Migration of Organophosphorus Insecticides Cyanophos and Prothiofos Residues from Impregnated Paper Bags to Japanese Apple-Pears (*Pyrus pyrifolia* Nakai Cv. Nijisseiki)

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Bags impregnated with the organophosphorus pesticides prothiofos and cyanophos in three levels were used to cover Japanese apple-pears to protect them from insects. The amounts of prothiofos residue in the bags collected 4 months after application ranged from 0 to 34% of the amounts (81, 148, and 333 μg) found in the bags prior to a bagging. The amounts of cyanophos residue in bags collected 4 months after application ranged from 11 to 29% of the amounts (335, 2860, and 3740 μg) present prior to a bagging. Amounts of prothiofos found in fruits after 4 months ranged from 0 to 0.076 ppm. Amounts of cyanophos found in fruits after 4 months ranged from 0 to 0.011 ppm. Results of the present study indicate that these two pesticides migrated from pesticide-impregnated bags to fruits during the growing season.

Keywords: *Cyanophos; pears; pesticide migration; prothiofos; Pyrus pyrifolia Nakai cv. Nijisseiki*

INTRODUCTION

In Japan, it is common practice in some orchards to cover individual peaches, pears, or bunches of grapes with a pesticide-impregnated bag. This practice prevents extensive insect damage to the fruit (Nishimura et al., 1976, 1977). A double bag impregnated on both surfaces with cyanophos is widely used for pears in Japan. In those limited areas where scale insect (family Pseudococcidae) outbreaks occur frequently, prothiofos-impregnated bags are used. These bags also prevent the occurrence of black spots (*Alternaria kikuchiana* Tanaka) on fruits under either dry or humid conditions and also protect fruit from hard rain. These pesticide-impregnated bags also protect fruits from exposure to pesticides sprayed in the orchards (Iuchi, 1983). However, the pesticides may migrate from the impregnated bag to the fruit (Iuchi et al., 1978). Therefore, it is important to know whether the pesticides impregnated in the bags actually do migrate into the fruits.

Japanese apple-pear (*Pyrus pyrifolia* Nakai cv. Nijisseiki) was originally developed in 1888 in Chiba, Japan. The trees have been commercially available in Japan since 1904. Initially, production of the Japanese apple-pear was limited due to damage by insect pests. It was also susceptible to a black spot. In 1926, the bagging method was developed, and since then production has increased steadily; it is now in excess of 72 000 tons annually. This makes it the third leading cultivar in Japan in 1997 (personal communication from Japan Ministry of Agriculture and Forestry, Information Center). Approximately 8000 tons were exported to Hong Kong, Singapore, the United States, and Canada in 1997.

The fruit of the Japanese apple-pear keeps well for over 2 weeks at room temperature and for 3−4 months in cold storage. The fruit has a medium apple shape (9−10 cm in diameter, 270−350 g) and its skin color is pale green to yellow. It is juicy and crisp with a mild flavor. The Japanese apple-pear is becoming popular in the United States because of its texture and sweet taste. In the present study, prothiofos and cyanophos residues in Japanese apple-pears that were cultivated in Japan and covered with pesticide-impregnated bags were analyzed using gas chromatography.

EXPERIMENTAL PROCEDURE

Chemicals. Cyanophos (*O*-4-cyanophenyl *O*,*O*-dimethyl phosphorothioate) and prothiofos (*O*-2,4-dichlorophenyl *O*-ethyl *S*-propyl phosphorodithioate) were purchased from Hayashi Pharmaceutical Industries, Co., Ltd. (Tokyo, Japan). All solvents (acetone, dichloromethane, and cyclohexane) were analytical grade.

Instruments. Gel-permeation chromatography was performed using a Shimazu LC-10A (Shimazu, Tokyo) equipped with a 50 cm × 22 mm i.d. Pyrex column (Tokyo, Japan) packed with Biobees S-X3 (200−400 mesh) (Biorade Co., Tokyo, Japan).

A Hewlett-Packard (HP) 5890 series II gas chromatograph (GC) equipped with a 15 m × 0.53 mm (d_f = 0.25 μm) DB-5 bonded-phase megabore column (J&W Scientific, Folsom, CA) and a flame photometric detector (FPD) was used for routine quantitative analysis. The oven temperature was programmed from 50 to 290 °C at 10 °C/min and held for 10 min; the detector and injector temperatures were 260 °C. The helium carrier gas flow rate was 10 mL/min.

Pesticide-Impregnated Bags. All pesticide-impregnated bags (Figure 1) were purchased from Kobayashi Seitai, Inc., in Nagano, Japan. The large outside bag (15 × 17.5 cm, 2.41 g) was made of wax paper, and the large inside bag (14.5 ×

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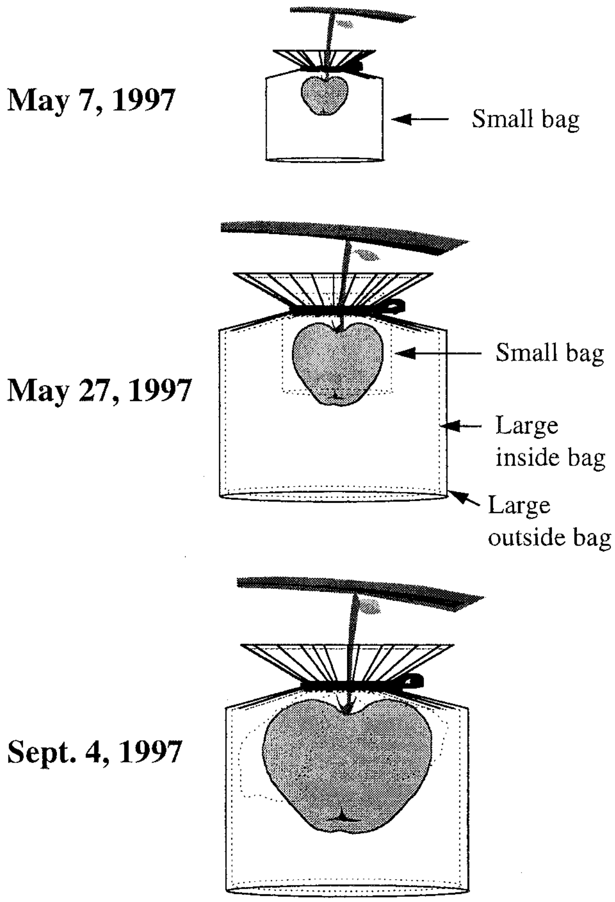

Figure 1. Systematic diagram of pesticide-impregnated bags.

17.0 cm, 1.51 g) was made of waterproof paper. The large outside and inside bags were impregnated with both prothiofos and cyanophos at three different levels (A = high, B = medium, C = low). The exact levels of pesticides applied by the manufacturer were not disclosed. Therefore, the pesticides levels of each bag were analyzed in our laboratory prior to application. No pesticide was impregnated in the small bag (8.5 × 11 cm, 0.77 g).

Fruit Bagging. Two 60-year-old Japanese pear trees planted 20 m apart were used. Each fruit was bagged with a small bag on May 7, 1997, when it was approximately 1 cm in diameter. Figure 1 shows the bags used and the method of fruit-bagging. The two large bags were applied on May 27 when the fruit size was 3−4 cm in diameter. Three branches were chosen from each tree, and 100 fruits from each branch were bagged with three different bags (A, B, and C).

Foliar Application of Prothiofos. A commercial prothiofos formulation (Nihon Bayer Agrochem Inc., Tokyo, Japan) was sprayed over one tree using a speed sprayer model 3WD-510 (Shoshin, Nagano, Japan) on June 9, 1997. A 500-L aqueous solution of prothiofos (32%) was diluted 800-fold with water, and then it was sprayed at 16 L/min. Only one tree was sprayed with prothiofos to compare prothiofos residue levels between bags and fruits from the sprayed tree and from the nonsprayed tree.

Analysis of Pesticides in Pesticide-Impregnated Bags. After removal from four pieces of fruit, four bags were cut into approximately 2-cm pieces and then placed into a 300-mL separatory funnel containing 100 mL of acetone. The separatory funnel was shaken for 5 min. The extraction was repeated three times. After the extract was filtered, the acetone was removed with a rotary flash evaporator at 40 °C. The residual materials were dissolved into a 5-mL dichloromethane/cyclohexane (1/1, v/v) solution. This solution (4 mL) was fractionated with GPC developed with a dichloromethane/cyclohexane (1/1, v/v) solution. The fraction eluted from 90 to 220 mL was condensed and subsequently dissolved into 4 mL of acetone. The acetone solution was analyzed for pesticides using GC/FPD.

Table 1. Amounts of Prothiofos Residues Found in the Pesticide-Impregnated Bags

level of pesticide impregnation	kind of bag	levels of residue (μg/bag)[a]: prior to bagging	4 months after bagging: prothiofos not sprayed	4 months after bagging: prothiofos sprayed
A (high)	outside	193 ± 4.8	32.1 ± 2.4	61.5 ± 3.5
	inside	140 ± 4.0	33.3 ± 3.0	57.6 ± 3.9
	small	*b*	2.7 ± 0.5	5.6 ± 0.9
	total	333	68.1 (20.5%)[c]	124.7 (37.4%)
B (medium)	outside	105 ± 3.4	25.2 ± 2.2	45.2 ± 3.7
	inside	42.7 ± 4.2	24.8 ± 2.6	41.2 ± 3.0
	small	*b*	2.9 ± 0.4	4.3 ± 0.6
	total	147.7	52.9 (35.8%)	90.7 (61.5%)
C (low)	outside	46.3 ± 4.4	*d*	14.2 ± 2.0
	inside	34.7 ± 4.0	*d*	14.4 ± 2.7
	small	*b*	*d*	0.7 ± 0.1
	total	81.0	*d*	29.3 (36.2%)

[a] Values are mean ± standard deviation (n = 4). [b] Not detected. [c] Percent of original. [d] Values are less than 0.5 μg/bag.

Analysis of Pesticides in Fruits. After stems and cores were removed from four pears, the fruit was cut into small pieces. The flesh pieces (20 g) were homogenized with 100 mL of acetone in a 200-mL stainless steel beaker using a high performance blender for 5 min. The homogenized materials were filtered using Cerite 545. The residual materials were eluted further with 50 mL of acetone, and the eluate was combined with the filtrate, and then the acetone was removed by a rotary flash evaporator. The residual viscous materials were mixed with 100 mL of a saturated sodium chloride solution and 100 mL of dichloromethane in a 300-mL separatory funnel. The funnel was shaken for 5 min and then was allowed to stand for 10 min. After the dichloromethane extract was removed, the solution was further extracted with 50 mL of dichloromethane, and the extracts were combined. The dichloromethane extract was dried over anhydrous sodium sulfate. After the sodium sulfate and dichloromethane were removed, the residual materials were dissolved into a 5-mL dichloromethane/cyclohexane (1/1, v/v) solution that was subsequently analyzed as described above.

RESULTS AND DISCUSSION

Cyanophos is used to control species of Aphidida, Coccidae, Diaspididae, Lepidoptera, and Margarodidae in cotton, fruit, and vegetables. It is also used to control locusts and pests such as Blattodea, Culicidae, and Muscidae (Tomlin, 1997a). Prothiofos is used to control leaf-eating caterpillars, *Pseudococcus* spp., thrips, cockchafer larvae, cutworms, etc., in a range of crops, including vegetables, fruit, maize, sugar carne, sugar beet, tea, tobacco, and ornamentals (Tomlin, 1997b).

There are no published reports describing analysis of cyanophos and prothiofos in any crops. The limits of quantitation for cyanophos and prothiofos were 0.002 and 0.010 ppm, respectively. The minimum detectable values for cyanophos and prothiofos were 0.001 and 0.005 ppm, respectively, in the present study. Recovery efficiencies of prothiofos and cyanophos from large bags were 100 ± 4.0 and 101 ± 6.0%, respectively. Recovery efficiencies of prothiofos and cyanophos from fruits were 99 ± 6.0 and 100 ± 9.0%, respectively.

Amounts of Pesticide Residues Found in the Bags. Table 1 shows the amounts of prothiofos found in pesticide-impregnated paper bags. Values are mean ± standard deviation (n = 4). The total amounts of prothiofos found in bags before use were 333.0 μg (A),

Table 2. Amounts of Cyanophos Residues Found in the Pesticide-Impregnated Bags

level of pesticide impregnation	kind of bag	levels of residue (μg/bag)[a] prior to bagging	4 months after bagging
A (high)	outside	2290 ± 64	841 ± 42
	inside	1450 ± 56	239 ± 24
	small	*b*	17.3 ± 2.1
	total	3740	1097 (29.3%)[c]
B (medium)	outside	1710 ± 50	541 ± 35
	inside	1150 ± 41	184 ± 11
	small	*b*	26.0 ± 2.5
	total	2860	751.0 (26.3%)
C (low)	outside	305 ± 9	36.8 ± 2.1
	inside	29.8 ± 3.0	*d*
	small	*b*	*d*
	total	334.8	36.8 (11.0%)

[a] Values are mean ± standard deviation ($n = 4$). [b] Not detected. [c] Percent of original. [d] Values are less than 0.5 μg/bag.

Table 3. Amounts of Prothiofos and Cyanophos Residues Found in the Bagged Pears

level of pesticide impregnation in bag	levels of residue (ppm)[a] prothiofos not sprayed	prothiofos sprayed	cyanophos
A (high)	0.050 ± 0.004	0.076 ± 0.006	0.011 ± 0.003
B (medium)	0.020 ± 0.002	0.037 ± 0.005	*b*
C (low)	*b*	0.020 ± 0.003	*b*

[a] Values are mean ± standard deviation ($n = 4$). [b] Values are less than 0.01 ppm.

147.7 μg (B), and 81.0 μg (C). The total amounts of prothiofos found in bags—from the tree where prothiofos was not sprayed—after 4 months (September 4, 1997) were 68.1 μg (A) and 52.9 μg (B); this is 20% of the original level for A and 34% of the original level for B. No prothiofos residues were detected in the C bags collected from either the sprayed or the unsprayed tree. On the other hand, 14.2 μg, 14.4 μg, and 0.7 μg of residue were found in the large outside bags, the large inside bags, and the small bags of C, respectively, from the tree sprayed with prothiofos. The results indicate that some prothiofos sprayed over the tree was retained in all the bags over a period of 4 months.

Table 2 shows the amounts of cyanophos found in pesticide-impregnated bags. Values are mean ± standard deviation ($n = 4$). The total amounts of cyanophos found in bags prior to bagging were 3740 μg from A, 2860 μg from B, and 334.8 μg from C. The amounts of cyanophos impregnated were approximately 10−45 times those of prothiofos. The total amounts of cyanophos residue in bags after 4 months were 29% of the original level for A, 26% of the original level for B, and 11% of the original level for C. Cyanophos residues were not found in the large inside bags or the small bags of C.

Prothiofos and cyanophos residues were found in the small bags that had no pesticide prior to bagging, indicating that both pesticides migrated from the large bags to the small bags over a period of 4 months. The results also indicate that prothiofos migrated from the outside bags to the inside bags.

Amounts of Pesticides Residues Found in the Fruits. Table 3 shows the results of pesticide analysis in the fruits collected on September 4. Values are mean ± standard deviation ($n = 4$). Amounts of prothiofos found were 0.05 and 0.02 ppm in pears bagged with A bags and B bags, respectively, whereas prothiofos was not detected in pears bagged with C bags. In the case of the pears from the tree where prothiofos was sprayed on June 9, the amounts of prothiofos found were 0.076, 0.037, and 0.020 ppm in the pears bagged with A bags, with B bags, and with C bags, respectively. The prothiofos residue in fruit increased when prothiofos was sprayed with the foliar application method. The pesticide residue levels in the fruit correlated with the levels of the pesticide impregnated in the bags. The levels of pesticide residues found in the pears were lower than the tolerance level (0.1 ppm) set by the Japan Ministry of Health and Welfare.

Cyanophos was found in a fruit bagged with an A bag at a level of 0.011 ppm, whereas it was not detected in a fruit bagged with a B bag or a C bag. The level found was much lower than the tolerance level (0.2 ppm) set by the Japan Ministry of Health and Welfare.

Even though the amounts of cyanophos impregnated in bags were approximately 10−45 times those of prothiofos, cyanophos residues found in fruits were much less than those of prothiofos. These results may be due to the relatively high vapor pressure (0.5 mPa at 20 °C) of cyanophos and its relatively high water solubility (46 mg/L at 30 °C). Therefore, cyanophos may escape from a bag by evaporation, degradation, and washing off with rain during a 4-month test. Prothiofos has been used only in limited areas in Japan due to its relatively high persistence in the environment.

Results of the present study show that varying amounts of pesticides migrated from pesticide-impregnated bags to fruit during the growing season. The pesticide levels were much less than the tolerance level set by the Japan Ministry of Health and Welfare. At the present time, cyanophos and prothiofos have not been registered in the United States, and their import tolerances have not yet been set.

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LITERATURE CITED

Iuchi, A. Influences of applying pesticide-impregnated paper bag on residue of pesticides in some fruit. *Tokushima Agric. Exp. Stat. Rep.* **1983**, *21*, 29−33.

Iuchi, A.; Nagai, Y.; Kuroshima, T. Persistence of organophosphorous insecticide residues in Japanese pears. *Tokushima Agric. Exp. Stat. Rep.* **1978**, *16*, 74−78.

Nishimura, T.; Tsuge, S.; Kawahara, T.; Suzuki, S. Disappearances of diazinon and chlorothalonil in pesticide impregnated paper bags and application of ^{14}C-lindane impregnated paper bag to a Japanese pear. *Bull. Agric. Chem. Insp. Stat.* **1976**, *16*, 45−49.

Nishimura, T.; Tsuge, S.; Kawahara, T.; Suzuki, S. Application of ^{35}S-captan impregnated paper bag to a Japanese pear. *Bull. Agric. Chem. Insp. Stat.* **1977**, *17*, 40−42.

Tomlin, C. D. S. In *The Pesticide Manual*, 11th ed.; Tomlin, C. D. S., Ed.; British Crop Protection Council: Farnham, UK, 1997a; p 282.

Tomlin, C. D. S. In *The Pesticide Manual*, 11th ed.; Tomlin, C. D. S., Ed.; British Crop Protection Council: Farnham, UK, 1997b; p 1043.

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