 270 nm for melamine. All chromatographic runs were performed in duplicate, and the reproducibility of retention times was ±0.5% or lower. The concentrations of cyromazine and melamine were calculated relative to an internal standard (atrazine, 5 mg L^{-1}). Atrazine was added to the final extract before analysis to compensate for variations during the injections. The above-reported analytical procedure was based on preliminary trials. Representative HPLC-UV DAD chromatograms of standard cyromazine and melamine solutions are given in **Figure 2**.

RESULTS

The medium and the high application doses of cyromazine (40 and 60 mg per plant, applied via the nutrient solution at concentrations of 80 and 120 mg L^{-1}, respectively) resulted in moderate and severe phytotoxicity symptoms, respectively, in the leaves of beans. However, the plants treated with the low dose were free of phytotoxicity symptoms. The symptoms of phytotoxicity appeared nearly 10–12 days after the application of cyromazine and mainly affected the older leaves in the form of chlorotic flecks, which subsequently turned into necrotic areas. Nevertheless, during weeks 3–8 after application, visible phytotoxicity symptoms also appeared in the younger leaves. After their appearance, the phytotoxicity symptoms on the leaves remained visible throughout the cropping period.

The concentrations of cyromazine in the fertigation effluents (drainage solution), which were running out of the substrate after each irrigation event, increased up to day 8 after application, regardless of the dose (**Figure 3**). After the eighth day after application, the cyromazine concentration in the drainage solution exhibited a rapid fall, but the rate of decline diminished with time. As a result, considerable cyromazine residues were detectable in the drainage solution even 99 days after application via the nutrient solution. Specifically, the cyromazine concentrations measured in the drainage solution 99 days after application at high, medium, and low doses were 7.7, 3, and 2 mg L^{-1}, respectively. The melamine concentrations in the drainage solution increased up to day 16 following the application of cyromazine, but after that they tended to gradually decrease. The melamine concentrations measured in the drainage solution 99 days after the application of cyromazine were 1.1, 0.6, and 0.3 mg L^{-1} in the treatments, corresponding to the high, medium, and low doses, respectively. As can be computed from the data shown in **Figure 3**, the cyromazine to melamine ratio in the drainage solution was approximately 4 one day after the application of the insecticide, but three days later it rose to a ratio of nearly 16. After that date, the cyromazine to melamine ratio exhibited a gradual decrease to a level of approximately 6 on day 99 after application of the insecticide. The cyromazine to melamine ratio was similar in all dose treatments at each sampling date.

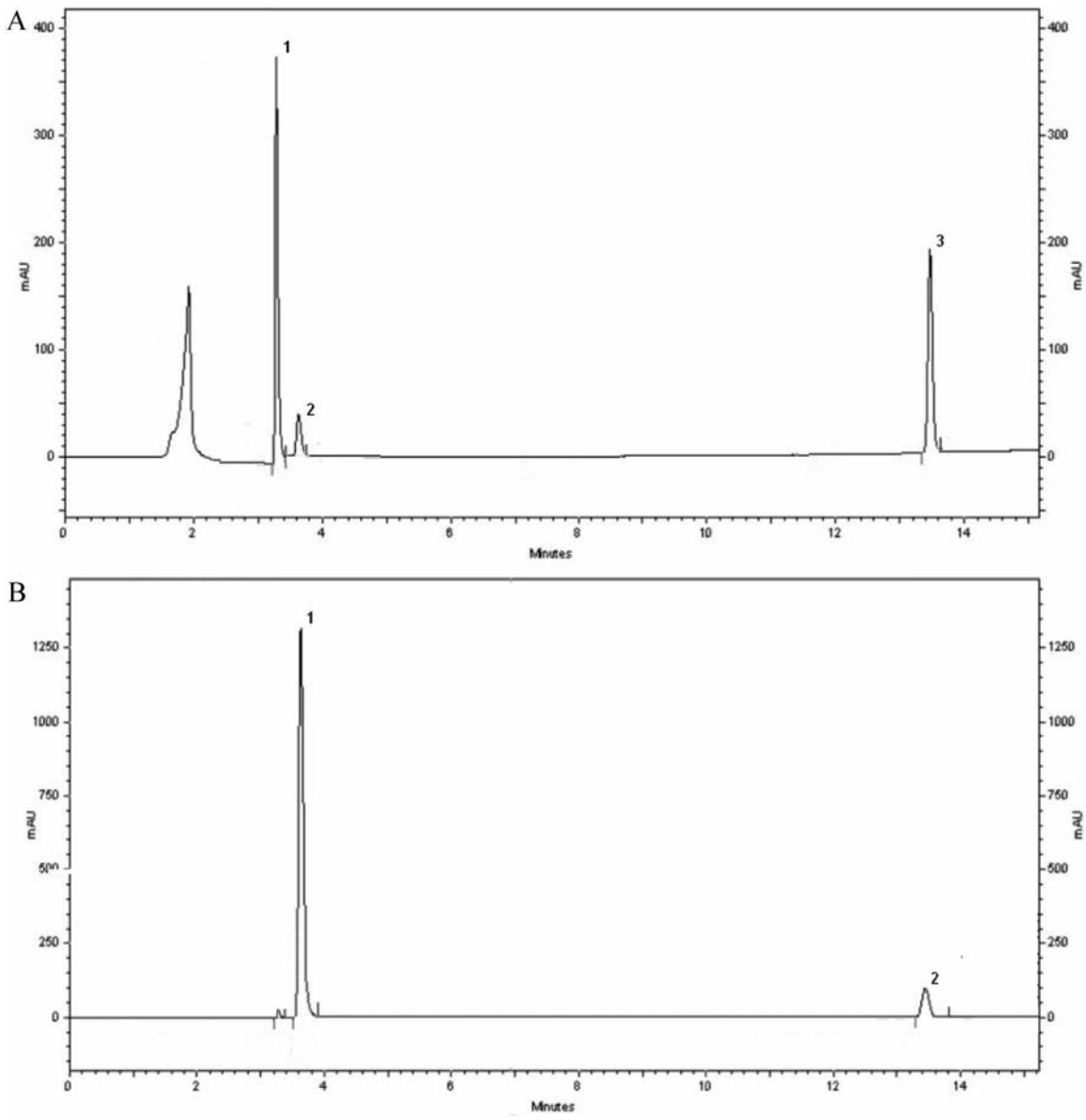

Figure 2. Representative HPLC-UV DAD chromatograms of standard solutions at a concentration of 5 mg L^{-1}: (**A**) at 215 nm: [1] cyromazine, [2] melamine, [3] atrazine (internal standard); (**B**) at 270 nm: [1] cyromazine, [2] atrazine (internal standard).

The amounts of cyromazine adsorbed by the growing medium (pumice) were assumed to be negligible (lower than 0.02 mg kg^{-1}) on the basis of the results of a previous study (*7*). The volume of nutrient solution retained by the substrate in each circuit at the time of drainage solution sampling was estimated on the basis of the water retention curve of 0–8 mm pumice grade, as suggested by Gizas and Savvas (*24*). Furthermore, because the volume of the drainage solution was also measured at each sampling time, it was possible to estimate the total amount of insecticide residues included in each closed system (circuit) at the time of sampling. This was the product of the insecticide concentration in the drainage solution on the particular sampling date and the volume of the nutrient solution included in the system (sum of drainage solution and solution retained in the substrate pores). However, the values measured during the first three sampling dates were not used for these quantitative calculations because, at that time, a homogeneous distribution of cyromazine in the closed system had obviously not been achieved. Indeed, during the initial three sampling dates, the concentration of cyromazine in the drainage solution was still relatively low and tended to increase. It is obvious that considerable amounts of the insecticide remained in the nutrient solution of the upper substrate layers during the initial three sampling dates. These amounts were gradually leached to the drainage, as new nutrient solution was supplied to the upper surface of the substrate via irrigation.

The amounts of cyromazine residues remaining in the closed hydroponic system at each sampling date were subsequently used to estimate the dissipation rate of cyromazine in each circuit by means of nonlinear regression analysis using the following equation as a model,

$$C = C_0 \, e^{-kt} \quad (1)$$

where t is the time in days from application via the nutrient solution, C is the total amount (g) of cyromazine in the system on day t, C_0 is the amount (g) of cyromazine initially supplied to the system, and k is a coefficient characterizing the dissipation rate. The best-fit curves for our data according to eq 1 and the corresponding values of C_0 and k are presented in **Figure 4**. The equations shown in **Figure 4** were used to estimate the half-life of cyromazine in the closed hydroponic system by

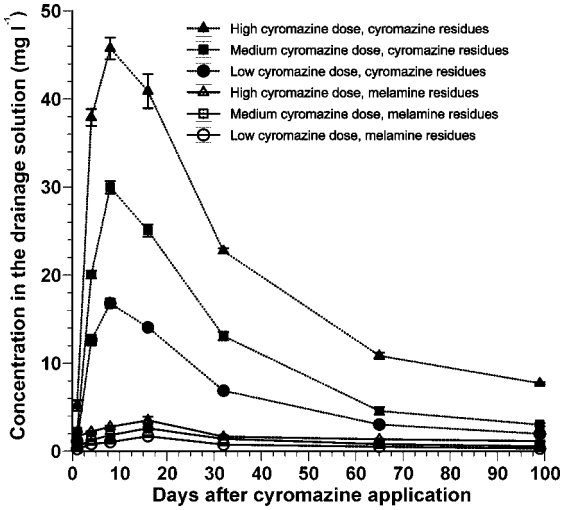

Figure 3. Cyromazine and melamine residue concentrations in the drainage solution over 99 days following a single application via the nutrient solution to a bean crop grown in a closed-cycle hydroponic system as influenced by application dose. Vertical bars indicate ± standard errors of means ($n = 4$).

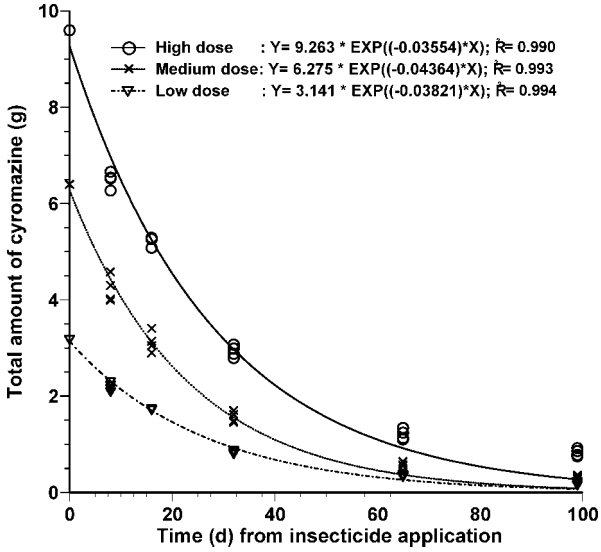

Figure 4. Dissipation rate of cyromazine in closed-cycle hydroponic systems following a single application via the nutrient solution to a bean crop as influenced by application dose. Symbols depict measured values and curves indicate values predicted by nonlinear regression analysis.

replacing C with $C_0/2$. The values obtained were 18.1, 15.9, and 19.5 days for the low, medium and high doses, respectively.

The concentration of cyromazine in the roots of beans increased gradually after addition to the recycled nutrient solution and reached a maximal level, depending on the dose treatment, 16 days after application (**Figure 5**). Specifically, the maximum cyromazine concentrations in the roots were 0.75, 1.67, and 2.4 mg kg^{-1} fresh weight (f. wt) for the low, medium, and high doses, respectively. Thereafter, the concentrations of cyromazine in the roots diminished. However, cyromazine residues were detectable in the roots even on day 99 after application. Specifically, the root cyromazine concentrations on day 99 after application were 0.18, 0.3, and 0.55 mg kg^{-1} f. wt for the low, medium, and high doses, respectively. The residues of melamine in the roots of bean followed a similar pattern to those of cyromazine but were much lower than the latter.

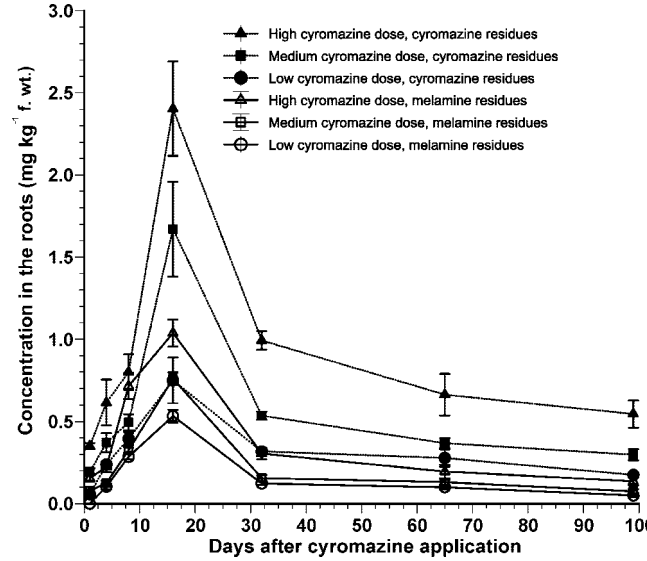

Figure 5. Cyromazine residue concentrations in roots of beans over 99 days following a single application via the nutrient solution in a closed-cycle hydroponic system, as influenced by application dose. Vertical bars indicate ± standard errors of means ($n = 4$).

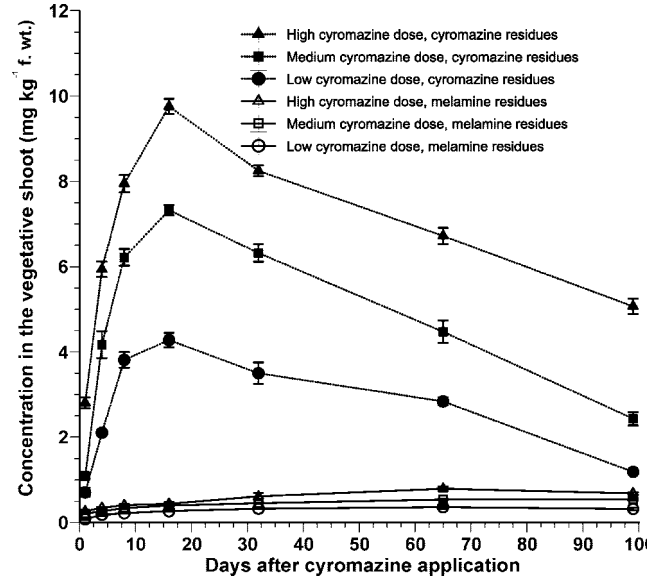

Figure 6. Cyromazine residue concentrations in the epigeous vegetative parts of beans over 99 days following a single application via the nutrient solution in a closed-cycle hydroponic system, as influenced by application dose. Vertical bars indicate ± standard errors of means ($n = 4$).

However, the cyromazine to melamine ratio was much lower than that found in the drainage solution (generally in the range of 2–4).

The concentrations of cyromazine in the shoots of beans were generally much higher than those measured in the roots for the same treatment and date. The highest cyromazine concentrations in the shoots (stems and leaves) of beans were measured 16 days after application and amounted to 4.3, 7.3, and 9.8 mg kg^{-1} f. wt for the low, medium, and high doses, respectively (**Figure 6**). Thereafter, the levels of cyromazine residues in the epigeous vegetative organs of bean gradually decreased, but the rate of decline was low in comparison with that observed in the roots. As a result, the residue concentrations of cyromazine in the shoots were still relatively high on day 99 after application, when the experiment was terminated. In contrast to cyromazine, the residues of melamine in the shoots of beans

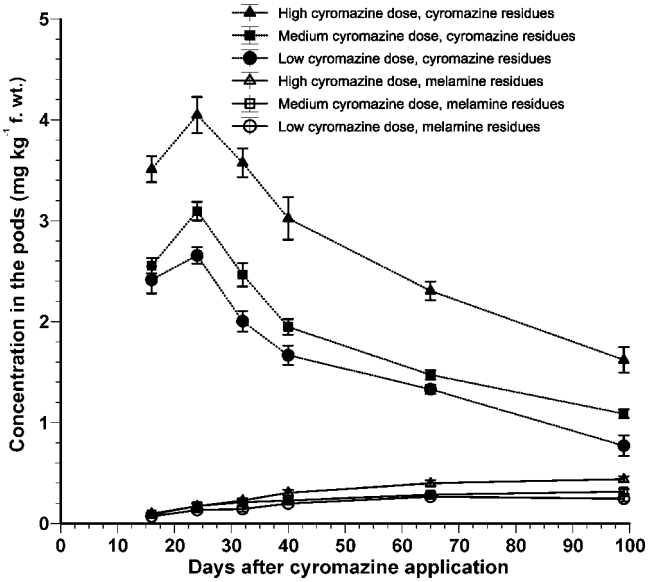

Figure 7. Cyromazine residue concentrations in commercially ripe pods of beans over 99 days following a single application via the nutrient solution in a closed-cycle hydroponic system, as influenced by application dose. Vertical bars indicate ± standard errors of means ($n = 4$).

were constantly increasing with time up to day 99 after application, whereas the application doses of cyromazine were clearly reflected in the measured shoot melamine concentrations.

The analysis of the bean fruits (pods) for cyromazine and melamine residues was commenced 16 days after the application of the insecticide via the nutrient solution when the first commercially acceptable produce was harvested. The cyromazine concentrations in pods ranged from 2.4 to 3.5 mg kg^{-1} f. wt 16 days after application and increased to 2.7–4 mg kg^{-1} f. wt 8 days later (**Figure 7**). After that date, the residues of cyromazine in the bean pods gradually decreased, but 99 days after application, the insecticide was still detectable at levels of 0.8–1.6 mg kg^{-1} f. wt. On all sampling dates, the differences in the cyromazine concentrations between the analyzed samples were commensurate with the differences in the dose treatments, although the differences between the high and medium doses were clearly larger than those between the medium and low doses. Unlike cyromazine, the melamine concentrations in the bean pods were low on the first sampling date (16 days after cyromazine application) and increased constantly up to levels ranging from 0.25 to 0.44 on day 99 after application. Overall, the residues of melamine in the bean pods were much lower than those of cyromazine on all sampling dates. Significant differences in melamine residue concentrations were observed only between the high dose and the two lower doses, whereas the values measured in samples from the medium dose did not differ significantly from those of the low dose treatment.

DISCUSSION

Our results revealed that the application of 20 mg of active ingredient per plant is roughly the maximum safe dosage with respect to phytotoxicity when cyromazine is applied via the nutrient solution to beans grown in closed-cycle hydroponic systems. Furthermore, our results indicate that mean cyromazine concentrations higher than 4–5 mg kg^{-1} f. wt in the shoot are associated with toxicity symptoms in the leaves of bean. In a previous experiment with gerbera grown in a closed-cycle hydroponic system (*7*), even higher dosages of cyromazine were applied via the nutrient solution (70 and 140 mg of active ingredient per plant, supplied at concentrations of 375 and 750 mg l^{-1}, respectively) in comparison with those tested in beans. The maximum levels of cyromazine residues that were determined in the foliage of gerbera after application of the insecticide at the above doses were 105 and 93 mg kg^{-1} f. wt, respectively. Despite these very high cyromazine doses and tissue concentrations, no phytotoxicity symptoms were observed in the gerbera plants. Hence, it seems that bean is susceptible to cyromazine toxicity, at least when this insecticide is applied via the root system.

In agreement with previous results (*7*), cyromazine was characterized by long persistence in both the nutrient solution and the plant tissues. This was partly because of the application of the insecticide in a closed-loop cultivation system with zero leaching of fertigation effluents. Furthermore, the cultivation of the plants in a chemically inert substrate (pumice), which is characterized by low specific surface (*25*), and hence negligible adsorption capacity, also contributed to the long persistence of cyromazine in the system. Hence, the dissipation of cyromazine in the recycled nutrient solution was the result of plant uptake, volatilization, and/or degradation originating from light, microorganizms, and abiotic factors other than light. The concentrations of cyromazine in the plant tissues of beans clearly show that plant uptake was indeed a factor that contributed to the dissipation of cyromazine from the recycled nutrient solution. Volatilization may also have partly contributed to the gradual dissipation of cyromazine in the nutrient solution too, as postulated by Lim et al. (*15*). However, the volatility of cyromazine is low (*13*), as also indicated by the low value of the Henry's Law Constant (*19*). Consequently, the contribution of volatilization to the dissipation of cyromazine in the nutrient solution presumably was small. Photodegradation of cyromazine is a well-known process resulting in dealkylation and the formation of its metabolite melamine (*15*). However, our results revealed that only a small fraction of cyromazine, roughly ranging from 0.05 to 0.15 of the total amount contained in the nutrient solution, was converted into melamine. Besides melamine, at least two more metabolites are implicated as being formed in the process of photochemical degradation of cyromazine (*13*). In addition to photodegradation and volatilization, microbial transformation may have also contributed to the disposition of cyromazine in the recycled nutrient solution. Nutrient solutions constitute an optimal medium for microbial activity (*26, 27*) and thus for quick degradation of diluted pesticides, as also suggested by Racke and Coats (*28*). The biodegradation of cyromazine is mainly ascribed to bacteria *Pseudomonas* sp., which utilize cyromazine as a nitrogen source (*29*). Nevertheless, the dissipation rate of cyromazine in the nutrient solution was much slower in the present study, in comparison with that observed in a previous experiment with gerbera. This is obvious from the half-life time, which is twice as high in the present study as that estimated by Karras et al. (*7*). The slower dissipation rate of cyromazine in the nutrient solution recirculating in the bean crop was presumably the result of a much slower plant uptake rate, as indicated by the appreciably lower tissue residue levels found in bean than in gerbera. However, the slower dissipation rate of cyromazine in the nutrient solution supplied to beans, in comparison with that reported for gerbera (*7*), may also be associated with a slower photodegradation rate. Indeed, in the present study, the hydroponic channels were covered with a black–white polyethylene sheet, which almost eliminated exposure of the slowly flowing drainage solution to light. In contrast, the channels used to collect the drainage

solution in the experiment with gerbera were uncovered; hence, the thin layer of the flowing drainage solution was directly exposed to sunlight for a long time (*7*). The above statement is supported by the results of Lim et al. (*15*), who found an appreciably lower disappearance rate of cyromazine placed on glass dishes when the latter were covered by aluminum foil, in comparison with direct exposure to sunlight.

The higher cyromazine concentrations in the epigeous vegetative shoot of beans in comparison with those measured in the root and the pods imply that cyromazine is readily transported to the foliage via the transpiration stream. However, cyromazine may also be retranslocated via the symplasm within the plant, as has been demonstrated in tomatoes using [^{14}C]cyromazine (*13*). The high mobility and translocation of cyromazine within the plant tissues is attributed to its relatively low lipophilicity, as indicated by its relatively low octanol–water coefficient (K_{ow}) (*17*).

The dissipation of cyromazine in plant tissues is anticipated to be mainly a result of plant metabolism and growth dilution because the volatility of cyromazine is low (*13*) and because microbial decomposition cannot occur in living plant tissues. However, the relatively low concentrations of melamine in the analyzed plant tissues, in comparison with those of cyromazine, imply that the rate of metabolic degradation in the plant tissues was low. A slow rate of metabolic degradation of cyromazine seems to be an explanation for its accumulation in the plant tissues at least up to day 16 after application (at least up to day 24 in the pods), although its concentration in the nutrient solution increased only during the initial 8 days after application. A very slow rate of cyromazine degradation in the plant tissues was also observed in gerbera after application via the nutrient solution (*7*), as well as in tomato (*13*) and potato (*30*) after application via foliar spray. In an experiment with potato (*30*), the highest residue levels of cyromazine within the plant were found one day after foliar application, followed by a consistent decrease thereafter. Hence, an increase of the residue concentrations of cyromazine in plant tissues for several days after application seems to characterize application via nutrient solution, particularly in closed hydroponics. Obviously, the influx of cyromazine into the plant tissues because of nutrient solution absorption in closed-hydroponics exceeds its degradation rate within the plants for several days after application.

The slow dissipation rate of cyromazine in the plant tissues may be an advantage with respect to plant protection efficacy. However, the slow dissipation rate results in the lengthy persistence of cyromazine in edible plant parts, which is a serious disadvantage from the point of view of consumer health. Nevertheless, the cyromazine residue concentrations found in the edible pods were in all treatments below the maximum residue levels (MRL) of 5 mg kg^{-1} that has been assigned by the European Commission (*31*) for beans with pods. Furthermore, the maximum residue level measured in pods from the lowest dose treatment was even lower than the MRL given for dry bean (3 mg kg^{-1}) by the U.S. Environmental Protection Agency (*32*). Hence, our results indicate that the application of cyromazine via the nutrient solution to beans at the doses tested in the present experiment do not pose a consumer health threat. Nevertheless, given that the plants of the lowest dose treatment were free of insect attacks throughout the cropping period, whereas the two higher doses caused phytotoxicity, a cyromazine dose of 20 mg per plant or even lower is recommended for applications via the nutrient solution to beans grown in closed-cycle hydroponic systems.

Based on our current knowledge, melamine is not considered to be a carcinogen in humans, although its toxicity is low and, therefore, it is no longer included as part of the tolerance expression for cyromazine residues (*11*, *12*). Nevertheless, the residues of melamine in the tested plant tissues were low, especially in the edible pods. Hence, melamine is not considered a residue of concern when cyromazine is applied via the nutrient solution in bean crops grown in closed-cycle hydroponic systems.

In the present study, only the lowest of the three tested application doses proved to be safe for the plants. On the other hand, the efficacy of the tested doses with respect to crop protection against leaf miners and other insects was not studied, but the plants remained free of any insect infection in all treatments. Hence, further studies are needed to test whether substantially lower application doses than those tested in the present study are capable of further reducing the residues of cyromazine in the bean pods while effectively protecting the crops against insect infections.

ACKNOWLEDGMENT

We thank LAVA S.A. for donating the substrate (pumice) and Prof. Harold-Christopher Passam from the Agricultural University of Athens for reviewing the English of the paper.

LITERATURE CITED

(1) Rouphael, R.; Colla, G.; Battistelli, A.; Moscatello, S.; Proietti, S.; Rea, E. Yield, water requirement, nutrient uptake and fruit quality of zucchini squash grown in soil and closed soilless culture. *J. Hort. Sci. Biotechn.* **2004**, *79*, 423–430.

(2) Stanghellini, C.; Kempkes, F.; Pardossi, A.; Incrocci, L. Closed water loop in greenhouses: Effect of water quality and value of produce. *Acta Hort.* **2005**, *691*, 233–242.

(3) Incrocci, L.; Malorgio, F.; Della Bartolla, A.; Pardossi, A. The influence of drip irrigation or subirrigation on tomato grown in closed-loop substrate culture with saline water. *Sci. Hort.* **2006**, *107*, 365–372.

(4) Savvas, D. Automated replenishment of recycled greenhouse effluents with individual nutrients in hydroponics by means of two alternative models. *Biosyst. Engin.* **2002**, *83*, 225–236.

(5) Zekki, H.; Gauthier, L.; Gosselin, A. Growth, productivity, and mineral composition of hydroponically cultivated greenhouse tomatoes, with or without nutrient solution recycling. *J. Amer. Soc. Hort. Sci.* **1996**, *121*, 1082–1088.

(6) Karras, G.; Savvas, D.; Patakioutas, G.; Pomonis, P.; Albanis, T. Fate of metalaxyl applied in nutrient solution to gerbera (*Gerbera jamesonii*) grown in a 'closed' hydroponic system. *J. Hort. Sci. Biotechn.* **2005**, *80*, 111–115.

(7) Karras, G.; Savvas, D.; Patakioutas, G.; Pomonis, P.; Albanis, T. Fate of cyromazine applied in nutrient solution to a gerbera (*Gerbera jamesonii*) crop grown in a closed hydroponic system. *Crop Prot.* **2007**, *26*, 721–728.

(8) Bel, Y.; Wiesner, P.; Kayser, H. Candidate target mechanisms of the growth inhibitor cyromazine: Studies of phenylalanine hydroxylase, puparial amino acids, and dihydrofolate reductase in dipteran insects. *Arch. Insect Biochem. Physiol.* **2000**, *45*, 69–78.

(9) Friedel, T.; Hales, D. F.; Birch, D. Cyromazine-induced effects on the larvae cuticle of the sheep blowfly, Lucilia cuprina: Ultrastructural evidence for a possible mode of action. *Pestic. Biochem. Physiol.* **1988**, *31*, 99–107.

(10) Sirota, J. M.; Grafius, E. Effect of cyromazine on larva, survival, pupation and adult emergence of the CO potato beetle (Coleoptera: Chrysomelidae). *J. Econ. Entomol.* **1994**, *87*, 577–582.

(11) Sancho, J. V.; Ibáñez, M.; Grimalt, S.; Pozo, Ó. J.; Hernández, F. Residue determination of cyromazine and its metabolite melamine in chart samples by ion-pair liquid chromatography

coupled to electrospray tandem mass spectrometry. *Analyt. Chim. Acta* **2005**, *530*, 237–243.
(12) Yokley, R. A.; Mayer, L. C.; Rezaaiyan, R.; Manuli, M. E.; Cheung, M. W. Analytical method for the determination of cyromazine and melamine residues in soil using LC-UV and GC-MSD. *J. Agric. Food Chem.* **2000**, *48*, 3352–3358.
(13) Root, D. S.; Hongtrakul, T.; Dauterman, W. C. Studies on the absorption, residues and metabolism of cyromazine in tomatoes. *Pestic. Sci.* **1996**, *48*, 25–30.
(14) Bardalaye, P. C.; Wheeler, W. B.; Meister, C. W. Gas chromatographic determination of cyromazine and its degradation product, melamine, in Chinese cabbage. *J. Assoc. Off. Anal. Chem.* **1987**, *70*, 455–457.
(15) Lim, L. O.; Scherer, S. J.; Shuler, K. D.; Toth, J. P. Disposition of cyromazine in plants under environmental conditions. *J. Agric. Food Chem.* **1990**, *38*, 860–864.
(16) Schwartz, L.; Wolf, D.; Markus, A.; Wybraniec, S.; Wiesman, Z. Controlled-release systems for the delivery of cyromazine into water surface. *J. Agric. Food Chem.* **2003**, *51*, 5972–5976.
(17) Tomlin, C. D. S., Ed.; The Pesticide Manual, 12th ed.; British Crop Protection Council: Farnham, Surrey, United Kingdom, 2000.
(18) Kaune, A.; Brüggemann, R.; Sharma, M.; Kettrup, A. Soil adsorption coefficients of *s*-triazines estimated with a new gradient HPLC method. *J. Agric. Food Chem.* **1998**, *46*, 335–343.
(19) The reconsideration of approvals of selected sheep ectoparasiticide products and their associated label; Australian Pesticides & Veterinary Medicines Authority: Canberra, Australia, 2006; Vol. 2 of 2, p 195,Electronic citation available at: http://www.apvma.gov.au/chemrev/downloads/ectoparsiticide_review_vol_2.pdf.
(20) U.S. Environmental Protection Agency. Cyromazine; Pesticide Tolerance. *Fed Regist.* **2003**, *68* (185), 55261–55269 http://www.epa.gov/fedrgstr/EPA-PEST/2003/September/Day-24/p24012.htm.
(21) Organization for Economic Co-operation & Development - SIDS. Melamine. Cas No: 108–78–1. United Nation Environment Programme (UNEP) Publications. Electronic citation available at: http://www.inchem.org/documents/sids/sids/108781.pdf.
(22) Hartley, D.; Kidd, H. Eds. The Agrochemicals Handbook, Royal Society of Chemistry, 2nd Edn. Nottingham, UK, 1988.
(23) Grusak, M. A.; Pomper, K. W. Influence of pod stomatal density and pod transpiration on the calcium concentration of snap bean pods. *J. Amer. Soc. Hort. Sci.* **1999**, *124*, 194–198.
(24) Gizas, G.; Savvas, D. Particle size and hydraulic properties of pumice affect growth and yield of greenhouse crops in soilless culture. *HortScience* **2007**, *42*, 1274–1280.
(25) Raviv, M.; Wallach, R.; Silber, A.; Medina, Sh.; Krasnovsky, A. The effect of hydraulic characteristics of volcanic materials on yield of roses grown in soilless culture. *J. Amer. Soc. Hort. Sci.* **1999**, *124*, 205–209.
(26) Brand, T.; Wohanka, W.; Alsanious, B. W. Enzyme activity assays for nutrient solutions from closed irrigation systems. *Sci. Hort.* **2001**, *92*, 329–338.
(27) Crum, S. J. H.; Runia, W. T.; Leistra, M.; Smelt, J. H. Behaviour of some pesticides in a nutrient-film and in a rock-wool system. *Sci. Hort.* **1985**, *25*, 1–9.
(28) Racke, K. D.; Coats, J. R. Enhanced biodegradation of pesticides in the environment. ACS Symposium, Series 426, Amer. Chem. Soc., Washington DC, 1990; pp 82–87.
(29) Cook, A. M.; Crossenbacher, H.; Hutter, R. Bacterial degradation of N-cyclopropylmelamine. *Biochem. J.* **1984**, *222*, 315–320.
(30) Weintraub, P. G. Effects of cyromazine and abamectin on the pea leafminer *Liriomyza huidobrensis* (Diptera: Agromyzidae) and its parasitoid *Diglyphus isaea* (Hymenoptera: Eulophidae) in potatoes. *Crop Prot.* **2001**, *20*, 207–213.
(31) European Commission. Commission Directive 2005/76/EC of 8 November 2005 amending Council Directives 90/642/EEC and 86/362/EEC as regards the maximum residue levels of kresoxim-methyl, cyromazine, bifenthrin, metalaxyl and azoxystrobin fixed therein. *Off. J. Eur. Union* **2005**, *293*, 2013–2022.
(32) U.S. Environmental Protection Agency. Cyromazine; Pesticide Tolerance. *Fed Regist.* **2002**, *67* (235), 72585–72593 http://www.epa.gov/fedrgstr/EPA-PEST/2002/December/Day-06/p30839.htm.

Received for review June 12, 2007. Revised manuscript received September 24, 2007. Accepted September 24, 2007. This Project is co-funded by the European Social Fund and National Resources (EPEAEK-II) ARCHIMEDES.

JF071726I