Dissipation of the Fungicide Tetraconazole from Field-Sprayed Sugar Beets

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The reduction of tetraconazole residue levels in field sugar beets (roots and foliage) was investigated, and the effectiveness of this new fungicide against the leaf spot disease of sugar beet was evaluated. The fungicide was used according to the recommended application procedures. Sugar beets received five applications of tetraconazole at rates of 0.05 and 0.10 kg of active ingredient/ha. A multiresidue method was adapted, and tetraconazole residues were determined with gas chromatography using a wide-bore column and an electron capture detector. Recovery of tetraconazole from sugar beet roots and leaves was found in the range of 86−111 and 78−103%, and the detection limits were estimated as 0.001 and 0.002 mg/kg, respectively. A relatively rapid dissipation rate of tetraconazole residues was found with a half-life of 5 days for either sugar beet roots or foliage.

Keywords: *Dissipation; tetraconazole; residues; analysis; sugar beet*

INTRODUCTION

Beet leaf spot caused by *Cercospora beticola* is one of the most serious foliage diseases of sugar beets in Greece. The effective control of this disease is vital for the survival of the crop, because yield loss may reach 40%, and at least four to six applications of fungicides per growing season are usually needed. The control of the disease is based mostly on the use of various mixtures of ergosterol biosynthesis inhibitors (EBIs) with nonsystemic fungicides, mainly maneb and chlorothalonil. There is a great possibility that resistance will be developed to EBIs, although resistant strains of *C. beticola* have not yet been reported in Greece (Skarakis et al., 1996). Steroid demethylation inhibitors (DMIs) instead of EBIs are used late in the growing season for disease control, to prevent disease resistance. Fungicides of the triazole group (DMIs) have shown excellent control of sugar beet spot (Ioannidis, 1994).

Tetraconazole is the common name of (±)-1-[2-(2,4-dichlorophenyl)-3-(1,1,2,2-tetrafluoroethoxy)propyl]-1*H*-1,2,4-triazole. It is a broad spectrum systemic triazole fungicide, which has recently been registered in various European countries, and its registration in Greece for use on vegetable crops, fruits, and vines is expected to be done soon. This fungicide is a steroid DMI, acting mainly on the vegetative stages of the fungi by blocking the mycelial growth both inside and outside the host plant. Tetraconazole is effective against a broad spectrum of diseases such as powdery mildew, brown rust, *Septoria*, and *Rhynchosporium* on cereals, powdery mildew and scab on apples, powdery mildew on vines and cucumbers, powdery mildew and rust on vegetables, and powdery mildew and beet leaf spot on sugar beets. Extensive metabolism of tetraconazole occurs in plants, and its identified metabolites are tetraconazole acid, tetraconazole alcohol, triazolylalanine, and triazolylacetic acid (British Crop Protection Council, 1995). Tetraconazole applied in field trials at either the recommended rates or at half-rate in mixtures with maneb and chlorothalonil showed excellent control of *C. beticola*, similar to that obtained by the other already commercialized triazole compounds (Ioannidis, 1994). The literature concerning the analysis of tetraconazole residues in different matrices is limited. An analytical method has been developed by the manufacturing company Isagro S.r.l. (Isagro, 1993) to determine tetraconazole residues in various agricultural products, using GC-AFID, after purification of acetone extract on alumina. However, recently a modified multiresidue method was reported by Khalfallah et al. (1998), which suggests silica gel-activated carbon for extract purification and gas−liquid chromatography with nitrogen phosphorus detection for determination of tetraconazole residues in cucumbers.

According to European Community legislation (EEC, 1991), data concerning the efficacy and residues of a new pesticidal product are needed to be derived under the environmental conditions of the interested country. Also, these data are required by EC procedure to set up the maximum residue limit (MRL) for the new product on each particular crop. In this study the persistence of tetraconazole residues in sugar beets was investigated in a field experiment conducted in central Macedonia, northern Greece, during the period of summer and autumn of 1996. Also, the efficacy of this new fungicide against sugar beet spot was evaluated during three years (1993−1995). The purpose of this work was therefore (a) to provide data on the disappearance of tetraconazole in sugar beets, roots, and foliage and (b) to adapt an existing rapid multiresidue method for the analysis of tetraconazole residues in sugar beets. Furthermore, the efficacy of tetraconazole to control sugar beet spot was evaluated.

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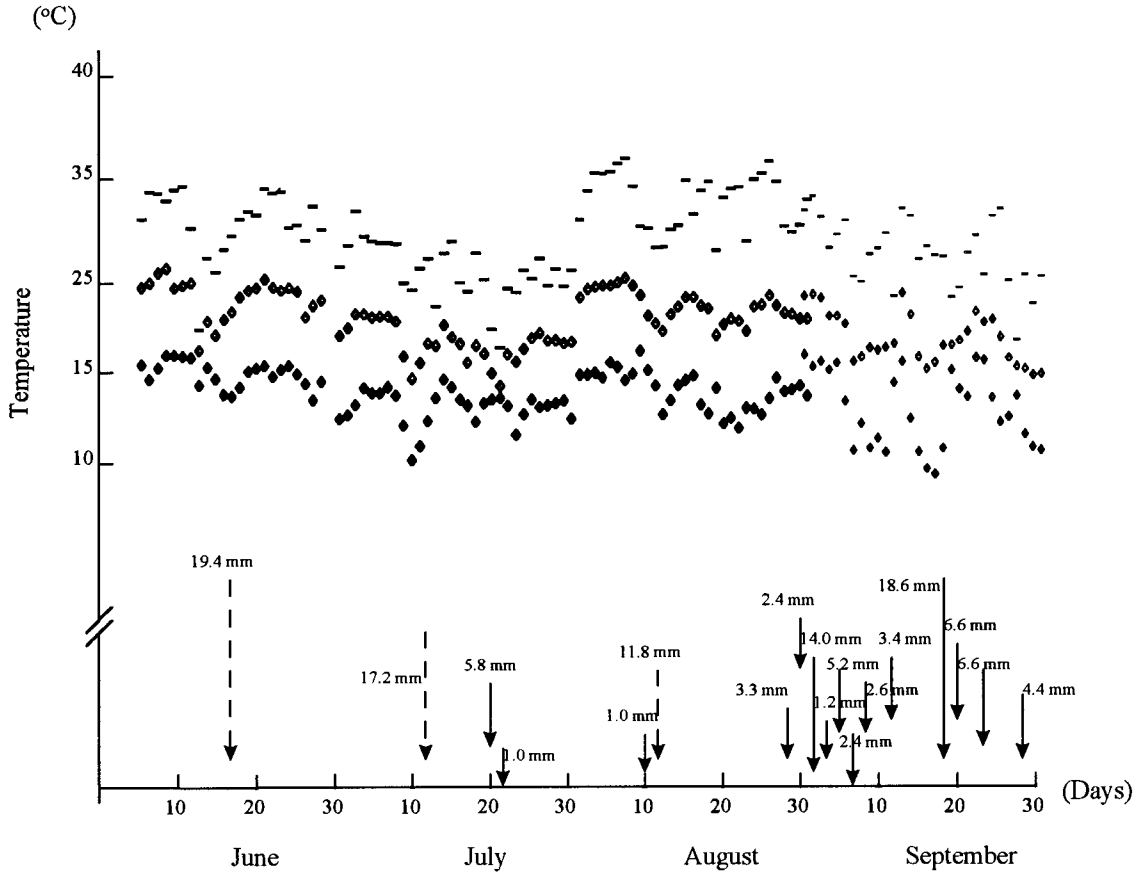

Figure 1. Daily maximum (-), average (◊), and minimum air temperature (♦), rainfall data (arrows), and irrigation (broken arrows) during the experimental period.

MATERIALS AND METHODS

Field Experiment. The trial was carried out at the experimental fields of the Hellenic Sugar Industry (HSI) located at Stavros, prefecture of Imathia, central Macedonia, Greece. The fields were sown with *Beta vulgaris* L. cv. Rizor, supplied by SES. The experimental area comprised 12 plots in a randomized complete block design with 4 replications. The size of each plot was 21.6 m^2 (2.7 m × 8 m). Herbicide and insecticide applications were performed according to the pest management program suggested by the HSI for sugar beet culture in northern Greece. The field was irrigated four times during the summer months (Figure 1) and fertilized with 100 kg of N and 50 kg of P/ha before crop sowing. Also, 52 kg of N/ha was broadcasted before the closing of the crop rows, along with foliar application of N, P, and K at 0.4, 0.3, and 0.2 kg/ha, respectively, in August. The environmental conditions that prevailed during the growing season were continuously recorded by an electronic meteorological station installed in the experimental area of the HSI (Figure 1). An aqueous emulsion of a 12.5% w/v tetraconazole formulation (Eminent 12.5EW, Isagro S.p.A., Novara, Italy) was applied at rates of 0.05 and 0.10 kg of active ingredient (ai)/ha, which correspond to the lowest (LRD) and highest (HRD) recommended application doses, respectively. Each dose was applied on four plots. The fungicide was applied with an AZO sprayer, at 5 atm spraying pressure, using cone disk D3 nozzles. The application was made with 360 L/ha water. The applicator used for his personal protection a cotton uniform, boots, gloves, and eye glasses. The first treatment was carried out on July 12, when the plants were ~3 months old and were 30−40 cm tall. The applications of tetraconazole were repeated later at 15−20-day intervals, on July 29, August 14, and September 4. The last application was performed on September 17, 1996, when the plants had reached the harvest stage. During the growing season, the average maximum daily temperatures ranged from 18.5 to 30.8 °C while the average night minimum temperatures were from 5.5 to 14.1 °C (Figure 1).

Sampling and Storage. Sampling was performed by collecting randomly 12 plants from each treated and control plot, of approximately the same size, according to FAO/WHO (1986) recommendations. The sampling was started 1 h after the last application, when the plants had dried (0 day), and was repeated 1, 3, 8, 15, and 23 days afterward to study the dissipation of the fungicide. In addition, samples were taken before the second, third, fourth, and fifth fungicide application to evaluate the possible accumulation effect of tetraconazole. The leaves with the top were separated from each beet and stored separately. Any adhering soil was removed from the root samples by light brushing with a soft brush, and the remaining soil was removed by rinsing gently the roots under running water. Field samples were put in bags and transported in ice boxes to the laboratory within 2 h from the time of collection. Each field sample was subdivided, chopped, and blended using a food cutter (laboratory sample of ~0.5−1 kg). The homogenized samples (analytical samples) were stored separately in a deep freezer at −20 °C until analysis. Two 50-g subsamples were analyzed within 2 months after sample collection.

Chemicals. Pesticide grade toluene, 2-propanol, and ethyl acetate were used as solvents (Riedel, Germany); anhydrous sodium sulfate and activated carbon were of analytical grade (Riedel); chromatography grade Celite (BDH) was used. Tetraconazole analytical standard and formulation (Eminent EW12.5) were provided by Isagro S.p.A., Milan, Italy. Stock standard solution was prepared at a concentration of 1 mg/mL in ethyl acetate; 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 according to a multiresidue method suitable for electron-captive compounds in nonfatty vegetable products (Ministry of Welfare, Health and Cultural

Table 1. Mean Recoveries (Percent)[a] and RSD of Tetraconazole from Sugar Beet Roots and Foliage at Various Fortification Levels

	sugar beet roots		sugar beet foliage	
concn (mg/kg)	recovery (%)	RSD	recovery (%)	RSD
0.001	111.1	19.1		
0.002			102.0	14.0
0.01	85.7	13.5	78.3	14.1
0.025	106.2	13.3	?	
0.05	107.0	10.5	97.2	0.43
0.1	93.2	11.3	93.3	17.0
1.0			103.3	7.7

[a] Three samples for each fortification level.

Affairs, 1988). Briefly, a 50-g portion of the frozen chopped tissue was transferred into a beaker, mixed for 3 min with 100 mL of toluene and 50 mL of 2-propanol, and homogenized with a Polytron homogenizer. The macerated mixture was filtered under vacuum through a Büchner funnel using a glass fiber filter, GF 52. The container and the filter cake were washed twice with toluene, and the combined extracts were collected and transferred into a separatory funnel, where they were washed with 2 × 250 mL of an aqueous solution of 2% Na_2SO_4. The two phases were separated after 1 min of shaking, and the aqueous phase was discarded. A 10-mL aliquot of the organic phase was transferred into a glass-stoppered cylinder, and 1 g of an absorbent mixture containing activated carbon and Celite (3:1 w/w) was added. After 1 min of shaking, the mixture was filtered off into a volumetric flask of suitable volume so that the final concentration of tetraconazole was brought into the linear range of the detector. The solution was ready for GC analysis. The stability of tetraconazole in both matrices (roots and leaves) during the storage was tested on spiked samples, stored under the same conditions, and analyzed at the end of the whole experimentation, when all of the samples had been processed.

Gas Chromatographic (GC) Analysis. A 5890 Hewlett-Packard Plus II gas chromatograph was used, equipped with an electron capture detector (ECD). The column was CP-Sil 8 wide-bore 15 m × 0.53 mm, film thickness 1.5 μm, connected with an HP 5 m × 0.53 mm fused silica deactivated capillary column for retention gap. A temperature program consisted of an initial temperature of 140 °C that was increased at a rate of 5 °C/min and then held at 200 °C for 6 min. The detector temperature was 300 °C, the injector temperature 220 °C, and the carrier gas helium flow rate 12 mL/min. The makeup gas was nitrogen at a flow rate of 40 mL/min. The retention time of tetraconazole at these operating conditions was 13.31 min.

Recovery Assays. Known quantities of tetraconazole dissolved in acetone were added to untreated samples of sugar beet roots and leaves at five fortification levels in the range of 0.001−0.1 and 0.002−1.0 mg/kg (Table 1), respectively. Tetraconazole was extracted as above-mentioned and analyzed. Recovery of the overall method was frequently checked. For this purpose, control and fortified with 0.025 or 0.05 ppm of tetraconazole subsamples of roots or leaves, respectively, were simultaneously processed with each batch of samples analyzed.

Efficacy Trials. Field experiments were established at two different locations, Platy and Alexandria, prefecture of Imathia, Greece, during the years 1993, 1994, and 1995. The experimental area was organized in a completely randomized block design with four to six replications. The size of each plot was 26.1 m^2 (2.7 m × 8 m). The plants received the common agricultural practices, as mentioned above. The fungicide was applied as an aqueous emulsion of 12.5% w/v tetraconazole formulation (M 14360 or Eminent 12.5EW, Isagro S.p.A.) at the rate of 0.1 kg of ai/ha. Also, mixtures of the same formulations of tetraconazole with maneb and chlorothalonil were applied at rates of 0.05/2.00 and 0.05/1.00 kg of ai/ha, respectively. There were four plots for each dose used. Efficacy of the fungicides was estimated using the Barrat−Horsfall scale, and the values were transformed and expressed as percentage of damaged foliage.

RESULTS AND DISCUSSION

Determination and Recoveries. The analytical method employed is a simple and rapid multiresidue method that is used in a routine scale in our laboratory for monitoring a variety of agricultural products. This method is suitable for the determination of a great number of various active ingredients in a number of different matrices. It allows the reliable determination of tetraconazole with very good precision and accuracy.

The response of the detector to tetraconazole concentration was linear, $y = 5.605 \times 10^6 x + 361.605$ in the range of 0.002−0.2 ng (0.001−0.1 μg/mL), with a very high correlation coefficient, $r = 0.999$. This linearity was checked with standard solutions of tetraconazole prepared by dilution either in ethyl acetate or in extracts of sugar beet roots and leaves from control samples. In the latter case, the response of the detector was checked again in the same range of 0.002−0.2 ng (0.001−0.1 μg/mL); the regression lines were $y = 6.381 \times 10^6 x + 2010.95$ and $y = 5.485 \times 10^6 x + 1179.09$ and the correlation coefficients were $r = 0.999$ and 0.997, respectively. Quantitation of tetraconazole in samples was made by comparing the detector response (area) for the sample to that measured for the calibration standard within the linear range. Blank samples fortified with similar concentrations of tetraconazole were injected between samples to confirm that the correct value had been estimated.

Recovery experiments were also performed to validate the method. Control samples were fortified with the fungicide at five fortification levels, covering the whole range of tetraconazole concentrations in the samples. The recovery data are summarized in Table 1. Average recovery percentages from spiked samples of sugar beet roots ranged from 86 to 111%, with relative standard deviations (RSD) from 10.5 to 19. Recoveries of the spiked sugar beet foliage samples were found in the range of 78−103%, with RSD from 0.43 to 17. Most of the samples obtained from the control plots were found free of tetraconazole, except for the samples taken from one control plot, where residues were detected. Estimations of the method's sensitivity, of detection limit (LDC), and of determination limit (LDM) were performed, for both matrices (roots and foliage), in accordance with the method of Thier and Zeumer (1987). The results of the recovery experiments were used to calculate the parameters of the regression line $\hat{y} = a_0 + \hat{S}_q$. In this equation $\hat{y}$ is the measured concentration of each fortification level q and the slope $\hat{S}$ is the estimated value of sensitivity S. LDC values are 0.0007 and 0.0017 mg/kg for the determination of tetraconazole in sugar beet leaves and roots, respectively. These values were calculated on the basis of standard deviations of the blanks at the lowest for each matrix fortification level, with $f = 6$ degrees of freedom at 95% confidence level. Consequently, the method's LDM values under these conditions were 0.002 and 0.001 mg/kg for the determination of tetraconazole in sugar beet leaves and roots, respectively. All values for both matrices were within the accepted range for residue determination, satisfying the three requirements LDM ≥ LDC, $S \geq 0.7$, and $V \leq 0.2$ (20%) (Thier and Zeumer, 1987). The values of LDM were well below the LDM of 0.01 mg/kg, which was reported by Khalfallah et al. (1998). Tetraconazole measurements of spiked samples stored at −20 °C showed that storage of both matrices

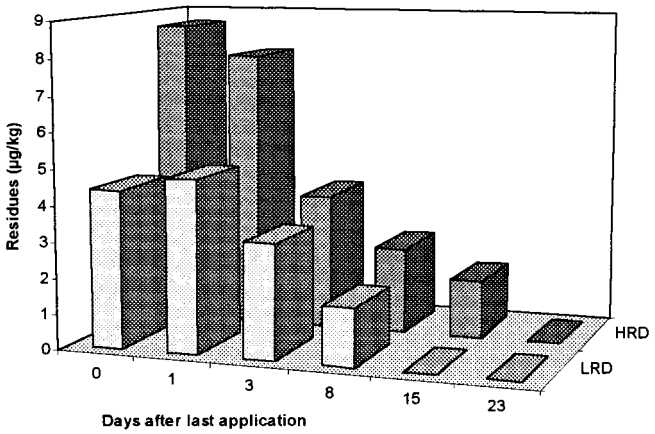

Figure 2. Tetraconazole residues (μg/kg) in sugar beet roots at various time intervals following the last application: LRD, 0.05 kg of ai/ha; HRD, 0.10 kg of ai/ha.

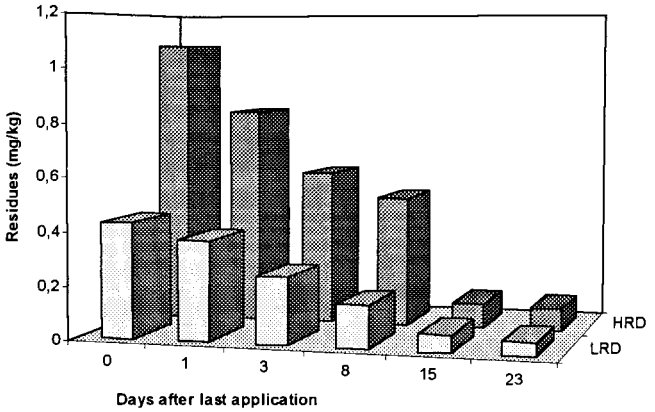

Figure 3. Tetraconazole residues (mg/kg) on sugar beet foliage at various time intervals following the last application: LRD, 0.05 kg of ai/ha; HRD, 0.10 kg of ai/ha.

under these conditions did not affect the amount of tetraconazole residues.

Disappearance of Residues. Most of the control samples were free of interference peaks or detectable tetraconazole residues. However, in some samples from one control plot residues were detected at amounts lower than the LDM. Residues of tetraconazole found in sugar beet roots and foliage following the last of five applications for both doses are shown in Figures 2 and 3, respectively. As shown in these figures, initial deposits of tetraconazole on sugar beet roots were 4.4 and 8.6 μg/kg for the LRD and the HRD, respectively, whereas in foliage 0.429 and 1.072 mg/kg tetraconazole were detected for the LRD and the HRD, respectively.

Tetraconazole residue in sugar beets declined with time. The amount of tetraconazole in roots was nondetectable 15 days after the last of five applications of the low dose treatment or 23 days after the high dose treatment. Also, 23 days after the last of five applications only 12 and 8% of the initial deposits were found in foliage samples obtained from the LRD and HRD treatments, respectively. Decline of pesticide residues in crops usually follows a pseudo-first-order model (Walter et al., 1993). Statistical interpretation of the tetraconazole dissipation data in all studied treatments was performed by assuming that the dissipation rate of the residues can be described by a pseudo-first-order reaction, according to the equation $R = R_0\, e^{-kt}$, where R is the amount of tetraconazole at t days after application, R_0 the amount of tetraconazole at time $t = 0$ days after application, and k the dissipation rate constant. The statistical parameters and the dissipation equations determined by the linear semilogarithmic regression analysis ($\log R = \log R_0 - kt$) are presented in Table 2. As shown in this table, the regression coefficient (r^2) ranges from 0.92 to 0.97. Tetraconazole half-life times ($T/2$) in either sugar beet roots or foliage were found to be 5.1−5.7 days for both application doses, indicating that it is independent of the initial deposit.

Table 2. Equations for Estimation of Half-Lives of Tetraconazole on Sugar Beet Foliage and in Sugar Beet Roots

	dose	k (days^{-1})	r^2	R_0	$T/2$ (days)
roots	LRD	−16.1483	0.96	11.211	5.7
roots	HRD	−23.099	0.92	19.847	5.2
foliage	LRD	−22.584	0.97	−9.335	5.7
foliage	HRD	−18.124	0.94	0.156	5.1

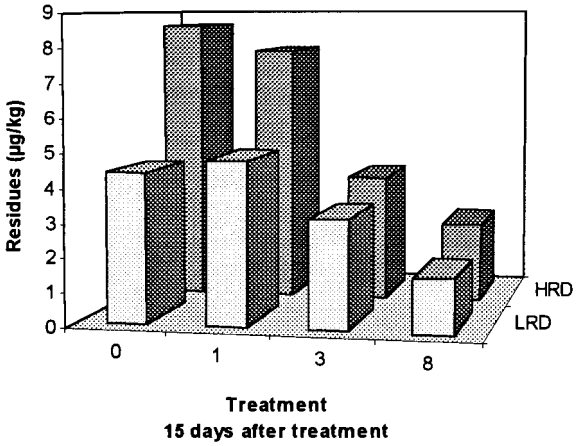

Figure 4. Tetraconazole residues (μg/kg) in sugar beet roots 15 days after each of four repeated, at 15-day intervals, applications.

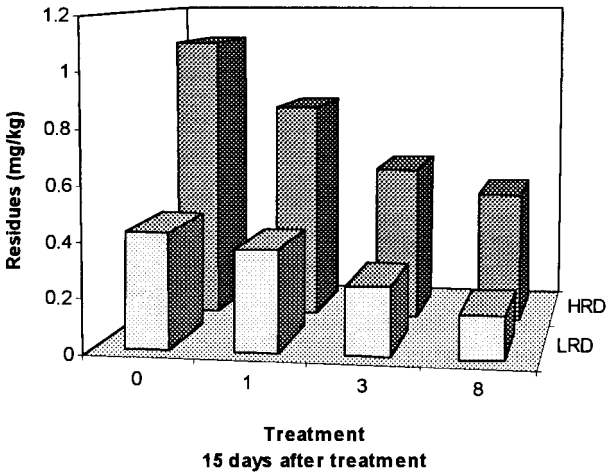

Figure 5. Tetraconazole residues (mg/kg) on sugar beet foliage 15 days after each of four repeated, at 15-day intervals, applications.

Figures 4 and 5 present the effect of five repeated applications at 15-day intervals on tetraconazole residues in sugar beet roots and leaves. As seen from the data, the amounts of tetraconazole residues in roots 15 days after each of the five repeated applications are almost similar, ranging from 4.5 to 10.5 μg/kg, indicating that accumulation did not occur. Only the first two applications of HRD resulted in higher amounts, ranging from 24 to 35 μg/kg. Sugar beet plants at that period were still young with no well-developed roots but with

foliage being at its higher density. In general, repeated applications of tetraconazole did not result in accumulation of its residue in sugar beets.

Tetraconazole efficacy to control sugar beet spot was evaluated during the growing seasons 1993, 1994, and 1995. Percentage of damaged foliage ranged from 6 to 8% (compared to the 100% damaged foliage of the untreated control) when the fungicide was applied at the recommended rate. However, when tetraconazole was applied at half-dose in a mixture with maneb or chlorothalonil, the percentage of damaged foliage ranged from 4.7 to 18.8% and from 5.4 to 22.0% for each mixture, respectively, compared to the 78−100% damaged foliage of the untreated control.

In conclusion, these results indicate that tetraconazole is an effective fungicide for beet leaf spot control, a serious disease of the sugar beet crop in Greece, which causes severe economic damage each year. The rapid multiresidue analytical method used allows the quantitative determination of parent compound residues down to 0.001 and 0.002 mg/kg in sugar beet roots and leaves, respectively. Tetraconazole dissipation from sugar beets was found to follow a first-order model for the duration of the experiment (23 days). Fifteen days after the last of the five consecutive applications was performed, residues of tetraconazole were not detected in roots. Tetraconazole residues on sugar beet foliage 23 days after the same treatments are reduced to ∼0.05 mg/kg, corresponding to ∼10% of the initial deposit. Finally, the results of this study allow us to conclude that tetraconazole used according to the Good Agricultural Practice (GAP) protocol did not leave detectable residues in roots at harvest time. Also, tetraconazole residues on foliage are not higher than the MRL. Consequently, tetraconazole is a promising compound of the triazole group that effectively controls *C. beticola* on sugar beets without leaving unacceptable residues in the crop.

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