Distribution of Folpet on the Grape Surface after Treatment

Paolo Cabras,*,† Alberto Angioni,† Pierluigi Caboni,† Vincenzo L. Garau,† Marinella Melis,† Filippo M. Pirisi,† and Franco Cabitza‡

Dipartimento di Tossicologia, Università di Cagliari, viale Diaz 182, 09126 Cagliari, Italy, and Centro Regionale Agrario Sperimentale, 09100 Cagliari, Italy

Field trials were carried out to evaluate whether folpet sprayed on grapevines penetrated the epicuticular wax and cell walls of grapes. Folpet showed poor penetration into the epicuticular wax; it was found almost totally on the surface. Despite its low solubility in water, perhaps due to the presence of adjuvants, its residues showed such a high resistance to washing that the action of rain was negligible in decreasing residues.

Keywords: *Folpet; grape surface; residues*

INTRODUCTION

Most pesticides are nonsystemic, because they cannot penetrate into the plant. After treatment, they form a deposit on the surface of leaves and fruits; in this way they can be adsorbed by surface dust and/or penetrate the epicuticular wax and subsequently the cuticle layer (Rieder and Schreiber, 1995). It was shown recently that the driving force for foliar penetration depends on the formulation and on the lipophilicity and concentration of the active ingredient (Baur et al., 1997; Marzouk et al., 1998).

Pesticide penetration into wax defends its residues from the effect of washing. This was confirmed in some studies, in which the residues of some pesticides present on fruit did not decrease even after prolonged washing under running water (Cabras et al., 1998a,b). Pesticide penetration into the epicuticular wax has an important practical implication: because it does not allow the residue to wash away, not even rain immediately after the treatment can decrease its efficacy. To our knowledge no field experiments have been carried out to assess whether sprayed pesticides can penetrate the wax and cell walls of the fruit, and, if so, how quickly it can penetrate. To answer these questions, we planned an experiment on grapevines using folpet.

MATERIALS AND METHODS

Field Trials. The trial was carried out in a white grape vineyard (cv. Nuragus), at Ussana, Cagliari, Italy. A random-block scheme was used with four replications per test, each block containing 100 plants. The treatment was carried out on September 1, 1998; Dipet (42.5% folpet) was a commercial formulation applied at the dose recommended by the manufacturer (250 g/hL; 6 hL/ha) with an F-320 portable motor sprayer (Fox Motori, Reggio Emilia, Italy). Samplings (on dry plants) started about 2 h after treatment and on days 2, 6, 10, 20, and 30. Random 2-kg samples of grapes were collected from each plot and immediately analyzed for fungicide residues. The environmental conditions were continuously recorded with an AD-2 automatic weather station (Silimet, Modena, Italy). During the experiments the total rainfall was 37.0 mm, with maximum values of 8.6 and 20.8 mm, respectively, on September 8 and 12. The maximum and minimum average temperatures were, respectively, 28.5 and 16.6 °C.

Chemicals. The pesticides were all analytical standards. Folpet and vinclozolin were purchased from Ehrenstorfer (Augsburg, Germany), and phthalimide was from Lancaster Synthesis (Muhlheim am Main, Germany). Acetone and methanol were HPLC grade solvents (Carlo Erba, Milan, Italy), whereas petroleum ether (Carlo Erba) was a special reagent for pesticide determination. Anhydrous sodium sulfate and sodium chloride were of analytical grade (Carlo Erba).

Stock standard solutions (~300 ppm each) were prepared in acetone for folpet and phthalimide. Working standard solutions were obtained by dilution with hexane containing vinclozolin at 0.3 mg/kg as internal standard (i.s.).

Residue Analysis. *Gas Chromatographic (GC) Determination.* An HRGC Mega 5160 (Carlo Erba, Milano, Italy) gas chromatograph was employed, fitted with an ECD 800 detector, an AS 800 autosampler (Carlo Erba), and a split−splitless injector, connected to an HP 3396-II reporting integrator (Hewlett-Packard, Avondale, PA). A Durabond fused silica column (30 m × 0.32 mm i.d.) (J&W Scientific, Folsom, CA) was employed, with a DB-5 MS liquid phase (film thickness = 0.25 μm). The injector and detector were operated at 250 and 320 °C, respectively. The sample (2 μL) was injected in the split mode (1:10), and the oven temperature was programmed as follows: 150 °C for 1 min, raised to 240 °C (5 °C/min), and held for 5 min. Helium was the carrier and makeup gas at 100 and 130 kPa, respectively. Calibration graphs for folpet and phthalimide were constructed with the internal standard (i.s.) method by measuring peak heights versus concentrations. Good linearities were achieved in the range 0−2.5 ppm, with correlation coefficients between 0.9994 and 0.9997.

Sample Preparation. *Washing.* To a 400-mL beaker containing 200 mL of distilled water was added 100 g of whole grapes with the pedicel. The beaker was placed for 5 min in an ultrasonic bath, and the grapes were then separated and strained. The same sample was washed repeatedly (five or six times) until all residues were removed.

Epicuticular Wax Extraction. Wax extraction was performed according to the method of McDonald et al. (1993). After washing, the same grape sample was drained with blotting paper and added to a 250-mL beaker containing 100 mL of chloroform. After 1 min exactly, the organic solvent was transferred to a screw-capped 200-mL flask. The grapes were transferred to a sheet of blotting paper, and the chloroform was allowed to evaporate in a fume hood.

Extraction Procedure. *Washing Water.* A 10-mL aliquot of washing water was added in a screw-capped 40-mL tube containing 10 mL of dichloromethane. The mixture was agitated in a vortex for 2 min. The phases were allowed to separate, and 1.0 mL of organic extract in a 2-mL vial was

† Università di Cagliari.
‡ Centro Regionale Agrario Sperimentale.

10.1021/jf990069u CCC: $19.00
Published on Web 02/03/2000

Table 1. Distribution of Folpet in Grapes after Treatment

days after treatment	distribution (mg/kg ± SD) grape surface	grape epicuticular wax	grape cell walls	grape pulp
0	1.26 ± 0.54	<0.01	<0.01	<0.01
2	1.13 ± 0.45	<0.01	<0.01	<0.01
6	0.94 ± 0.38	0.02 ± 0.01	<0.01	<0.01
10	0.54 ± 0.19	0.05 ± 0.02	<0.01	<0.01
20	0.32 ± 0.07	0.06 ± 0.02	<0.01	<0.01
30	0.12 ± 0.01	0.04 ± 0.02	<0.01	<0.01

dried under nitrogen stream. The residue was taken up with 0.5 mL of hexane containing i.s. vinclozolin and injected for GC analysis.

Epicuticular Wax. In a 2-mL vial, 1 mL of chloroformic extract was dried under a nitrogen stream. The residue was taken up with 1.0 mL of hexane containing i.s. vinclozolin and injected for GC analysis.

Cell Walls. The same grape sample that had been washed and wax extracted was placed in a 250-mL screw-capped flask containing 100 mL of an acetone/petroleum ether mixture (1:1, v/v) and 10 g of NaCl. The flask was then agitated in a shaker for 15 min, and 25 mL of organic extract was dehydrated with 5 g of anhydrous sodium sulfate. In a 2-mL vial, 1 mL of this extract was dried under a nitrogen stream. The residue was taken up with 1 mL of hexane containing i.s. and injected for GC analysis.

Pulp. The sample that had been submitted to washing, wax extraction, and residue extraction from the cell walls was chopped and homogenized with a food cutter. The obtained sample was extracted as described above for the cell walls.

Recovery Assays. Water, wax of untreated grape, and untreated grape samples were fortified with folpet and phthalimide and processed according to the above-described procedure. Recovery assays, carried out at 0.01, 0.50, and 3.00 ppm, showed values obtained from four replicates ranging between 90 and 110%, with a maximum coefficient of variation (CV) of 9%.

RESULTS AND DISCUSSION

The solubility of folpet in water at room temperature is 0.8 mg/L (Tomlin, 1997). Because the residue amount on grapes after treatment was 1.26 mg/kg (Table 1) and 100 g of grapes was dipped in 200 mL of water, only one washing should have been enough to solubilize the entire deposit.

Nevertheless, the grapes had to be washed five or six times before water without residues was obtained. Folpet's resistance to solubilization in water could be attributed to the action of the adjuvants in the commercial formulation that links the pesticide molecule to the cuticular lipid layer and decreases its solubilization in water. The presence of residues in the epicuticular wax at very low levels (0.02 mg/kg) after 6 days of treatment showed that folpet had a low tendency to penetrate into the wax. Subsequently, the residue increased slightly in the wax, but it never exceeded 5% of the initial deposit. No residue was found in the cell walls or in the pulp, thus confirming the poor ability of this pesticide to penetrate the fruit. During the experiment it rained heavily on two occasions, one after 8 days and the other after 12 days, with 8.6 and 20.8 mm of rain, respectively. To assess whether these rainfalls affected residue washing, we reported the decrease in residue, expressed as ln [M] ([M] = molar concentration) against time (Figure 1). The correlation coefficient was 0.993. The obtained straight line showed that these rainfalls did not affect the residue amount. The decrease in residue follows a pseudo-first-order kinetics with a half-life of 8.9 days. Phthalimide (a metabolite formed on grapes from folpet) residues were present in the washing water but only in small amounts (<0.03 mg/kg). In a previous paper (Cabras et al., 1997) phthalimide residues determined in grapes were higher, but this was due to the degradative action of must acidity during sample preparation, which degraded folpet rapidly and mainly yielded phthalimide.

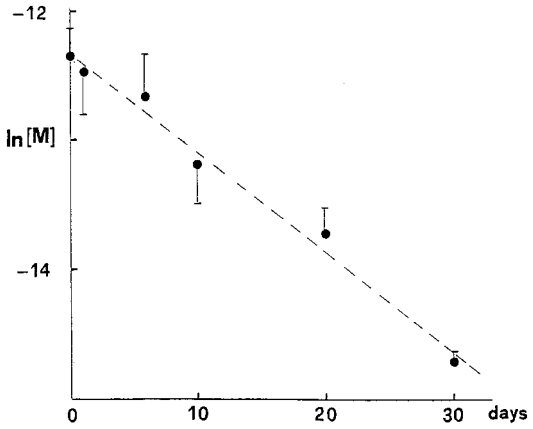

Figure 1. Decrease of folpet (expressed as ln M) in grapes after treatment.

CONCLUSION

Folpet settled on the fruit after treatment showed poor penetration into the epicuticular wax and remained deposited almost totally on the surface. Folpet residues showed such high washing resistance, perhaps due to the presence of its adjuvants, that the action of rain was considered to be negligible as a residue-decreasing factor.

LITERATURE CITED

Baur, P.; Marzouk, H.; Schönherr, J.; Grayson, B. T. Partition coefficients between plant cuticle and adjuvants as related to foliar uptake. *J. Agric. Food Chem*. **1997**, *45*, 3659−3665.

Cabras, P.; Angioni, A.; Garau, V. L.; Melis, M.; Pirisi, F. M.; Farris, G. A.; Sotgiu, C.; Minelli, E. V., M. Persistence and metabolism of folpet in grapes and wine. *J. Agric. Food Chem*. **1997**, *45*, 476−479.

Cabras, P.; Angioni, A.; Garau, V. L.; Melis, M.; Pirisi, F. M.; Cabitza, F.; Cubeddu, M. Pesticide residues on field-sprayed apricots and in apricot drying process. *J. Agric. Food Chem*. **1998a**, *46*, 2306−2308.

Cabras, P.; Angioni, A.; Garau, V. L.; Melis, M.; Pirisi, F. M.; Cabitza, F.; Pala, M. Pesticide residues in raisin processing. *J. Agric. Food Chem*. **1998b**, *46*, 2309−2311.

McDonald, R. E.; Nordby, H. E.; McCollum, T. G. Epicuticular wax morphology and composition are related to grapefruit chilling injury. *Hortic. Sci*. **1993**, *28*, 311−312.

Marzouk, H.; Baur, P.; Schönherr, J. Relative solubilities of bifenox and 1-naphthylacetic acid (NAA) in plant cuticles and in selected pure or aqueous glycol additives. *Pestic. Sci*. **1998**, *53*, 278−284.

Riederer, M.; Schreiber, L. Waxes−The transport barriers of plant cuticles. In *Waxes: Chemistry, Molecular Biology and Functions*; Hamilton, R. J., Ed.; The Oily Press: Dundee, Scotland, 1995; Vol. 6, pp 131−156.

Tomlin, C. D. S. *The Pesticide Manual*, 11th ed.; BCPC: Farnham, U.K., 1997.

Received for review January 20, 1999. Revised manuscript received December 1, 1999. Accepted December 13, 1999.

JF990069U