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# Uptake of Cyantraniliprole into Tomato Fruit and Foliage under Hydroponic Conditions: Application to Calibration of a Plant/Soil Uptake Model

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Supporting Information

**ABSTRACT:** Measured uptake of cyantraniliprole (3-bromo-1-(3-chloro-2-pyridinyl)-*N*-[4-cyano-2-methyl-6-[(methylamino)-carbonyl]phenyl]-1*H*-pyrazole-5-carboxamide) into tomatoes following hydroponic exposure allowed calibration of a novel soil uptake model. The total mass of plant parts in treated plants was derived from the weights of successively harvested control plants (no cyantraniliprole provided) over 18 days following the first sampling of ripe tomatoes. Transpired water measured during plant growth was coupled with the calculated increase in plant mass to determine a transpiration coefficient constant (L/kg plant fresh weight) for use in the model. Cyantraniliprole concentrations in mature fruit, fresh foliage, and plant uptake solutions were used as the basis for a nonlinear least-squares optimization that consistently resolved to values that were empirically valid compared to metabolism studies in whole plants. This calibrated reference model adequately described uptake from soil pore water into plant fruit, and served as the basis for describing residues in fruit following commercial greenhouse growing conditions.

**KEYWORDS:** *crop protection, residue prediction, plant uptake, optimization*

## INTRODUCTION

Crop Protection Product (CPP) national registrations and international trade require magnitude of residue data for treated crop commodities.[1−3] These data are used to assess human dietary risk and establish legal limits (maximum residue limits, MRLs) for traded produce. Typically, magnitude of residue field trials are required prior to obtaining national permissions for use of a particular crop protection chemical. However only when the residue data are obtained is a registrant able to assess dietary exposure considerations with actual residues versus toxicological end points. The ability to predict residues based on a more limited data set prior to conduct of these supervised residue trials affords business value in order to foretell any dietary risk considerations that might hinder the total market share for a specific chemical. In addition, the ability to model residues under different use scenarios other than those tested during supervised residue trials can provide flexibility in predicting residues to meet specific regulatory needs or secondary regulatory needs.[4]

Previously, we had evaluated the utility of predicting residues following foliar application of the crop protection chemical.[5] These prediction techniques utilized geometrical considerations of the treated produce, growth characteristics of the produce, and an understanding of the level of chemical deposited following application. This predictive tool requires the calibration of the model using actual residue data, which allows for empirical determination of mass fluctuations in chemical residues during plant growth.

Availability of the soil uptake model utilized in this current work to predict uptake into plants based on physical parameters and simple lab experiments could also have the potential of guiding initial pesticide development. Direct application of the crop protection chemical to the soil or root zone, rather than to the foliage, is gaining popularity.[6] For example, efficacious soil and/or root zone delivery imparts advantage by avoiding unnecessary wetting of plant surfaces.[7] Thus, this application mode is important in regions where strict water use is necessary for the economical production of the crop, or where the grower desires to minimize any operator or worker exposure to the crop protection chemical. This type of direct soil application can occur in a number of ways, such as drip irrigation, flooding, or similar means. Modeling of uptake of residues into the plant following soil application, however, is inherently more difficult than modeling residues following foliar application. One must consider a host of factors other than deposition, plant growth, and plant metabolism. These include the chemical's behavior in the soil, concentration in soil water, movement across root surfaces in the plant, and movement in the plant until eventually residues appear in the commodities of interest.[8]

The soil uptake model developed originally by Trapp and since modified into the cascade model is a useful model to evaluate for predicting plant uptake of residues from soil.[9,10] The current available model is a Microsoft Excel version of a dynamic mathematical model that predicts uptake of neutral chemicals by roots and translocation to leaves and fruit. Data

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input requirements are extensive. Some inputs are available as a result of work conducted for regulatory studies for crop protection products, while others have been calculated or assumed. In the present manuscript we attempt to use a simple greenhouse/laboratory plant uptake model to obtain, empirically, these previously calculated or assumed inputs to the cascade model to better evaluate the model's utility in crop protection product development decisions.

Cyantraniliprole is a novel crop protection chemical that has physical properties that make it an attractive candidate for drip irrigation and other direct soil application practices. Specifically, DuPont measurements of an acid dissociation constant ($pK_a$) of 8.8 and a lipophilicity ($\log K_{ow}$) of 1.94 for cyantraniliprole at 20 °C are collectively indicative of xylem mobility.[8] Thus, this active ingredient is an appropriate test compound to use for model calibration. Furthermore, anticipated use of cyantraniliprole for soil application provides the opportunity of future utility in addition to a more theoretical application for a variety of crops, including tomatoes.

Tomatoes have commercial systemic application uses such as drip irrigation, are an extensively traded commodity, and represent a crop in which cyantraniliprole is effective against a number of insect pests.[11,12] Tomatoes were chosen for the preliminary model studies based on the availability of metabolism data and residue data generated under controlled, greenhouse conditions using systemic delivery of cyantraniliprole. Following the outcome of the "reference model" experiments under hydroponic conditions, subsequent greenhouse "evaluation tests" were identified to potentially build on the reference model knowledge as a means to gain insight into chemical uptake and movement within the plant under independent conditions.

## MATERIALS AND METHODS

**Plant Experimental Model.** Tomato plants used in these experiments were Pixie Hybrid II, an orange fruited variety of tomatoes (*Solanum lycopersicum*). Plants are 46 cm tall at maturity and typically bear 3 cm yellow-orange fruits with meaty orange flesh within 52 days of planting.

**Test Plant Growth, Treatment, and Sampling.** Plants were germinated in Grodan rock wool (C. J. Klep B. V., Etten-Leur, The Netherlands) prior to transplanting into Penn Perlite TCBP perlite growth medium (Pennsylvania Perlite Corporation, Bethlehem, PA, USA). Plants were watered twice daily with a modified Hoaglund's solution [ 0.4 $NH_4H_2PO_4$; 2.4 $KNO_3$; 1.6 $Ca(NO_3)_2$; 0.8 $MgSO_4$; 0.1 $Fe^{3+}$ as Fe-chelate; 0.023 $B^{3+}$ as $B(OH)_3$ [boric acid]; 0.0045 $Mn^{2+}$ as $MnCl_2$; 0.0003 $Cu^{2+}$ as $CuCl_2$; 0.0015 $Zn^{2+}$ as $ZnCl_2$; 0.0001 $Mo^{6+}$ as $MoO_3$ or $(NH_4)_6Mo_7O_{24}$; $Cl^-$ as chlorides of $Mn^{2+}$, $Zn^{2+}$, and $Cu^{2+}$ (all concentrations in units of mmol/L); providing 200 mg/L nitrogen]. Plants were watered with this solution as described until flowering commenced. Control plants continued to receive an unamended solution as described, while treated plants received nominally 0.4 mg/L or 3 mg/L cyantraniliprole (in the modified Hoaglund's solution) as derived through inclusion of suspension concentrate formulated product (DuPont Crop Protection Cyantraniliprole 200 g/L SC, see Figure 1). The pH of the prepared control and cyantraniliprole treatment solutions were adjusted from initial pH values of approximately 7 to approximately pH 5.8 through addition of sulfuric acid, to maintain cyantraniliprole stability over the course of its administration to the plants. Samples of the treatment solution were retained for analysis to verify the applied dose. Calcium (100 mg/L) was added to the treatment solution of both control and cyantraniliprole treated plants to remedy an incidence of blossom end rot found in cyantraniliprole-treated tomatoes prior to the tomatoes maturing.

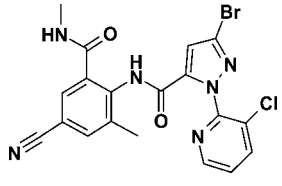


**Figure 1.** Chemical structure of cyantraniliprole.

The entire set of experimental plants included four each at both the low and high (replicates 1 and 2) cyantraniliprole treatment levels, and four untreated control plants. Temperature and relative humidity were monitored throughout the study by means of an automated data collection system (Argus Control Systems, Ltd., White Rock, BC, Canada, running Argus Control Software Firmware Version 12.19). Set points of 24 to 27 °C were imposed on the greenhouse, with supplemental temperature reduction achieved through use of evaporative cooling as well as through the use of 50% shade cloth.

When the initial fruit set became ripe (day 0), entire mature fruit and representative foliage samples were taken nonsystematically from each set of 4 plants comprising a cyantraniliprole treatment group. All fruit and foliage samples per treatment regimen were combined at each sampling point to enable an average representation of residues per treatment. This approach is consistent with the targeted business utility of the model such as to assess likelihood of residue value compliance with MRLs, where official compliance-testing methods are focused on the average residues of a produce lot.[13]

Thereafter and similarly, the entire amount of treated ripe fruit and a corresponding portion of the available foliage from representative areas of each plant were sampled on a weekly basis. Fruit collection continued for four further sampling periods (days 7, 14, 21, and 28). Applications of cyantraniliprole to replicate 2 of the high treatment group were discontinued following the day 14 sampling to allow for the potential dissipation of residues.

One control plant was sampled at the beginning, one at the end, and one at each of two other times evenly distributed within the sampling range of the treated plants. Specifically, at each sampling point of control plants (days 0, 9, 18, and 28 days) following collection of the initial ripe tomato samples, one entire plant was removed and separated into ripe fruit, unripe fruit, stem, and leaves. The weights of fruit and foliage were obtained and recorded, as well as the number of fruits obtained. Control plant part weights (days 0, 9, 18, and 28) were thus available for use to derive the total mass of treated plant parts (days 0, 7, 14, 21, and 28) by interpolation, as needed.

At the fifth ripe treated fruit collection (day 28 after start of sampling), the entire plant from each treatment regime was sampled by cutting the stem at the perlite surface and separating each plant into immature fruit, mature fruit, leaves, and stems. Like samples from like treatment scenarios were combined, weighed, and frozen.

The volume of added solution, as well as that which eluted out of the bottom of each of the pots during watering (pass-through water), was measured. A sample of the treatment solution and the pass-through water was individually collected and analyzed for cyantraniliprole levels during the first three weeks of exposure of the plants. A separate set of pots to determine water requirements in the absence of growing plants was established for pots containing only perlite. For both perlite-containing pots without growing plants and those with growing plants, the volume of treatment solution was administered until seepage out of the bottom of the pot was visible. Difference in the net volume retained for both the plant-containing and perlite-only containing pots allowed determination of the volume of treatment solution transpired by each plant.

**Independent Evaluation Tests: Test Plant Growth, Treatment, and Sampling.** Independently, residue data from commercially grown greenhouse grown tomato plants were used for comparison with residue values determined with the model; data was obtained from two test sites in The Netherlands. Characteristics of the test varieties, conditions, and sampling can be found in the Supporting Information.

## SAMPLE ANALYSIS

**Reagents and Analytical Standard Materials.** All reagents and solvents used were obtained from commercial sources, except the analytical standards. Analytical standard of cyantraniliprole was synthesized by DuPont Crop Protection, Global Technology Division, E. I. du Pont de Nemours and Co.

**Extraction and Sample Preparation.** The entire collected sample from the reference model experiment was frozen after collection, and was maintained at nominally −20 °C prior to homogenization under cryogenic (dry ice) conditions using a Robot Coupe commercial food processor (Robot Coupe USA, Inc., Scientific Industrial Division, Ridgeland, MS, USA), according to the manufacturer's instructions. Samples were removed from the freezer and approximate 1 g samples weighed into individual 25 mL plastic sample vials. The samples were thawed at room temperature for approximately 1 h, at which time three 0.95 cm diameter carbon steel beads were added to each sample vial. Vials were placed on a 2000 Geno/Grinder high-speed extractor (SPEX CertiPrep, Inc., Metuchen, NJ, USA) for 1 min at 1500 beats per min (bpm), after which time exactly 5 mL of high-performance liquid chromatography (HPLC) grade acetonitrile was added to each vial prior to continued grinding for 1 min at 1500 bpm. Approximately 2 mL of each extract was removed and placed into a Bio Plas siliconized polypropylene 2.0 mL conical microcentrifuge tube (Bio Plas, Inc., San Rafael, CA, USA) and centrifuged for 5 min at 14000 rpm on an Eppendorf 5415C microcentrifuge (Eppendorf, Enfield, CT, USA) . Tomato fruit and stem sample vials were capped with rubber septa for direct analysis from the centrifuge tube. An aliquot volume of 20 $\mu$L for each leaf extract was mixed with HPLC-grade acetonitrile to a volume of 2000 $\mu$L to achieve a 100× dilution. The untreated blank leaf matrix was processed in the same manner prior to preparing the analytical standards.

Tomato fruit samples from independent (Netherlands glass house) evaluation tests were similarly macerated while frozen, while mixing extensively during the grinding process to ensure homogeneity. The dry ice was allowed to sublime, and then a 10 g subsample was transferred into a 50 mL plastic centrifuge bottle and extracted with acetonitrile/water, 9/1, v/v. Extracts were semipurified by chromatography on a Varian PN12256013 strong anion exchange (SAX) solid phase extraction (SPE) cartridge with elution with solvent of acetonitrile/water composition.

**Sample Fortifications.** A recovery assay was conducted for each matrix per sample set from final harvest with two levels to bracket the respective residues in the treated plant parts and thereby evaluate the efficiency of the analytical procedures. Samples of untreated tomato matrices (stems, fruit, and leaves) were fortified with cyantraniliprole standard solution to obtain nominal levels of 0.05 and 3.0 mg/kg (fruit), 0.5 and 30 mg/kg (stems), and 5.0 and 300 mg/kg (leaves). The fortified samples were then processed according to the extraction procedure for each matrix.

For the independent (Netherlands glass house) evaluation tests, untreated control tomato fruit samples were fortified with 0.010−1.0 mg/kg cyantraniliprole to allow subsequent analysis concurrently with the treated samples to verify analytical method performance.

**Preparation of Analytical Standards.** Intermediate stock solutions in acetonitrile were used to prepare analytical standards in matrix (control plant extracts) in HPLC vials containing limited volume inserts. Standards were prepared in blank matrix to compensate for any matrix effects during the analysis. Standards ranging in concentration from 0.0005 mg/L to 5 mg/L were prepared in this manner.

Analytical standard solutions of cyantraniliprole were similarly prepared for use in the analysis of the independent evaluation tests while using methanol:water (50:50 v/v) as the diluent to cover the residue levels from 0.10 to 20 ng/mL.

**Analytical Methods.** *Analysis of Stock Hydroponic Solution and Growth Pot Pass-Through.* Samples from the bulk watering solution containing modified Hoaglund's and cyantraniliprole were filtered using a "Spin-X" Costar 8170 centrifuge tube filter utilizing a Pall Corporation 0.45 $\mu$M nylon filter (Pall Corp., Exton, PA, USA) into a 2 mL tube. Samples were analyzed on an Agilent 1100 series HPLC (Agilent Technologies, Wilmington, DE, USA) with chromatography conducted on a 4.6 mm × 250 mm Phenomenex Luna C18 5 $\mu$M column (Phenomenex, Torrance, CA, USA) utilizing a stepwise gradient of solvent A (0.1% formic acid in water) and solvent B (0.1% formic acid in acetonitrile) for a 30 min run time. The gradient consisted of 62% solvent A:38% solvent B to 0% solvent A:100% solvent B over 18 min, holding at this concentration until 23 min, at which time initial solvent conditions were reestablished, to achieve a retention time of cyantraniliprole of 13.65 min for a flow rate of 1 mL/min. Quantitation was achieved by UV detection at 254 nm. Analyses were performed using 4- to 7-point calibration curves.

*Analysis of Residues of Cyantraniliprole in Calibration Plant Tissue.* Plant tissues sample extracts were analyzed on a Waters Alliance 2795 HPLC (Waters Corp., Milford, MA, USA) coupled with a Micromass Quattro Micro API mass spectrometer (Waters Corp., Milford, MA, USA). HPLC chromatography used a 2.1 × 50 mm Zorbax SB C18 column, with a 5 $\mu$M particle size, utilizing a steep gradient from 5% acetonitrile in water to 90% acetonitrile over 1.5 min, holding at this concentration until 2.0 min, at which time initial solvent conditions were reestablished. Both the water and acetonitrile were amended with 0.1% formic acid. The column temperature was maintained at 40 °C, with a 1.0 mL/min flow rate and a 10 $\mu$L injection volume. Cyantraniliprole was quantified in electrospray positive mode while monitoring transitions of 472.9 to 283.9 amu, and from 474.9 to 285.9 amu.

Cyantraniliprole residues in leaf and fruit samples collected during the in-life phase were quantified using a 4-point calibration curve. Upon whole-plant sacrifices at the end of the experiment, cyantraniliprole residues were quantified using a 5-point calibration curve for each matrix analyzed, with the range of standards bracketing the residues found per matrix.

*Analysis of Tomato Fruit from the Independent Evaluation Tests.* Extracts were similarly analyzed by reverse phase chromatography using a Perkin-Elmer series 200 HPLC (Perkin-Elmer, Inc., Buckinghamshire, U.K.), with a Phenomenex C18 guard column (4.6 × 2.0 mm) with 3 $\mu$M particle size and a Phenomenex Aqua C18 analytical column (150 mm × 2.0 mm) (Phenomenex UK Limited, Cheshire, U.K.) with 3 $\mu$M particle size, with a 40 °C column temperature, 0.25 mL/min flow rate, a 25 $\mu$L injection volume, and an approximate 9.3 min retention time, followed by Applied Biosystems API 5000 triple quadrupole mass spectrometry (MS/MS) (Applied Biosystems, Cheshire, U.K.) detection. HPLC chromatography used solvent A (2 mmol of formic acid and 0.001 mmol of ammonium formate added to 1 L of HPLC-grade water) and solvent B (2 mmol of formic acid and 0.001 mmol of

ammonium formate added to 1 L of methanol) in a gradient. The gradient consisted of 60% solvent A:40% solvent B to 1% solvent A:99% solvent B over 12.1 min, holding at this concentration until 14.1 min, at which time initial solvent conditions were reestablished. Cyantraniliprole was quantified using an electrospray positive mode while monitoring the transition from 475.0 to 444.0 amu.

The limit of quantitation for analysis of cyantraniliprole was defined as the lowest tomato fruit fortification level for which recoveries routinely fell in a range of 70−110%, with a relative standard deviation less than or equal to 20%. Cyantraniliprole residues per tomato fruit sample were quantified using an 8-point calibration curve. The slope and intercept from the corresponding curve (constructed via weighted linear regression $1/x$) were used to calculate the analyte concentration (ng/mL) in the sample.

**The Cascade Model for Data Entry.** The cascade model is named to reflect the sequential flow of plant uptake from the growth medium to roots, roots to stem, and stem to leaves and fruits in parallel.[10] The model as applied to the current system considers the processes of each pulse input to the perlite compartment (pore water), loss from perlite and all plant compartments to air, uptake into roots with the transpiration water, translocation from roots to stem and from stem to leaves and fruit with the transpiration stream, transport to fruit with phloem, growth dilution and metabolism in all plant compartments, passage of solution through pot base, degradation of cyantraniliprole in the perlite compartment, and calculation of transpiration from the transpiration coefficient and plant masses at various time points.[10]

**Physical Constants and Growth Conditions.** Physical chemical characteristics of cyantraniliprole used in populating the cascade model prior to optimization were obtained from DuPont internal measurements and are provided as Supporting Information. Generalized representations of the plant part surface area per unit mass, water content, and lipid content were supplied for the root, stem, leaf, and fruit portions and were entered into the model according to Legind et al., 2011.[10] Water content of the growth medium (178 kg/m$^3$) was calculated as the difference between the Pennsylvania Perlite Corporation (Bethlehem, PA, USA) manufacturer-supplied wet and dry bulk densities. Occupied pot size for the plants of the experiment was estimated as 1720 mL based on physical measurement of the pot and perlite depth within the pot. Daily greenhouse temperature and relative humidity values were obtained as described above and used as inputs to the cascade model. The mass of chemical applied was determined by multiplying the average measured concentration of cyantraniliprole in the application solution at each treatment level by the volume of aqueous solution transpired by the plant. The volume of solution transpired by the plant was calculated by subtracting the volume of solution collected from the base of the pots from the volume of solution added to each pot per each application event, as well as subtracting the net volume of solution retained by perlite only (no plant) pots.

**Plant Growth Characteristics.** The general shape of the leaf and stem weight growth curve incorporated, in part, measurements available through the literature.[14] To represent the actual contour of the leaf and stem growth curves for this variety of tomatoes in this experiment, individual plant part weights from an entire control plant harvested at each of four time points was used as well as the mass of entire treated plants at harvest. For fruits, the shape of the growth curve for modeling purposes was based on that described in Gustafson, 1926.[15] The published growth information was adapted to the current experiment by digitizing the growth information found in the published reference, using the digitization software GetData (GetData Graph Digitizer Version 2.22), which was then scaled to reflect fruit development time in the current experiment and actual fruit mass at harvest. This approach assumed that the shape of the growth curve was generally characteristic of the crop and could be linearly scaled to mimic varietal differences. Plant part weights were interpolated for entry into the reference model to reflect nominally half-day time periods in alignment with the twice daily dosing for each cyantraniliprole treatment group. Model operation currently requires that mass in each plant part compartment be increasing, however infinitesimal. Thus, even before fruit has formed, a very small, increasing weight (e.g., $10^{-21}$ kg/model time interval) was represented for the fruit.

It was not readily possible to separate roots from the perlite growth medium in order to determine their mass. Root mass growth information was estimated based on literature leaf to root fresh weight ratios of approximately 5.6 that are given for tomatoes grown in glasshouses by Mohammed et al., 2009.[16]

**Measured Residues.** The cyantraniliprole residues measured in leaf and fruit samples at up to three of the sample points (prior to plant senescence) were entered as values to which model output concentrations were to match. In addition, the cyantraniliprole concentration in the solution eluted from the base of pots by application solutions (corrected for the cyantraniliprole in the application/elution solution) was used to calculate the cyantraniliprole concentration in the chemically inert perlite substrate. Thus, measurements in a total of three model compartments were used for model optimization purposes.

**Transpiration Coefficient.** The transpiration coefficient [L of transpired water/increase in plant fresh weight (kg)] was determined over the 18 day period from start of sampling to onset of senescence based on measured water use, and the difference in linear-fitted plant weights between 0 and 18 days after start of sampling. The resultant transpiration coefficient per cyantraniliprole treatment group was entered as an initial value in the cascade model, prior to model optimization.

**Optimization of the Cascade Model.** A nonlinear least-squares optimization routine was used to optimize (i.e., calibrate) the model against residue measurements representing the perlite, leaf, and fruit model compartments at multiple time points. The Solver Add-in within Microsoft Excel was used to perform the optimizations. The following example conditions were used: Max Time, 100 s; Iterations, 200; Precision, 0.000001; Tolerance, 5%; Convergence, 0.00001; Assume Non-Negative; Estimates, Tangent; Derivatives, Forward; Search, Newton. The sum of the squares of the 1/(residue concentration)$^2$ − weighted residuals (the difference between the measured and calculated residues) was minimized by changing the values for the mass based loss rate constants for the growth medium, root, leaf, stem, and fruit compartments, as well as for the transpiration coefficient. Initial values for rate constants were set to no decline (e.g., $1.00 \times 10^{-12}$), whereas the initial value for the transpiration coefficient was based on the experimentally determined value. Optimizations were repeated to assess global versus local minima.

The model was further evaluated with respect to residues in independently grown greenhouse tomatoes using rock wool as a growth substrate with supplemental irrigation and cyan-

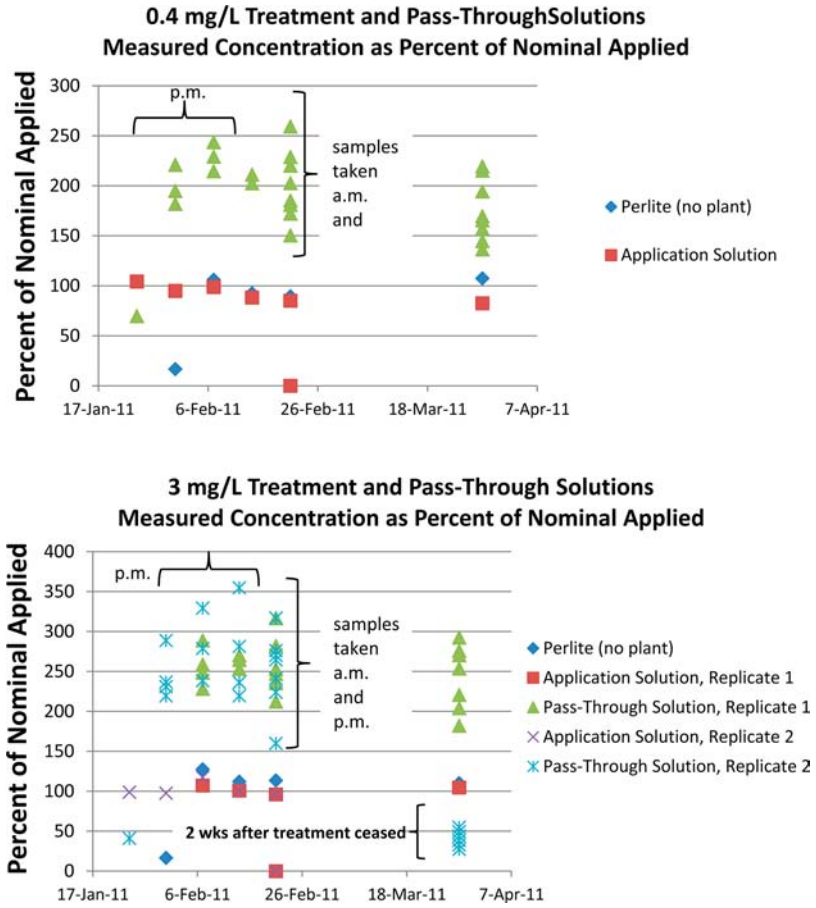


**Figure 2.** Concentration of cyantraniliprole in perlite "pass-through" water in comparison to initial concentration of solution applied.

traniliprole application (DuPont confidential information). Several critical inputs were unknown for the independently conducted tests. Thus, these data were used to qualitatively assess relative differences in predictive capability of the current model based on whether optimization of the reference hydroponic model was performed using perlite, fruit, and leaf residue measurements or performed using only fruit and leaf measurements. Relevant trial characteristics for each evaluation test were entered into the cascade model as previously optimized against measured residues in the fruit, leaf, and/or perlite compartments through the reference hydroponic experiment. These evaluation test characteristics were such features as the substrate (rock wool) depth and density, the average daily values of temperature and relative humidity, the product application scenario, and the relevant rock wool and plant compartment masses. The previously optimized rate constants per reference model compartment were used unchanged. The resultant model-calculated residues for the fruit and leaves were compared to those measured through conduct of the independent evaluation tests.

## RESULTS

**Exposure to Cyantraniliprole.** The use of perlite as a chemically inert growth substrate yielded the null hypothesis that the concentration of cyantraniliprole in the application solution would be the same as the cyantraniliprole concentration in the pass-through solution. Experimental findings (Figure 2) confirmed the null hypothesis for unplanted perlite pots only. For planted pots, the concentration in the freshly watered pot "pass through" was not at the treatment concentration, but was actually appreciably higher. We attribute this to the selective relative concentration of cyantraniliprole due to preferential uptake of water by the plants versus cyantraniliprole uptake. Such preferential plant uptake of water is consistent with previous work that indicated a ratio of approximately 0.06 for [$\mu$g(leaves + stems):$\mu$g(hydroponic solution)] (DuPont measurements for cyantraniliprole in young tomato plants).

This phenomenon is analogous in principle to documented behavior in closed hydroponic systems where drainage nutrient solutions are reused. Over time, the nutrient composition in the drainage solutions is substantially different from that of the solution supplied to the crop due to the variation in efficiencies with which individual solution components are taken up by the plant.[17]

Thus, the concentration for further use in the reference model was assumed to be the concentration originally present in the uptake solution prior to adding to the pots. The volume of uptake solution was carefully determined by measuring both the amount added to each pot and the amount that eluted from each pot after watering. The net volume of solution retained for pots containing growing plants was compared to that with pots containing the same amount of perlite, but no plants. The difference between these values is the volume of treatment solution transpired by each plant. These data were used to calculate an estimated transpiration coefficient when dividing by the total plant fresh weight at the end of the experiment. These results can be seen in Table 1.


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**Table 1. Inputs Used in Calculation of a Plant Transpiration Coefficient[a]**

| attribute | control | 0.4 mg/L | 3 mg/L rep 1 | 3 mg/L rep 2 |
|---|---|---|---|---|
| cumulative water transpired (mL) | 39643 | 42394 | 41835 | 42598 |
| [all fruit ripe] + [green fruit at sacrifice] (g) | 947 | 592 | 693 | 686 |
| leaves at sacrifice (g) | 241 | 371 | 241 | 234 |
| stem at sacrifice (g) | 253 | 407 | 355 | 332 |
| estimated root (g) | 49 | 63 | 43 | 42 |
| plant part weight sum (g) | 1490 | 1434 | 1332 | 1293 |
| calcd transpiration coeff (L/kg) | 27 | 30 | 31 | 33 |

[a]Does not account for transpiration occurring prior to applications, which began at 18 days after transplanting.

**Residues in Fruit and Leaves.** The concentration of cyantraniliprole in the mature tomatoes, stems, and leaves can be found in Table 2. Residue concentrations on a fresh weight basis were found to progressively increase generally by an order of magnitude between fruit, stems, and leaves, respectively. Root tissues were not analyzed since it was not possible to remove the perlite growth media from the root tissue. Thus, it was not possible to get an accurate weight and concentration measurement for the roots.

**Table 2. Concentration of Cyantraniliprole in Mature Tomatoes, Leaves, and Stems[a]**

| dose | sample timing (days) | cyantranilprole concen (μg/g tissue) leaf | fruit | stem |
|---|---|---|---|---|
| 0.4 mg/L | 0 | 6.8 | 0.065 | |
| | 7 | 11 | 0.069 | |
| | 14 | 9.8 | 0.076 | |
| | 21 | 8.4 | 0.063 | |
| | 28 | 6.0 | 0.078 | 0.60 |
| 3 mg/L | | | | |
| rep 1 | 0 | 110 | 0.44 | |
| | 7 | 150 | 0.34 | |
| | 14 | 140 | 0.56 | |
| | 21 | 130 | 0.56 | |
| | 28 | 110 | 0.46 | 6.8 |
| rep 2 | 0 | 150 | 0.66 | |
| | 7 | 200 | 0.57 | |
| | 14 | 150 | 0.45 | |
| | 21 | 160 | 0.45 | |
| | 28 | 89 | 0.56 | 2.7 |

[a]Cyantraniliprole residues are expressed to two significant figures according to the error analysis of the analytical methodology used.

Demonstrated method limits (signal-to-noise ratios of 3 or higher) for the analytical methodology used to measure residues in the reference hydroponic experiment were 0.002, 0.02, 0.2 mg/kg for fruits, stems, and leaves, respectively. Adequate method performance is supported by the concurrent recovery assay yielded recoveries from the extraction process, ranging from 79 to 103%. The calibration curve coefficients of determination ($r^2$) per matrix were ≥0.94 for analyses across plant parts, as well as for the application and pass-through solutions. The mean relative percent deviation ($P$) for calibration curves ranged from 5 to 8% for plant part analyses and from 1 to 6% for application and pass-through solutions, where

$$P = \left(100/n\right) \sum \left(|\text{measured} - \text{fitted}|/\text{measured}\right)$$

for $n$ standards comprising a calibration curve. Thus, adequate linearity was demonstrated.

For the independently conducted evaluation tests, the demonstrated limits of detection and quantitation for the methodology were 0.003 and 0.010 mg/kg, respectively. All calibration curves showed sufficient linearity, as reflected by correlation coefficient, $r$, values ≥0.99 and 2% mean relative percent deviation. The calculated concentrations for standards incorporated into calibration curves met specific acceptance criteria, being within ±15% of the actual concentration (within ±20% of the actual concentration at the lower end of linearity). Untreated control samples fortified with 0.010−1.0 mg/kg cyantraniliprole were analyzed concurrently with the treated samples' verified method performance, as reflected by mean recovery values (±standard deviation) of samples fortified over the range 0.010 mg/kg to 1.0 mg/kg for cyantraniliprole being 91% ± 12.

**Optimization of the Cascade Model.** Mathematically optimized loss rates per model compartment as expressed as half-life values are given in Table 3. The degradation half-life values found through model optimization for the fruit compartment and the transpiration coefficients showed reasonable agreement across treatment groups. The degradation half-life values found for the leaf compartment show reasonable agreement between the low treatment and the first replicate of the high treatment groups. For the second replicate of the high treatment group, the model simulation period was shortened relative to the other two treated groups due to earlier

**Table 3. Optimized Values of Half-Lives per Perlite and Plant Part Compartments**

| For Cascade Model Optimization Relative to Measured Values for Perlite, Leaf, and Fruit Compartments | | | |
|---|---|---|---|
| | degradation half-life (days) at 20 °C | | |
| | | high rate (3 mg/L) | |
| compartment | low rate (0.4 mg/L) | rep 1 | rep 2 |
| fruit | 2 | 2 | 4 |
| leaf | 8 | 19 | no decline |
| stem | no decline | no decline | 1 |
| root | no decline | no decline | no decline |
| perlite | no decline | 2 | 3 |
| transpiration coeff (L/kg fresh wt) | 30 | 31 | 33 |
| **For Cascade Model Optimization Relative to Measured Values for Leaf and Fruit Compartments** | | | |
| | degradation half-life (days) at 20 °C | | |
| | | high rate (3 mg/L) | |
| compartment | low rate (0.4 mg/L) | rep 1 | rep 2 |
| fruit | 4 | 2 | 4 |
| leaf | 20 | 22 | no decline |
| stem | no decline | no decline | 3 |
| root | no decline | no decline | no decline |
| perlite | 1 | 1 | 1 |
| transpiration coeff (L/kg fresh wt) | 30 | 31 | 33 |


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onset of apparent senescence of the tomato plants as evidenced by decreasing plant part weights which limited useful data for modeling purposes to the day 0 and day 7 samplings (per model requirement for increasing plant part weights).

Model-calculated residue concentrations as a percent of measured concentrations are given in Table 4. The percentages found indicate model convergence with reasonable agreement between calculated and measured residues per compartment.

**Table 4. Model Calculated Concentration as a Percent of Measured Concentration**

| For Cascade Model Optimization Relative to Measured Values for Perlite, Leaf, and Fruit Compartments | | | |
|---|---|---|---|
| | model calcd concn as percent of measd concn | | |
| | | high rate (3 mg/L) | |
| compartment | low rate (0.4 mg/L) | rep 1 | rep 2 |
| fruit | 114 | 104 | 96 |
| | 103 | 115 | 105 |
| | 76 | 63 | *a* |
| leaf | 132 | 118 | 109 |
| | 74 | 90 | 92 |
| | 71 | 91 | *a* |
| perlite | 67 | *a* | 83 |
| | 107 | 104 | 111 |
| | 95 | 64 | 65 |
| | 126 | 145 | 149 |
| For Cascade Model Optimization Relative to Measured Values for Leaf and Fruit Compartments | | | |
| | model calcd concn as percent of measd concn | | |
| | | high rate (3 mg/L) | |
| compartment | low rate (0.4 mg/L) | rep 1 | rep 2 |
| fruit | 116 | 103 | 94 |
| | 103 | 116 | 104 |
| | 76 | 61 | *a* |
| leaf | 128 | 116 | 108 |
| | 80 | 91 | 92 |
| | 86 | 93 | *a* |

[a]Measurement not available.

The independently conducted Netherlands greenhouse evaluation tests were used to qualitatively assess the predictive capability of the cascade reference hydroponic model. The resultant comparison of nearness of predicted residues to actual residues for the evaluation tests is given in Table 5. The comparison shows that the residues for the evaluation tests were best estimated when only the measured leaf and fruit residues were used in calibrating the reference model.

## DISCUSSION

Certain modifications to data input were made that were both necessitated and enabled due to the study design of the reference hydroponic experiment that was unique compared to previous use of the model.[10] Growth medium (perlite) characteristics had a much greater pore water component than that of soil. However, measured concentrations before addition to the systems and the pot pass-through solution indicated potentially more complexity than a purely hydroponic design that contained no solid medium. The current design did allow plant growth configuration geometry to be accurately calculated from pot dimensions, and did better mimic an actual soil system where cyantraniliprole moved both into and out of

**Table 5. Independent Cross-Check of Predicted vs Actual Residues**

| calibration scenario description | | | | predicted residues compared to actual residues at longest sampling time of indep cross-check test[b] | | |
|---|---|---|---|---|---|---|
| | | | | | high rate (3 mg/L) | |
| leaf[a] | fruit[a] | perlite[a] | indep cross-check trial no. | low rate (0.4 mg/L) | rep 1 | rep 2 |
| X | X | X | 1 | near | below | near |
| X | X | X | 2 | above | near | above |
| X | X | NA | 1 | below | below | below |
| X | X | NA | 2 | near | near | near |

[a]X denotes measurement entered for optimized model output to match during model calibration; NA = not applicable. [b]Relative ratios of predicted to actual residues found for independent cross-check tests: above (ca. 3), near (ca. 1−2), below (less than 1).

the system. Environmental conditions were precisely monitored using the in-place greenhouse monitoring systems. Plant part masses were extrapolated from a combination of actual measured plant part masses coupled with standard growth curves available from the literature. Measured residues of cyantraniliprole in the various compartments allowed a direct comparison to modeled values. Precise water balance measurements during the experiment coupled with the extrapolated (and measured) plant part mass increases allowed an accurate determination of the actual transpiration coefficient.

**Calculated Transpiration Coefficient.** A transpiration coefficient of approximately 30 L/kg fresh weight of plant growth was calculated from the measured cumulative water use by control plants, divided by the difference in linear-fitted plant weights, between 0 and 18 days after the start of sampling, prior to the onset of senescence. This calculation method was tested against calculation of the cumulative volume transpired over the entire plant dosing period, divided by the total plant fresh weight at the end of the experiment. This alternate calculation approach also yielded approximately 30 L/kg fresh weight of growth, when computed for both control and treated plants (Table 1). Further, corroboration is found through consistency of the calculated values of the transpiration coefficient with literature-based determinations, where an average of 0.38 L of water was found to be taken up per gram of dry weight of untreated tomato plant controls across four cultivars.[18] Coupling this value with a separately published value of 7.6% dry matter in tomato plants yields 29 L of water transpired per kilogram of tomato plant fresh weight.[19]

**Measured Concentrations in Hydroponic Grown Tomato Tissue.** Residues in stem, leaves, and fruit are roughly proportional to concentration of cyantraniliprole in the treatment solution and may provide residue predictive utility outside the calibration of the cascade reference hydroponic model. Residues on a dry weight basis are comparable for stems and fruit within a treatment group. Residues between leaves and fruit within a treatment group show leaf residues to be higher (dry weight basis: ca. 30× on average across treatments). The higher residues in leaves may be explained by the relative position and flow velocity for each respective plant part within the transpiration stream. Leaves (leaf compartment) are represented in the cascade model in parallel with the fruit (fruit compartment), in the position of farthest upward-transport along the path from the pore water of the growth


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medium to roots, to stem, and ultimately to leaves and fruit.[10] Relative surface areas of leaves and fruits that potentially drive transpiration to these farthest positions suggest that the xylem flux for leaves can be expected to be significantly larger than for fruits.[10] Correspondingly, relative flow velocities, $Q$ (transpiration, L/day), calculated in the reference model for each individual time period show appreciable differences for leaves and fruits ($Q_{leaves}/Q_{fruit}$ ratios of roughly 50). These large ratios are reasonable when considering the xylem flux to leaves and fruits is computed as transpiration for roots ($Q_{roots}$ = transpiration coefficient × root mass change/period length) multiplied by the fraction of the surface area each commodity represents of the total combined surface area of leaves (6 $m^2$/kg) and fruit (0.16 $m^2$/kg).

Further, Legind et al., 2011, reported that the relative total transpiration for leaves and fruits in the case of a related solanacea vegetable, peppers, is higher for leaves when additionally considering the phloem flux from leaves to fruits.[10] For cyantraniliprole specifically, the physical-chemical properties of the molecule [acid dissociation constant ($pK_a$) of 8.8 and a lipophilicity (log $K_{ow}$) of 1.94, at 20 °C] correlate to poor phloem translocation.[8,12] Thus, primarily xylem flux led to concentrations of cyantraniliprole in each plant part that were concurrently subject to reduction through growth dilution and metabolism.

The study design, including twice-daily cyantraniliprole application over a sustained period, did not allow evaluation of establishment of a "plateau" level in tomato fruit, but rather provided four iterations of the "same" experiment. Changes in solution concentration in this perlite "model" system during a day/night cycle speak to the complexity of modeling soil residue uptake into plants.

**Cascade Model Optimization and Validation.** The optimization consistently resolved to values that were empirically valid compared to chemical mass dissipation rates found for radiolabeled metabolism studies in whole plants (DuPont confidential information). Further consistency is evidenced by model calculated residues as a percent of measured residues of 97% ± 19% for fruit, 97% ± 22% for leaves, and 101% ± 30% for the perlite compartment when optimization was conducted relative to measured values for the perlite, leaf, and fruit compartments. Model calculated residues as a percent of measured residues were 97% ± 20% for fruit and 99% ± 17% for leaves when optimization was conducted relative to measured values for only the leaf and fruit compartments.

Plant masses for the whole plant system increase to a point, as fruit are produced through successive inflorescences, and eventually begin to decrease.[20] Thus, the current cascade model requirement for continually increasing plant part masses (as the basis for assessing transpiration) limited the time period for which residue measurements could be used for model optimization purposes.

Residues measured through independently conducted greenhouse tomato trials were best approximated with the cascade model when optimized using only leaf and fruit residues. Optimization that included perlite compartment residues and associated additional inputs resulted in modeled residue values more distant from the measured residue values. This finding may be indicative of the need to account for input variability when assessing model utility as suggested by Warren-Hicks et al., 2002.[21] These authors propose that, particularly for complex models, probabilistic Monte Carlo analysis enables a more appropriate assessment of model performance than does the simple one-to-one comparison between measurements and predictions as were conducted in this in-life and modeling experiment. The authors' recommendation for incorporation of model input ranges with appropriately assigned distributions may potentially address the limitation of key model input parameters not being known for the independently conducted evaluation tests in this work.

Learnings on the eventually decreasing fruit load for a continuously ripening crop are translatable to soil application-related business questions for this type of produce production scenario. The cascade model is responsive to the inputs and converges to a mathematical solution per treatment regime. Additional assessment of key issues such as local minima, sensibility of optimized parameters (transpiration coefficient, compartment rate constants), accounting for input variability, and future experimental designs is needed.

## ■ ASSOCIATED CONTENT

### Supporting Information

Supporting tabulations inclulding characteristics of independently conducted evaluation tests and physical-chemical properties for test chemical as relevant to the cascade model. This material is available free of charge via the Internet at http://pubs.acs.org.

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### Notes

The authors declare no competing financial interest.

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