Regular Article

Comparison of adherence tendencies of pesticide residues sprayed on small-, medium-, and large-sized tomatoes

Takuya Nagata,* Hikari Dobashi, Kazuaki Iijima and Kazutoshi Ohyama

The Institute of Environmental Toxicology (IET), 4321, Uchimoriya-machi, Joso-shi, Ibaraki 303–0043, Japan

🅢 *Supplementary material*

Crop field trials were conducted to investigate the residues of sprayed pesticides on the different sizes of tomatoes. Pesticide residue data in tomatoes varied due to different locations of the three crop fields selected and/or physicochemical properties of the three pesticides tested. The pesticide residue levels in the medium- and small-sized tomatoes were 1.5 and 2.4 times higher than the level in large-sized tomatoes under similar spray conditions, whereas amount of pesticides adhered per unit surface area were approximately equal among all three sizes of tomatoes. The results of this study suggested that the differences in pesticide residue levels were due to differences in the degree of specific surface area of each tomato size. Resultant residue data of medium-sized tomatoes demonstrated a proportional relationship between pesticide residue levels and the specific surface area of tomatoes.
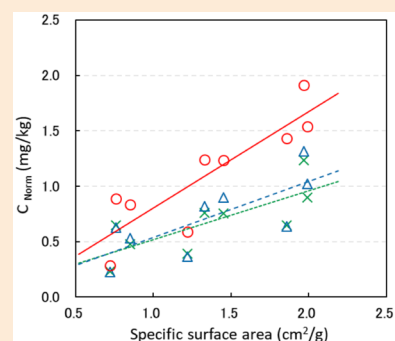
Introduction

The tomato is one of the most popular and widely grown vegetables in the world. According to the Food and Agriculture Organization of the United Nations,[1] approximately 182 million tons of tomatoes were produced globally in 2018, more than any other vegetable. Tomatoes of different sizes are consumed, from small tomatoes, called "cherry or mini tomatoes," to large tomatoes that are common in Japan. Therefore, many researchers have investigated and reported on the residue-forming tendencies of pesticides in tomatoes.[2–7] In 2017, the production ratio of mini tomatoes to the total tomato production in Japan was 16%.[8]

The amount of pesticide residue on the crop surface is affected by various factors, such as the physicochemical properties of pesticides, the nature of the crop surface, the growth stage, the crop leaf canopy, and the application method.[9] Furthermore, the commodity weight, size, and shape also play important roles in determining the residue levels. Several studies on the tomato reported that pesticide residue levels in small-sized fruits are significantly higher as compared to those of large-sized fruits.[4–7] However, few studies have comprehensively discussed the relationship between different tomato sizes and pesticide residue levels.

To investigate the relationship between pesticide residue levels and fruit size, crop field trials on large-, medium-, and small-sized tomatoes were simultaneously conducted under normal agricultural practice at three test fields in Japan. Three pesticides with a wide range of physicochemical properties were selected for the trials. Dinotefuran, a systemic neonicotinoid insecticide, has a relatively low n-octanol/water partition coefficient (log P_{OW}) of -0.549 and a high water solubility of 39.8 g/L.[10] Pyridalyl is an insecticide that has a relatively high log P_{OW} of 8.1 and a low water solubility of 0.15 μg/L.[10] Fludioxonil is a phenylpyrrole fungicide with an intermediate log P_{OW} of 4.12 and a water solubility of 1.8 mg/L.[10] The aqueous photolysis half-life of dinotefuran is 3.8 hr, whereas those of pyridalyl and fludioxonil are 3.6 and 10 days, respectively.[10] This study provides information valuable for understanding the relationship between different

tomato sizes and the surface formation/adherence of pesticide residues.

Materials and methods

1.　Field experiments
Field experiments on large-, medium-, and small-sized tomatoes were simultaneously conducted in January and February, 2018, at Ibaraki, Kochi, and Miyazaki greenhouses in accordance with the Japanese guidelines for crop field trials.[11] Cultivars of large-sized tomatoes were: *CF Momotaro Haruka*, *House Momotaro*, and *Momotaro Peace* in the Ibaraki, Kochi, and Miyazaki fields, respectively. The medium-sized tomato cultivar was *Red Ore* in all crop fields. Cultivars of small-sized tomatoes were *Pepe* in the Ibaraki field, and *Carol7* in the Kochi and Miyazaki fields.

Three pesticide formulations were mixed in a tank. Albarin® water-soluble granules of dinotefuran, 20.0% active ingredient (a.i.) (Mitsui Chemicals Agro, Tokyo, Japan); Savior® flowable 20 with fludioxonil, 20.0% a.i. (Syngenta Japan, Tokyo, Japan); and Pleo® flowable with pyridalyl, 10.0% a.i. (Sumitomo Chemical, Tokyo, Japan) were diluted with water in a ratio of 1:1000 (Savior® and Pleo®) or 1:2000 (Albarin®). These pesticide formulations were sprayed twice, using backpack sprayers connected to cone nozzles, with 7 to 8 days between applications, which were uniformly distributed on each tomato plant, including fruits. Notably, to meet the study objectives, the pre-harvest intervals (PHIs) after the final application and the number of pesticide applications were optimized rather than applying the maximum amounts of pesticide.

Samples of tomatoes were harvested at PHI of 0 (approximately 4 hr), 1, 3, 7, and 14 days. Each sample was harvested randomly from the test field, more than 2 kg for large and medium sizes, and more than 1 kg for small sizes. Samples were subsequently shipped to our institute by a commercial shipping service, and the temperature was maintained below 10°C.

2.　Sample preparation
The sample weight and size (diameter and length) of each tomato were measured at their receipt; thereafter, each stem was removed. Each sample was homogenized using a blender (Blixer-5 Plus; Robot Coupe, USA) to prepare the sample homogenate. These samples were preserved at −20°C until analysis.

3.　Residue analysis
An analytical method was optimized for the rapid analysis of each pesticide and applied to every tomato size, as described below.

3.1.　Chemicals and reagents
Analytical standards of dinotefuran (purity 99.8%), fludioxonil (purity 100%), and pyridalyl (purity 99.2%) were purchased from FUJIFILM Wako Pure Chemical (Japan). Pesticide analysis-grade acetone, acetonitrile, and methanol; LC-MS-grade methanol; and analytical-grade ammonium formate and formic acid were purchased from FUJIFILM Wako Pure Chemical. Water used in the experiments was purified using a PURELAB Flex-UV system (Veolia Water Technologies, France).

Standard stock solutions (200 mg/L) of each pesticide were prepared separately with acetone. An aliquot of the dinotefuran stock solution was diluted with methanol/water (1:4, v/v) to prepare standard solutions with concentrations ranging from 0.05 to 2 μg/L. Aliquots of the fludioxonil and pyridalyl stock solutions were mixed and diluted with methanol to prepare standard solutions with concentrations ranging from 0.05 to 2 μg/L. These standard solutions were used for preparing calibration curves.

3.2.　Extraction
A portion (20 g) of the homogenate sample was weighed into an Erlenmeyer flask and extracted with 100 mL of acetonitrile (dinotefuran) or acetone (fludioxonil and pyridalyl) by shaking for 30 min in a reciprocal shaker. The mixture was subsequently filtered by vacuum suction, and the residue cake was washed with 50 mL of the corresponding solvent and filtered again. The filtrates were combined, and the volume was made up to 200 mL with the respective solvent.

3.3.　Cleanup
3.3.1.　Dinotefuran
A portion of the acetonitrile extract (0.5 mL, equivalent to 0.05 g of the sample) was cleaned using a graphite carbon black cartridge (500 mg/6 mL, InertSep GC; GL Science, Japan). After adding 5 mL of 0.1% aqueous formic acid solution, the extract mixture was added to the cartridge, which was pre-conditioned sequentially with 5 mL of acetonitrile containing 0.1% formic acid and 0.1% aqueous formic acid solution. The cartridge was washed with 5 mL of acetonitrile/water/formic acid (20:80:0.1, v/v/v), and the eluate was discarded. Thereafter, 5 mL of acetonitrile/water/formic acid (40:60:0.1, v/v/v) was passed through the cartridge, and the eluate was collected. The eluate was evaporated in a water bath at 40°C, and the residue was dried under a low-flowrate nitrogen stream and subsequently dissolved in a suitable volume (5 to 50 mL) of methanol/water (1:4, v/v).

3.3.2.　Fludioxonil and pyridalyl
A portion of the acetone extract (1 mL, equivalent to 0.1 g of the sample) was cleaned using a graphite carbon black cartridge (pre-conditioned with acetone). The acetone extract was added to the cartridge. The cartridge was washed with 10 mL of acetone, and the eluate was discarded, after which 20 mL of acetonitrile/toluene (3:1, v/v) mixture was passed through the cartridge, and the eluate was collected. The eluate was evaporated in a water bath at 40°C, and the residue was dried under a low-flowrate nitrogen stream. The residue was dissolved in a suitable volume (10 to 250 mL) of methanol.

3.4.　LC-MS/MS analysis
A liquid chromatography (LC, ACQUITY UPLC I-Class pumping system; Waters, USA) and a tandem mass spectrometer (MS/MS, Xevo TQ-S Triple Quadrupole Tandem Mass Spectrometer; Waters) equipped with an electrospray interface operating in positive (for dinotefuran and pyridalyl) or negative (for fludioxonil) ion mode were used. The data were processed using MassLynx software (version SCN876/904, Waters). LC separa-

tion was performed on an ACQUITY UPLC CSH Phenyl-Hexyl column (100 mm×2.1 mm i.d., 1.7 µm; Waters) at 40°C.

The LC-MS/MS conditions for dinotefuran were as follows: methanol and 5 mmol/L aqueous ammonium formate solutions were used as the mobile phase at a flowrate of 0.3 mL/min. In the gradient elution analysis, the initial mobile phase consisted of 10% methanol (maintained for 1 min), after which the percent volume of methanol linearly increased to 30% for 3 min. The retention time was 3.1 min. The MS/MS parameters were as follows: capillary voltage, 0.6 kV; cone voltage, 20 V; desolvation temperature, 500°C; source temperature, 130°C; cone gas (nitrogen gas), 150 L/hr; desolvation gas (nitrogen gas), 800 L/hr; and the collision gas for MS/MS analysis was argon. The monitoring ion (precursor ion>product ion) in the multiple-reaction monitoring (MRM) mode had an *m/z* of 203.1>114.0 (collision energy 10 eV). An aliquot (2 µL) of the test solution was injected into the LC-MS/MS system.

The LC-MS/MS conditions for fludioxonil and pyridalyl were as follows: methanol and 5 mmol/L aqueous ammonium formate solutions were used as the mobile phase at a flowrate of 0.3 mL/min. In the gradient elution analysis, the initial mobile phase consisted of 50% methanol (maintained for 0.5 min), after which the percent volume of methanol linearly increased to 90% for 3 min (maintained for 3 min). The retention times for fludioxonil and pyridalyl were 3.2 min and 5.3 min, respectively. The MS/MS parameters were as follows: capillary voltage, 0.6 kV; cone voltage, 50 V for fludioxonil and 35 V for pyridalyl; desolvation temperature, 500°C; source temperature, 130°C; cone gas, 150 L/hr; desolvation gas, 800 L/hr, and collision gas (argon gas). The monitoring ions had an *m/z* of 247.0>179.9 for fludioxonil (collision energy 30 eV) and an *m/z* of 491.8>163.9 for pyridalyl (collision energy 35 eV). An aliquot (2 µL) of the test solution was injected into the LC-MS/MS system.

4. Data processing

To correct the differences in the application rates of the examined pesticides, the normalized residue levels (C_{Norm}) to an application rate (1 kg a.i./ha) were calculated from the measured residue data. The normalized factors derived from the application conditions (a.i. content, dilution factor, application volume, number of applications) for dinotefuran and pyridalyl ranged from 1.79 to 2.02, and the normalized factors for fludioxonil ranged from 0.90 to 1.01.

The half-life of the pesticide in each residue data was calculated by the following equation: $\ln (C_{Norm}(t_i)/C_H) = -kt$, where $C_{Norm}(t_i)$ is the residue level of pesticide at time t of PHI, C_H is the highest residue level of each pesticide, and *k* is the decline rate constant. The decline curve of the residue ratio ($C_{Norm}(t_i)/C_H$) to the PHI was generated by the linear regression curve, and the slope (denoted as *k*) of the linear curve was determined. Thus, the half-life ($T_{1/2}$) was calculated by the following equation: $T_{1/2} = \ln 2/k$.

Results

1. Validity of the analytical method

Results of the recovery and stability tests for large-, medium-, and small-sized tomatoes are summarized in Supplemental Table S1. The accuracy and precision of the analytical methods were evaluated by recovery tests, which were conducted using each size of tomatoes at more than two fortified levels, including the limit of quantification (0.01 mg/kg) and levels above the highest residue levels. The mean recoveries of the fortified samples measured in quintuplicate ranged from 80 to 115%, and their relative standard deviations were ≤15.3%. The selectivity of the analytical method was evaluated by analyzing duplicate blank samples harvested from each crop field. No interference peak was observed around the retention time for each pesticide in the chromatograms of the blank samples.

For the internal quality control of residue analysis, additional recovery samples (mixed with 0.1 mg/kg for each pesticide) and blank samples were simultaneously analyzed with every batch of test samples. All of the concurrent recoveries (*n*=36) were within the acceptable range (70 to 120%). No interference peak was observed around the retention time for each pesticide in chromatograms of simultaneous analysis results of blank samples.

Pesticide stability tests were performed for each test field sample (fortified at 0.5 mg/kg) stored for 30 (Ibaraki), 29 (Kochi), or 18 (Miyazaki) days at −20°C. All recoveries from the stability samples (*n*=54) were within the acceptable range of 70 to 120%.

These results indicated that the analytical methods applied to this study generated adequate data to meet the present study objectives.

2. Fruit size of examined tomatoes

Information pertaining to tomato sample measurements, such as unit weight, diameter, length (height), surface area, and specific surface area, is summarized in Table 1. The mean unit weights of tomatoes in this study ranged from 166 to 215 g for the large, from 34.6 to 40.5 g for the medium, and from 10.1 to 13.0 g for the small sizes. The values for the large- and small-sized tomatoes were within the weight range reported for tomato samples in Korea[4] and Italy[6,7] (large-sized tomato, 96 to 307 g; small-sized tomato, 9.6 to 19 g). The weights of the medium-sized tomatoes were between the weights of the large- and the small-sized tomatoes, which were adequate for this study.

The cultivars in this study were empirically selected with different residue levels in the three tomato types. The mean diameters of tomatoes ranged from 7.9 to 8.0 cm for the large, from 4.1 to 4.3 cm for the medium, and from 2.6 to 2.8 cm for the small sizes. The mean lengths (heights) of tomatoes ranged from 5.8 to 6.2 cm for the large, from 3.6 to 3.8 cm for the medium, and from 2.7 to 2.9 cm for the small sizes. The mean density of the tomatoes calculated from the fruit diameter, length, and unit weight was 1.09, indicating that each measured value was appropriate. The shape of each tomato was predominately flattened for the large, slightly flattened or circular for the medium, and cir-

Table 1. Information pertaining to the harvested tomatoes

Fruit size, test field	Unit weight, g[a]	Diameter, cm[a]	Length, cm[a]	Surface area, cm²[b]	Specific surface area, cm²/g[c]
Large,					
Ibaraki	166	8.0	5.8	148	0.89±0.05
Kochi	215	8.0	6.2	158	0.74±0.02
Miyazaki	200	7.9	5.9	151	0.76±0.01
Medium,					
Ibaraki	35.3	4.3	3.7	51.3	1.45±0.04
Kochi	40.5	4.3	3.8	51.3	1.27±0.04
Miyazaki	34.6	4.1	3.6	46.8	1.36±0.03
Small,					
Ibaraki	10.1	2.6	2.7	21.6	2.14±0.22
Kochi	13.0	2.8	2.9	25.7	1.98±0.09
Miyazaki	12.6	2.8	2.8	24.8	1.97±0.06

[a] Mean values of unit weight and size.
[b] Surface area calculated by the formula of $4\pi r^2$, where fruit radius (r) is half of mean of diameter and length, assuming each fruit is a sphere.
[c] Specific surface area (±standard deviation) calculated as surface area divided by unit weight.

cular or elliptical for the small sizes.[12] The differences among tomatoes from the three test fields in shape, weight, and diameter for each fruit size were insignificant.

The specific surface area of each tomato, assumed as a sphere, was calculated using the mean of the diameter and length of fruits. The calculated specific surface area of tomatoes ranged from 0.74 to 0.89 cm²/g for the large, from 1.27 to 1.45 cm²/g for the medium, and from 1.97 to 2.14 cm²/g for the small sizes. No difference in fruit surface texture was observed for the tomatoes when examined visually. These tomatoes could be considered to be representative commodities, which can be obtained from the local agricultural market and were appropriate test samples for this study.

3. Residue data
The pesticide residue data are summarized in Supplemental Table S2. The highest residue levels measured for dinotefuran, fludioxonil, and pyridalyl were 0.62, 1.32, and 0.96 mg/kg, respectively, obtained from the small-sized tomatoes of Ibaraki. Although each tomato sample was harvested approximately 4 hr after the final application, residue levels determined in this study

were lower than the maximum residue levels (MRLs) specified by the Japanese Food Sanitation Law[13] for tomatoes (dinotefuran, 2 mg/kg; fludioxonil, 5 mg/kg; pyridalyl, 5 mg/kg). The lowest residue levels for dinotefuran, fludioxonil, and pyridalyl were 0.10, 0.24 (PHI of 14 days), and 0.15 mg/kg (PHI of 7 days), respectively, obtained from the large-sized tomatoes of Kochi. All three pesticides examined in this study were detected in all test samples obtained from the three crop fields.

Although it is difficult to specify the cultivation conditions of tomatoes, the residue level and declining tendency of fludioxonil in this study were similar to those reported by Garau[3] but higher than the result reported by Cabizza.[6] There were no prior studies for dinotefuran and pyridalyl.

Pesticide residue data in this study varied due to differences in the three crop fields and physicochemical properties of the three pesticides. It is well known that the pesticide residue data varies with different crop fields[9,14] and the physicochemical properties of pesticides. Therefore, all residue data were used without rejection to evaluate the adherence potency of pesticides on different sizes of tomatoes, as discussed in the following section.
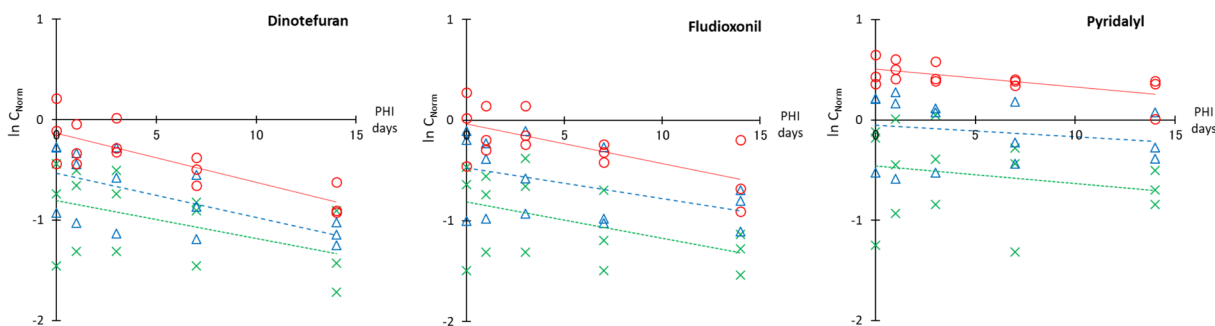
Fig. 1.   Plots of normalized residue levels of dinotefuran, fludioxonil, and pyridalyl in small- (○: solid line), medium- (△: dashed line), and large- (✕: dotted line) sized tomatoes *versus* days of PHI

Table 2. Half-life (in days) of pesticides estimated for each residue data of tomato sizes and crop fields

Test field, fruit size	Dinotefuran	Fludioxonil	Pyridalyl
Ibaraki			
large	13 (R^2=0.880)	17 (R^2=0.786)	27 (R^2=0.561)
medium	11 (R^2=0.935)	18 (R^2=0.861)	108 (R^2=0.335)[a]
small	9 (R^2=0.906)	10 (R^2=0.932)	32 (R^2=0.885)
Kochi			
large	28 (R^2=0.727)	62 (R^2=0.348)[a]	NA[b]
medium	35 (R^2=0.799)	NA[b]	NA[b]
small	22 (R^2=0.846)	72 (R^2=0.293)[a]	235 (R^2=0.707)
Miyazaki			
large	19 (R^2=0.794)	12 (R^2=0.675)	18 (R^2=0.721)
medium	14 (R^2=0.904)	11 (R^2=0.851)	17 (R^2=0.844)
small	21 (R^2=0.656)	18 (R^2=0.624)	24 (R^2=0.816)

The half-life ($T_{1/2}$) was calculated by the following equation: $T_{1/2}=\ln 2/k$. Where the decline curve of the normalized residue level (determined as Ct_{norm}/C_H) to the PHI was generated by the linear regression analysis, and the slope of them was determined as the decline rate constant of k.
[a] Reference value because of no significant correlation.
[b] Not analyzed because of poor decline tendency.

Discussion

1.   Comparing the tendency of residue formation by pesticide

In general, the pesticide residue level depends on its application rate.[2,5] To negate the influence of the different application rates of the examined pesticides, the residue levels normalized to 1 kg a.i./ha were calculated and used to evaluate the residue tendency in the subsequent discussion (Supplemental Table S2). Among the three pesticides, the highest normalized residue levels were observed for pyridalyl, when comparing same-sized tomatoes from each crop field. The total amount of a.i. for pyridalyl was half that of fludioxonil, and equal to that of dinotefuran. This result indicated that the highest adhesion potency was observed for the non-polar pesticide pyridalyl among the three examined pesticides.

Figure 1 shows plots of the normalized residue levels of dinotefuran, fludioxonil, and pyridalyl in small-, medium-, and large-sized tomatoes *versus* the days of PHI. The decline curves in the figure present differences in the declining tendency of each pesticide in different-sized tomatoes. For all three pes-

ticides, the residue level was in the descending order of small->medium->large-sized tomatoes based on pesticide evaluation. The differences in the y-intercepts among the three decline curves generated for each fruit size were relatively high for pyridalyl, which was the most non-polar among the three examined pesticides. On the other hand, the difference in the decline curves of dinotefuran, which was the most polar of the three examined pesticides, was relatively low, particularly between those for medium- and small-sized tomatoes.

The decline tendency of residue levels for dinotefuran clearly showed a decrease with days of PHI for all sizes and crop fields (R^2>0.6, Table 2). From the result, the degradation and/or dissipation rate of dinotefuran was considered to be relatively higher than those of the other two pesticides. On the other hand, the declining tendencies of fludioxonil and pyridalyl were not evident in three of the nine residue datasets.

2.   Comparison of residue tendency by crop field

Figure 2 presents the normalized residue level plot of pesticides in different sizes of tomatoes *versus* days of PHI at each
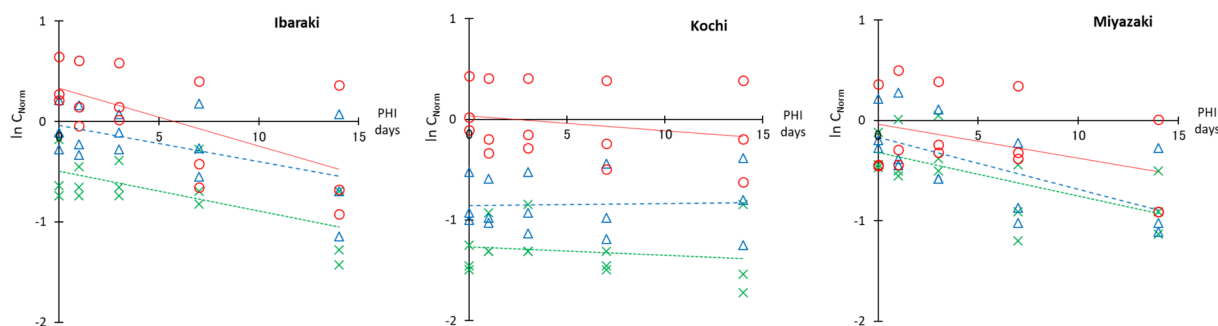
Fig. 2.   Plots of normalized residue levels of pesticides in small- (○: solid line), medium- (△: dashed line), and large- (×: dotted line) sized tomatoes *versus* days of PHI at Ibaraki, Kochi, and Miyazaki fields

Table 3. Statistical significance of the residue levels in large-, medium-, and small-sized tomatoes under the same cultivation conditions

	PHI, days				
	0	1	3	7	14
Dinotefuran	NS (3.82)	NS (4.73)	NS (4.62)	NS (4.66)	NS (4.54)
Fludioxonil	NS (4.36)	NS (5.07)	NS (4.62)	NS (4.36)	* (6.01)
Pyridalyl	* (6.01)	NS (5.96)	NS (5.96)	* (6.49)	* (6.54)

Result of statistical analysis by the Kruskal–Wallis test ($p<0.05$, $\chi^2_{0.05}=5.99$) is expressed as NS, no significant difference or *, significant difference. Estimated statistic KW value is expressed in parentheses.

crop field. This figure shows the differences in pesticide residue tendencies in small-, medium-, and large-sized tomatoes, depending on the cultivation conditions at each crop field. The pesticide residue levels in large- and medium-sized tomatoes from the Miyazaki field were similar. This tendency is probably because the pesticide residue levels in large-sized tomatoes in the Miyazaki field samples were comparatively higher than those in the other two crop fields. Except for the aforementioned example, the residue level was evidently in a descending order of small->medium->large-sized tomatoes based on field evaluations.

The declining tendency of pesticide residue levels in the Kochi samples was obscure as compared to those of the other two crop fields. Although the half-life of dinotefuran can be determined with relatively higher correlations for every tomato size from Kochi, there are no significant correlations to estimate the half-life of the other two pesticides (Table 2). The half-lives of dinotefuran calculated from residue datasets of the Kochi field (22 to 35 days) were evidently longer than those of the Ibaraki and Miyazaki fields (9 to 21 days). The influence of a leaf canopy (preventing sunlight exposure) and/or the slow growth of tomatoes may explain this incomprehensible declining tendency on the residue datasets from the Kochi field.

3. Comparison of residue data in large-, medium-, and small-sized tomatoes

For the residue data among the large-, medium-, and small-sized tomatoes, the results of the statistical analysis by the Kruskal–Wallis test ($P<0.05$) varied for each pesticide (Table 3). For dinotefuran, the difference was insignificant in each residue data of the three tomato sizes at all PHIs. For fludioxonil, differences were insignificant in the residue data at four of five PHIs (except for PHI of 14 days). For pyridalyl, the difference was insignificant in the residue data at 1 and 3 days of PHI but was observed in the residue data at 0, 7, and 14 days of PHI.

Ratios of the residue levels in medium- and small-sized tomatoes to those in the large-sized tomatoes are calculated and summarized as the mean of the three fields and pesticides in Table 4. The mean ratios of pesticide residue levels in small-sized tomatoes to those in large-sized tomatoes ($n=15$) ranged from 1.4 (Miyazaki) to 3.6 (Kochi), and the overall mean ratio was 2.4. These results were within the reported range of residue level ratios in tomatoes between large and small sizes (maximum ratio: 7 times).[4–7] The mean ratios of residue levels in medium-sized tomatoes to those in large-sized tomatoes were 1.1 (Miyazaki) and 1.6 (Ibaraki and Kochi), and the overall mean ratio was 1.5. All mean ratios for small-sized tomatoes were higher than the ratios for medium-sized tomatoes. From this study, an MRL of pesticides may be evaluated for tomatoes regardless of their size. The widest range of residue levels for the examined pesti-

Table 4. Effect of the unit size of tomato fruits on pesticide residues

	Residue level[a]		Amount per surface area[b]	
	Medium/Large	Small/Large	Medium/Large	Small/Large
Mean ratio on field base ($n=15$)				
Ibaraki	1.6±0.2	2.2±0.5	1.0±0.1	0.9±0.2
Kochi	1.6±0.3	3.6±0.9	0.9±0.2	1.4±0.3
Miyazaki	1.1±0.2	1.4±0.4	0.6±0.1	0.5±0.1
Mean ratio on pesticide base ($n=15$)				
Dinotefuran	1.3±0.2	2.0±0.9	0.8±0.2	0.8±0.3
Fludioxonil	1.5±0.3	2.4±1.1	0.9±0.2	0.9±0.4
Pyridalyl	1.6±0.4	2.8±1.3	0.9±0.2	1.1±0.5
Overall mean ratio ($n=45$)				
	1.5±0.3	2.4±1.1	0.9±0.2	0.9±0.4

[a] Ratio of pesticide residue levels in small- or medium-sized tomatoes to the levels in large size fruits.
[b] Ratio of pesticide residue amounts per surface area on small- or medium-sized tomatoes to the amounts on large size fruits.
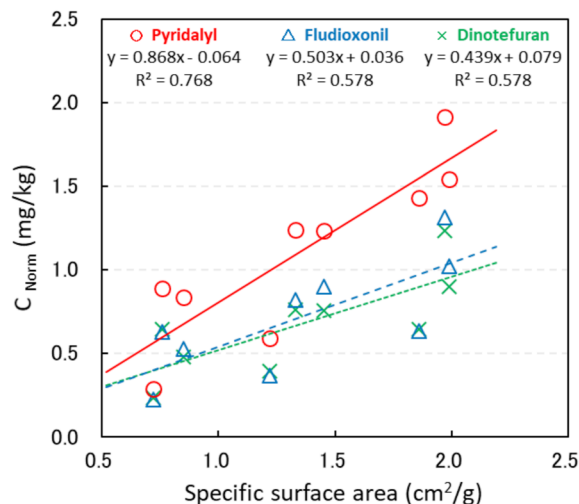
Fig. 3. Plots of normalized residue levels (to application rate=1 kg a.i./ha) of dinotefuran (×), fludioxonil (△), and pyridalyl (○) *versus* specific surface area of tomatoes harvested on the day of final application (PHI of 0 days)

cides in this study was within five times, which is an index of the CODEX MRLs in estimating residue levels for the same commodity group.[15]

4. Effect of the specific surface area on pesticide residue levels

Theoretically, residue data obtained from the samples immediately harvested after the final application would have a minimal effect on pesticide loss (decomposition and/or volatilization) and dilution (growth dilution of fruits). Therefore, the residue data obtained immediately after pesticide application is the best dataset for evaluating the adherence potency of pesticides sprayed on different sizes of tomatoes. Figure 3 shows the relationship between pesticide residue levels and specific surface areas of tomatoes, obtained from the residue data of harvested tomatoes on the final day of pesticide application (PHI of 0 days). All three pesticides had good correlations between the

specific surface area and residue levels ($R^2>0.57$). These results implied that the difference in residue levels of different-sized tomatoes was primarily due to the difference in their specific surface area.

Table 5 summarizes the parameters of the regression curves between the specific surface areas of tomatoes harvested at 0, 1, 3, 7, and 14 days of PHI, and the residue levels of three pesticides. Except for the residue data at 14 days of PHI for dinotefuran and fludioxonil, the specific surface area of the tomatoes and the pesticide residue levels were highly correlated ($R^2>0.51$). The slope of the regression curve of each pesticide corresponded to the adherence potency of pesticides, in the order of pyridalyl >fludioxonil>dinotefuran. In addition, each slope of the regression line for each pesticide to PHI corresponded to the declining tendency of pesticides. The results demonstrated that the variations in the amounts of pesticides adhering to tomatoes depended on differences in the polarity of each pesticide.

5. Comparison of the amounts of pesticides adhering to tomatoes

The adhered amounts calculated per unit surface area of tomatoes are summarized in Supplemental Table S3, whereas the ratios of the values obtained from medium- and small-sized tomatoes to the large-sized tomatoes are summarized in Table 4. The overall mean ratios of the amounts of pesticide residues per unit surface area calculated for small- and medium-sized tomatoes to the large-sized tomatoes were similar at 0.9. This result indicated that differences in adhered amounts per unit surface area were approximately equal in every size of tomatoes except for the following partial exceptions. On the residue dataset in the Miyazaki samples, the ratios of the pesticide residue amounts per unit surface area of medium- and small-sized tomatoes to the large-sized tomatoes were 0.6 and 0.5, respectively. These were evidently smaller than the above-mentioned overall mean value (0.9), since the difference in pesticide residue levels was minimal in different tomato sizes from the Miyazaki field. On the contrary, in the residue data from the Kochi field, the ratio of the pesticide residue amount per unit surface area of small-sized to-

Table 5. Proportionality relational expression between pesticide residue levels and the specific surface area of tomato fruits

PHI	Dinotefuran	Fludioxonil	Pyridalyl
0 days	$y=0.439x+0.079$	$y=0.503x+0.036$	$y=0.868x-0.064$
	$R^2=0.578$	$R^2=0.578$	$R^2=0.768$
1 day	$y=0.286x+0.216$	$y=0.423x+0.083$	$y=0.870x-0.057$
	$R^2=0.512$	$R^2=0.652$	$R^2=0.761$
3 days	$y=0.350x+0.115$	$y=0.406x+0.107$	$y=0.758x+0.008$
	$R^2=0.577$	$R^2=0.562$	$R^2=0.713$
7 days	$y=0.105x+0.093$	$y=0.328x+0.079$	$y=0.736x-0.069$
	$R^2=0.626$	$R^2=0.650$	$R^2=0.857$
14 days	$y=0.059x+0.094$	$y=0.213x+0.134$	$y=0.567x+0.060$
	$R^2=0.433$[a]	$R^2=0.440$[a]	$R^2=0.879$

PHI: pre-harvest interval days after the final application.
[a] Reference value because of no significant relationship.

matoes to that in large-sized tomatoes was 1.4, which was higher than the overall mean value. This difference in pesticide residue levels was largely dependent on the fruit size of the tomatoes in the Kochi sample.

On the other hand, the difference based on the pesticide evaluation was insignificant. The ratio of the pesticide residue amounts per unit surface area for small- and medium-sized tomatoes to large-sized tomatoes, calculated for each pesticide, ranged from 0.8 to 1.1. These results indicated that the differences in adhered amounts per unit surface area were approximately equal in three pesticides with a wide range of physicochemical properties.

The test conditions of this study were designed to compare the pesticide residue formation in fruits of different sizes. In particular, the test conditions in each crop field were carefully maintained, all tomato plants were simultaneously cultivated in the same greenhouse, and pesticides were applied with a tank mix using the same sprayer. The comparison of the residue data for each field was expected to appropriately reflect the adhesion behavior of each pesticide according to the tomato fruit size. However, the variations in the cultivation fields[15] strongly affected the residue tendencies of pesticides for tomatoes as compared to the variations in the physicochemical properties of pesticides. These results suggested that it is necessary to consider the effect of the plant growth stage and/or the leaf canopy of crops because of variations in the cultivation fields on the evaluation of adhesion potency of pesticide residues in tomatoes.

Conclusions

In conclusion, the residue level in each tomato increased in the order of small->medium->large-sized tomatoes, whereas the residue amounts per unit surface area of tomatoes were approximately equal. In addition, for the three pesticides, there were good correlations between the specific surface area and residue levels in all tomatoes. These results indicated that pesticide residue levels in tomatoes may have a proportional relationship with the specific surface area of each tomato when pesticides are sprayed in similar conditions. The differences in pesticide residue levels may have been caused by a difference in the degree of the specific surface area of each tomato. The pesticide residue data of this study, including medium-sized tomatoes, demonstrated a proportional relationship between pesticide residue levels and various sizes of tomatoes. This result may be utilized to determine the relationship among residue data in various tomato sizes and may be useful in evaluating various residue data in tomatoes.

Electronic supplementary materials

The online version of this article contains supplementary materials (Supplemental Table S1, S2 and S3), which are available at https://www.jstage.jst.go.jp/browse/jpestics/.

References

1) http://www.fao.org/faostat/en/#data (accessed December 10, 2020)
2) S. Sadło: Quantitative relationship of application rate and pesticide residues in greenhouse tomatoes. *J. AOAC Int.* **83**, 214–219 (2000).
3) V. L. Garau, A. Angioni, A. A. Del Real, M. Russo and P. Cabras: Disappearance of azoxystrobin, pyrimethanil, cyprodinil, and fludioxonil on tomatoes in a greenhouse. *J. Agric. Food Chem.* **50**, 1929–1932 (2002).
4) H. Kwon, J. Kim, H. Lee, Y. Ihm, K. Kyung, I. Park and J. Choi: Estimate of pesticide residues in tomato varieties using ratio of surface area to weight. *Korean. J. Pestic. Sci.* **8**, 30–37 (2004) (in Korean).
5) D. J. Maclachlan and D. Hamilton: A new tool for the evaluation of crop residue trial data (day-zero-plus decline). *Food Addit. Contam., Part A* **27**, 347–364 (2010).
6) M. Cabizza, F. Dedola and M. Satta: Residues behavior of some fungicides applied on two greenhouse tomato varieties different in shape and weight. *J. Environ. Sci. Health B* **47**, 379–384 (2012).
7) F. Dedola, M. Cabizza and M. Satta: Determination of 28 pesticides applied on two tomato cultivars with a different surface/weight ratio of the berries, using a multiresidue GC-MS/MS method. *J. Environ. Sci. Health B* **49**, 671–678 (2014).
8) https://www.maff.go.jp/j/tokei/ (accessed December 10, 2020) (in Japanese)
9) Food and Agriculture Organization of the United Nations: "FAO Plant Production and Protection Paper 197, Submission and Evaluation of Pesticide Residues Data for the Estimation of Maximum Residue Levels in Food and Feed," Rome, Italy, 2009.
10) C. D. S. Tomlin (eds.): "The Pesticide Manual 18th Edition" British Crop Protection Council, Hampshire, U.K., 2018.
11) OECD Test Guideline No. 509, Crop Field Trial; The Organization for Economic Co-operation and Development, Paris, France, 2009.
12) http://www.hinshu2.maff.go.jp/info/sinsakijun/kijun/1392.pdf (accessed December 10, 2020) (in Japanese)
13) https://db.ffcr.or.jp/front/ (accessed December 10, 2020) (in Japanese).
14) T. Yajima, M. Fujita, K. Kondo, K. Iijima, K. Sato and Y. Kato: Factors affecting field-to-field variation in pesticide residue levels in Chinese cabbage and spinach. *J. Pestic. Sci.* **38**, 200–207 (2013).
15) http://www.fao.org/fileadmin/templates/agphome/documents/Pests_Pesticides/JMPR/Report13/JMPR_2013_Report__2013__web__.pdf (accessed December 10, 2020)