Dissipation Study of the Fungicide Tetraconazole in Greenhouse-Grown Cucumbers

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The fate of tetraconazole residues, applied according to the recommended procedures on greenhouse cucumbers, was studied. Cucumbers received three applications of the fungicide at rates of 4.0 and 8.0 g of active ingredient/100 L. Residues were determined by a gas chromatographic method using NPD detector. Recovery of tetraconazole from cucumbers was in the range of 96−103% with a limit of determination 0.01 mg/kg. Tetraconazole residues dissipated relatively rapidly, with a half-life of 7 days.

Keywords: *Tetraconazole; residues; dissipation; cucumbers*

INTRODUCTION

Powdery mildew is the most common disease of the plant family Cucurbitaceae, and cucumber is one of the most sensitive species. The disease can cause considerable damage to growing plants unless proper treatment is carried out at the right time. Control of the disease in greenhouse-grown cucumber includes the use of various, preferably with systematic action, protective fungicides. Application of fungicides for powdery mildew control is regularly employed in integrated control programs upon the first appearance of disease symptoms.

Tetraconazole, (±)-1-[2-(2,4-dichlorophenyl)-3-(1,1,2,2-tetrafluoroethoxy)propyl]-1*H*-1,2,4-triazole, is a broad spectrum systematic triazole fungicide. It has recently been registered in various European countries, while its registration in Greece for use on fruit, vine, and vegetable crops is pending. This fungicide is a steroid demethylation inhibitor, acting mainly on the vegetative stages of fungi by blocking the mycelial growth either inside or on the surface of the host plant. Tetraconazole is effective in controlling a broad spectrum of diseases such as powdery mildew and scab on fruits, powdery mildew on vines and cucumbers, powdery mildew and rust on vegetables, and powdery mildew, brown rust, *Septoria*, and *Rhynchosporium* on cereals. Tetraconazole is extensively metabolized in plants; its identified metabolites are tetraconazole acid, tetraconazole alcohol, triazolylalanine, and triazolylacetic acid (British Crop Protection Council, 1995). There is no available literature on the analysis of tetraconazole residues in different matrices, except that provided by the manufacturing company Isagro S.r.l. (Isagro, 1993).

According to European Community legislation (EEC, 1991), data concerning the efficacy and residues of a new pesticidal product are (a) to be derived under the environmental conditions of the interested country and (b) required to set the maximum residue limit (MRL) for tetraconazole on this particular crop. In this study the persistence of tetraconazole residues in cucumbers was investigated in a greenhouse experiment conducted during the spring of 1995 in Macedonia (northern Greece). The aim of the work was twofold: (a) to adapt an existing multiresidue method for the analysis of tetraconazole parent compound residues and (b) to obtain data on the dissipation behavior of tetraconazole in cucumbers.

MATERIALS AND METHODS

Field Experiment. The trial was carried out in a commercial 1000 m^2 greenhouse, located at Angelohori, 30 km east of Thessaloniki, Macedonia (northern Greece). The experimental area comprised 12 plots, where a random block scheme was established with 4 replications. Each plot consisted of 3 rows of 12 plants, the distance between rows being 0.8 m and between plants 0.4 m. The plants received routine horticultural practices in accordance with an integrated pest management program. An aqueous emulsion of a 10% (w/v) tetraconazole formulation (Domark 10EC, Isagro S.p.A., Italy) was applied at rates of 4.0 g of active ingredient (ai)/100 L of water (lowest recommended application dose, LRD) and 8.0 g of ai/100 L of water (highest recommended application dose, HRD). The fungicide was applied to runoff (~0.23 L of emulsion/plant) with a hand-driven knapsack sprayer. A cotton uniform, glasses, gloves, and boots were used by the applicator for his personal protection. First treatment was carried out on April 6, when plants were ≈2 months old and cucumbers 4−6 cm in length. Application was repeated at 10-day intervals, on April 16 and 26, 1995, when fruits were in the growing stage. During the experiment no extreme weather conditions were recorded outside the greenhouse, with average maximum daily and average minimum night temperatures ranging from 20 to 25 °C and from 14−18 °C, respectively.

Sampling and Storage. Sampling was performed by randomly collecting 12 cucumbers of approximately the same size from each treated and control plot, according to FAO/WHO (1986) recommendations. Sample collection was started 1 h after the last application, when the plants were dry (0 day),

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Table 1. Mean Recoveries (Percent)[a] and Relative Standard Deviations (RSD) of Tetraconazole at Various Fortification Levels

tetraconazole concn (mg/kg)	recovery (%)	RSD (%)
0.01	96.0	9.0
0.03	102.7	2.3
0.05	99.8	2.2
0.20	103.0	7.3
0.60	98.0	1.1

[a] Four samples for each fortification level.

and repeated 1, 3, 7, 10, and 15 days afterward to study the dissipation of the fungicide. In addition, samples were taken before the second as well as the third application to evaluate any accumulation effect of tetraconazole. Field samples were put in bags and transported to the laboratory in an ice chest within 2 h from collection. Each field sample was subdivided, and ~1 kg was chopped and blended using a food cutter. At least 250 g of this homogenized sample (laboratory sample) was stored in glass jars at −20 °C until analysis (analytical sample). Two 30-g subsamples were analyzed within 1 month after sample collection. A control sample fortified with 0.05 ppm of tetraconazole was also stored at the same conditions to verify storage influences on the quantity of tetraconazole residues in cucumbers.

Chemicals. Dichloromethane and acetone were of p.a. grade (Riedel, Germany) and glass distilled before use; ethyl acetate was of pesticide grade (Riedel); anhydrous sodium sulfate, granular for residue analysis, was purchased from Merck; sodium chloride, activated carbon, and silica gel were purchased from Riedel. Tetraconazole analytical standard and formulation (Domark 10EC) were provided by Isagro S.p.A., Milan, Italy. Stock standard solution was prepared at a concentration of 1 mg/mL; working standard solutions were obtained by proper dilution of the stock solution with ethyl acetate.

Extraction and Cleanup Procedure. Sample Processing. All samples were analyzed with a method suggested by the manufacturing company modified properly and in accordance with a multiresidue method suitable for nitrogen-containing compounds (Thier and Zeumer, 1987a). A 30-g portion of the frozen chopped tissue was transferred into a blender glass jar and homogenized with 100 mL of acetone for 3 min. The macerate was filtered under vacuum through a Büchner funnel using a GF/C filter disk. The container and the filter cake were washed twice with 100 mL of acetone, and the combined extracts were collected in a 500-mL round-bottom flask. The acetone was removed under reduced pressure on a rotary evaporator with a water bath temperature not exceeding 40 °C. The aqueous residue was transferred into a separatory funnel with 80 mL of water and 100 mL of dichloromethane. After 5 min of shaking, 25 mL of saturated aqueous solution of sodium chloride was added and the mixture allowed to separate for 15 min. The aqueous phase was drained off, and the organic phase was filtered through 20 g of anhydrous sodium sulfate and evaporated to just dryness under reduced pressure. The residue was dissolved in 10 mL of dichloromethane and transferred to a 20 mm i.d. glass chromatographic column to separate interfering substances. The column was prepared in dichloromethane/acetone (75:25, v/v) with 5 g of silica gel and 15 g of activated carbon/silica gel mixture (1:15, w/w). The column was subsequently eluted with 140 mL of dichloromethane/acetone (75:25, v/v) eluting mixture. The eluate was rotary evaporated to ~2 mL and then to dryness under a nitrogen stream. The residue was dissolved in 2 mL of ethyl acetate, thus being ready for GC analysis.

Gas Chromatographic Analysis. A 5890 Hewlett-Packard Plus II gas chromatograph, equipped with a nitrogen−phosphorus detector (NPD) and connected to a Hewlett-Packard model 3396 integrator, programmed for external standardization using peak area, was used. The column was HP-1 methyl silicone gum, 15 m × 0.53 mm × 0.5 μm film thickness, and the injection port was splitless with a temperature of 230 °C. The column temperature was 170 °C and the detector temperature 280 °C. The carrier gas was helium at a flow rate of 18 mL/min, the detector gases were air and hydrogen at 100 and 3.5 mL/min, respectively, and the makeup gas helium at 20 mL/min flow rate. At these operating conditions the retention time of tetraconazole was 2.7 min.

Recovery Efficiency Studies. Known quantities of tetraconazole dissolved in acetone were added to untreated samples of cucumbers at five fortification levels (ranging from 0.01 to 0.6 mg/kg; Table 1). Recovery of the overall method was frequently checked by simultaneous processing with each batch of samples analyzed control and fortified with 0.05 ppm of tetraconazole subsamples. Tetraconazole was extracted and analyzed as above.

RESULTS AND DISCUSSION

Determination and Recoveries. The analytical method employed is simple and allows the reliable determination of tetraconazole with very good precision and accuracy. The initial method suggests use of alumina grade II−III according to the Brockmann method for the cleanup step and hexane as the eluting solvent. The behavior of this adsorbent must be checked for every new batch of alumina. In a previous work for the determination of tetraconazole residues in peaches and grapes, we found great differences between different batches of alumina with poor recoveries (unpublished data). Thus, presently and in order to adapt the method to a multiresidue one, a mixture of activated carbon/silica gel (Thier and Zeumer, 1987a) was used for the cleanup of the sample, and tetraconazole was eluted with a mixture of dichloromethane/acetone. This purification technique, although requiring longer elution times, utilizes an absorbent (silica gel) with a more constant behavior; nevertheless, in every new study, GLP guidelines require a procedural recovery test to be run concurrently with sample analysis, regardless of the type of adsorbent used. The response of the detector for tetraconazole was linear in the range 0.2−2 ng (0.1−1 μg/mL), the regression line was $y = 840 + 265 \times 10^3 x$, and the correlation coefficient was $r = 0.998$. Quantitation of tetraconazole in samples was performed by comparing the detector response (area) for the sample to that for the calibration standard within the linear range. Standard solutions were injected between sample injections.

The method was validated by performing recovery experiments. Blank samples, free of tetraconazole residues, were fortified with the fungicide at five fortification levels, covering the whole range of tetraconazole concentrations in samples. The recovery data are summarized in Table 1. Average recovery percentages ranged from 96 to 103, with relative standard deviations (RSD) from 1.1 to 9. Samples from control plots were found free of tetraconazole residues. Estimations of the method's sensitivity, of detection limit (LDC), and of determination limit (LDM) were performed in accordance with Thier and Zeumer (1987b).

From recovery results, the parameters of the regression line $\hat{y} = a_0 + \hat{S}q$ were computed to be 0.0018 for a_0 and 0.98103 for $\hat{S}$. Therefore, the estimated value of sensitivity was $\hat{S} = 0.98$, and that of detection limit was 0.0024 mg/kg. The latter was calculated on the basis of the standard deviations of the blanks and the lowest fortification level $q = 0.01$ mg/kg with $f = 6$ degrees of freedom at 95% confidence level. At the lowest fortifica-

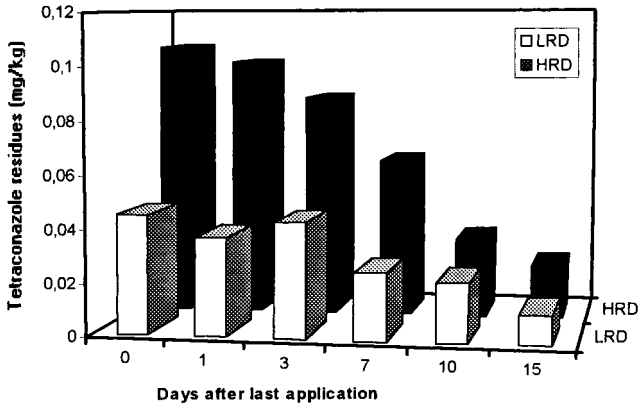

Figure 1. Tetraconazole residues (milligrams per kilogram) in cucumbers at various time intervals following last application: LRD, 4 g of ai/100 L; HRD, 8 g of ai/100 L.

tion level of 0.01 mg/kg, the coefficient of variation V was calculated as 0.09, in other words, <0.2. Also at this concentration the recovery was 96%, while the concentration of the fortified samples was 0.01 mg/kg, greater than the limit of detection (LDC) = 0.0024. Consequently, the method's limit of determination under these conditions was 0.01 mg/kg. All values were within the accepted range for residue determination, satisfying the three requirements LDM ≥ LDC, S ≥ 0.7, and $V \leq 0.2$ (20%). Storage of samples at −20 °C did not affect the amount of tetraconazole residues.

Disappearance of Residues. All cucumber samples from control plants collected at various sampling dates were found free of detectable tetraconazole residues. Residues of tetraconazole detected in cucumbers following the last of three applications for either of the two doses are shown in Figure 1. As shown in this figure initial deposits of tetraconazole in cucumbers were 0.045 and 0.106 mg/kg for the LRD and HRD, respectively. Tetraconazole residues in cucumbers declined with time, and 15 days after the last application 24% and 19% of the initial deposits were found in samples for the LRD and HRD treatments, respectively.

Tetraconazole half-life in cucumbers was evaluated with the Student t value at 95% confidence level and for four degrees of freedom (Snedecor and Cochran, 1980). The equations of regression lines were $\log C = -1.3277 - 0.0388t$ and $\log C = -0.9407 - 0.0509t$ with correlation coefficients $r = 0.969$ and 0.984 for the results of the two experiments, respectively. From these dissipation lines, the half-life of tetraconazole in cucumbers was found to be 7 days for both application doses, indicating that it is independent of the initially deposited quantities. These data show a linear decline of residues with time in either treatment, which is described by a first-order dissipation model. At the time of the third application cucumber plants were almost mature and therefore tetraconazole dissipation could be attributed to degradation mainly by chemical mechanisms and less by growth dilution effects.

Since tetraconazole is a new fungicide, its MRL has not as yet been established in Greece. Tetraconazole is already registered for use on cucumber in Italy and Spain, and the MRL is 0.2 mg/kg with a preharvest interval of 7 days. As seen from Figure 1, the remaining tetraconazole residues in cucumbers on this date were 0.025 and 0.061 mg/kg for the two application doses, respectively. Fifteen days following the last LRD application residues reached the LDM (0.01 mg/kg), while for the HRD spraying remained at 0.02 mg/kg.

Table 2. Tetraconazole Residues (Milligrams per Kilogram) at All Sampling Times

sampling time	residue (mg/kg) for application dose of: 4 g of ai/100 L (LRD)	8 g of ai/100 L (HRD)
10 days after 1st application	ND	ND
10 days after 2nd application	ND	ND
0 days after 3rd application	0.045	0.106
1 day after 3rd application	0.037	0.100
3 days after 3rd application	0.043	0.086
7 days after 3rd application	0.025	0.061
10 days after 3rd application	0.022	0.030
15 days after 3rd application	0.011	0.020

[a] ND, not detected (LDC = 0.0024 mg/kg).

Table 2 presents also the effect of three repeated applications at 10-day intervals on tetraconazole residues in cucumbers. As seen from the data, the first two applications had no effect on tetraconazole residues. This is attributed to the fact that, by that time, cucumber fruits were at the growing stage and there was, therefore, a high dilution effect. Moreover, as biomass increases at the beginning of vegetative period, residues in general have been observed to dissipate intensively (Spynu, 1989). Cucumber plants in particular continue exhibiting a relatively high vegetative growth while producing fruits. However, following the third application, when fruits reached the harvesting point and were mature, an accumulation effect was evident for both application doses and may be more significant if more applications are performed by farmers. Indeed, residue levels and disappearance rates are basically affected by frequency of treatment and plant vegetative stages (Spynu, 1989).

In conclusion, the analytical method described for tetraconazole determination permits the quantitave determination of parent compound residues down to the 0.01 mg/kg level. Tetraconazole dissipation from cucumbers follows a first-order model for the duration of the experiment (15 days). Fifteen days after the last of three consecutive applications, tetraconazole residues are reduced to 0.01 mg/kg, corresponding to the limit of determination. The results, related to the cumulative effect of tetraconazole, also indicate that tetraconazole should be used with care, avoiding repeated sprayings, especially on mature cucumbers. In any case, adequate time should elapse before harvesting.

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