Journal of Asia-Pacific Entomology 12 (2009) 227–232

Contents lists available at ScienceDirect

Journal of Asia-Pacific Entomology

journal homepage: www.elsevier.com/locate/jape

Effect of nitrogen levels of two cherry tomato cultivars on development, preference and honeydew production of *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae)

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ARTICLE INFO

Article history:
Received 5 February 2009
Revised 16 April 2009
Accepted 18 April 2009

Keywords:
Koko
Pepe
Development
Chemical contents
Honeydew
Choice and non-choice tests

ABSTRACT

Two cherry tomato plant cultivars (*Lycopersicon esculentum* Miller, cultivars 'Koko' and 'Pepe') were supplied with high (395 ppm), medium (266 ppm) and low (199 ppm) concentrations of nitrogen to determine the influence of nitrogen fertilization on development, cultivar preference and honeydew production by greenhouse whiteflies, *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae). The nitrogen, protein, and chlorophyll contents of tomato leaves were higher in the high nitrogen supplied plants than in the medium or low nitrogen supplied plants, but the sugar content showed an inverse relationship. The developmental times of eggs and nymphs decreased as the nitrogen concentrations increased in both cultivars. The preference of *T. vaporariorum* was compared by counting the number of eggs deposited on leaves in choice and non-choice tests. In the non-choice test, no significant nitrogen treatment effects were observed but the upper plant stratum was preferred for egg laying. In the choice test, there were significant main effects of cultivar and nitrogen concentration. *T. vaporariorum* laid eggs more on leaves of plants with higher nitrogen at the upper stratum. In both experiments, *T. vaporariorum* preferred the 'Koko' cultivar to the 'Pepe' cultivar. The honeydew production of *T. vaporariorum* nymphs increased with decreasing nitrogen treatment concentrations. The largest honeydew production was detected in the 'Pepe' cultivar grown at low nitrogen concentration. It is concluded that cultivar 'Pepe' had an advantage over 'Koko' in term of *T. vaporariorum* management program in tomato greenhouses.

Introduction

Greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae), is a common insect pest of many ornamental and vegetable crops around the world (Mound and Halsey, 1978). *T. vaporariorum*, like all insect species with sessile immature stages, is subjected to strong selective pressure to choose host species or plant parts that are most suitable for feeding and oviposition (van Lenteren and Noduls, 1990).

Leaf nitrogen content is an indicator of food quality and a factor affecting host selection by phytophagous insects (McNeil and Southwood, 1978; Mattson, 1980). It influences the survival, development, and reproduction of insects (van Emden, 1966; Auclair, 1976; McClure, 1980; Minkenberg and Fredrix, 1989). Jauset et al. (1998) reported that the nitrogen content of plants is directly related to the level of nitrogen fertilization, and that it affects among- and within-plant distribution of *T. vaporariorum* adults on tomatoes. *T. vaporariorum* females aggregated and laid more eggs on leaves and on plants with the highest nitrogen and water content. The effects of plant nitrogen fertilization on honeydew production of whiteflies have been reported by several researchers (Bentz et al., 1995; Rubeiz et al., 1995). Blua and Toscano (1994) indicated that subtly different levels of cotton nitrogen fertilization could affect honeydew production of *Bemisia argentifolii* Bellows and Perring. Whitefly larvae started to produce honeydew earlier on plants treated with higher nitrogen than those on plants treated with medium or low concentration of nitrogen, but subsequently generated fewer droplets (Blua and Toscano, 1994).

The effects of nitrogen fertilization on cherry tomato-whitefly interactions in greenhouse conditions have not been investigated. In Korea, cherry tomato acreage has expanded quickly because of high and stable market prices. The main cultivars for commercial production are 'Koko' and 'Pepe' which are produced by Dakii Nursery Company of Japan. The fruits have a high Brix degree and long shelf life.

The objective of this study was to compare the influence of nitrogen treatment levels in two cherry tomato cultivars, 'Koko' and 'Pepe', on the development time of immatures, cultivar preference for

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1226-8615/$ – see front matter
doi:10.1016/j.aspen.2009.04.004

oviposition, and honeydew production of *T. vaporariorum* under greenhouse conditions.

Materials and methods

Whitefly and host plants

The *T. vaporariorum* population was obtained from the National Institute of Agricultural Science Technology, Suwon, Korea, and maintained on tomato plants in an insect rearing room. The host plants were two cherry tomato (*Lycopersicon esculentum* [Miller]) cultivars, 'Koko' and 'Pepe'. Tomato seeds were sown in Rockwool® plugs (20×27 mm) filled with a recommended nutrient solution (pH = 5.5 and electrical conductivity (EC) = 2.3–2.6) for cherry tomatoes (Ohta et al., 1993). Vermiculite was used as an inert rooting substrate.

The plants were grown in the laboratory, free of whiteflies, at room temperature of 26.5 ± 2.5 °C and under a photoperiod of 18:6 [L:D]) h until ready for the experiments. When the seedlings were at the 2- or 3-leaf stage, they were transferred individually to a Rockwool® block (100×100×65 mm) filled with nutrient solution (pH = 5.5 and EC = 3.0). Plants were grown until the 4-leaf stage with a recommended nitrogen concentration (266 ppm) for cherry tomatoes and then were randomly divided into three groups and each assigned to a different concentration of nitrogen treatment. Tomato plants used in the experiments had 12 fully expanded leaves. The leaves of each plant were numbered from top (the 1st leaf) to bottom (the 12th leaf), and assigned to three different strata, i.e., upper (leaves 1st–4th), middle (5th–8th), and lower (leaves 9th–12th).

Nitrogen treatments

Three different nutrient solutions containing high (395 ppm), medium (266 ppm) or low (199 ppm) nitrogen were supplied to randomly chosen plants. The concentrations of nitrogen were within the normal range of tomato fertilization (Mason and Wilcox, 1982). Interactions of nitrogen uptake and EC value in hydroponic nutrient solution have been reported for greenhouse tomatoes (Dorai et al., 2001). Therefore, the concentrations (in ppm) of the remaining macronutrients were modified slightly to adjust EC = 3: P, 33; K, 449; Mg, 64; Ca, 224; S, 270 for the high nitrogen concentration, P, 33; K, 457; Mg, 65; Ca, 228; S, 157 for the medium nitrogen concentration and P, 33; K, 461; Mg, 65; Ca, 230; S, 229 for the low nitrogen level. One hundred ml of each nutrition solution were applied to each treatment three times per week.

Effects of nitrogen fertilization levels on cherry tomato cultivars

For all experiments, leaves from 12-leaf stage plants were used. Plants were divided into 3 strata and three leaves from each stratum were detached for further chemical analysis. Leaf area was measured using a Matrox Inspector Image Analysis Software (Matrox Electronic System Ltd, Canada). Leaves from each stratum were digitally scanned and digital images were produced (Hewlett Packard Scanjet 5300C). Leaf areas (cm^2) within the same plant stratum were pooled, and mean areas were calculated.

Total nitrogen content was measured using the micro-Kjedahl procedure (Jones, 1984). Chlorophyll concentrations were determined using the method described by Arnon (1949). Tomato leaves (0.2 g fresh weight) was ground in liquid nitrogen and pigments were extracted at 4 °C in 80% acetone. Chlorophyll concentrations were measured spectrophotometrically at 663 and 645 nm. Total protein was assayed using the Bradford method modified for plant protein by Jones et al. (1989). Leaves (0.2 g fresh weight) were ground in liquid nitrogen and homogenized using a tissue grinder in phosphate buffer (25 mM NaH_2PO_4, 25 nM Na_2HPO_4, pH 7.5). After 30 min, the homogenate was centrifuged at 3000 *g* for 15 min. An aliquot (0.3 ml) of supernatant was added to a cuvette containing 3 ml Biorad solution with polyvinylpyrolidine (3 mg/ml). Absorbance was read at 595 nm within 20 min. A standard curve was constructed using a ribulose 1, 5-diposphate carboxylase–oxygenase. Protein levels are expressed as percent protein (wet weight).

Total soluble sugars were measured using the Anthrone method (Siddigi et al., 1998). The filtrate (15 ml), henceforth referred to as "pulp", was placed in a pre-weighed centrifuge tube, diluted in 10 ml distilled water and weighed. After centrifugation at 1000 *g* for 10 min, the supernatant was collected in a test tube and its volume and weight were determined. After appropriate dilution of the supernatant, soluble sugars were measured colorimetrically by adding concentrated HCl, 45% formic acid, and anthrone/sulphuric acid solution. Absorbance was measured at 630 nm.

Effects of nitrogen levels on development time of T. vaporariorum

Five plants of each treatment (total 30 plants; two cultivars and three concentrations of nitrogen contents) were randomly distributed in a greenhouse in which whiteflies were continuously maintained, and allowed to become infested for 48 h. After removing all the adults from the plants, the plants were screened with a fine mesh fabric (10×20 holes/cm^2) to keep the plants free from new adult whiteflies. The ave-rage temperature during this study was 26.5 ± 2.5 °C. Relative humidity was not measured. The eggs on the top three leaves were counted and leaves with more than 20 eggs were marked with a white tape near petiole. For each treatment, at least six leaves were observed to study the egg and nymphal development of whiteflies. The selected leaves were observed daily with a hand lens to detect the appearance of the different stages.

Effects of nitrogen levels on the cultivar preference of T. Vaporariorum

Oviposition preference of *T. vaporariorum* in the two cherry tomato cultivars with different concentrations of nitrogen treatments was determined by offering whiteflies each treatment alone (a non-choice test), and all combinations of treatment (a choice test) in a greenhouse. The preference was judged by counting the number of eggs laid on each treatment.

In non-choice test, one treatment was offered to whiteflies at a time, and the number of eggs laid was counted. Three tomato plants of a single treatment were placed inside an acryl cage (40×40×50 cm), and 50 adult whiteflies were allowed to lay eggs for 48 h. Adults were collected from the *T. vaporariorum* infesting greenhouse using an automatic aspirator (Hausherr's Machine Works, NJ, USA). In choice experiments, adult whiteflies were offered all treatments at once. Six plants (one of each treatment) were placed inside an acryl cage (60×60×50 cm), and 50 adult whiteflies were allowed to lay eggs for 48 h.

After 48 h, all the adults inside the cage were completely removed from both experiments. Five days after exposure, the numbers of whitefly eggs were counted on all leaves. Each test was repeated three times on different dates. Because leaf areas differ among plant positions, the preference data (mean egg numbers) was standardized on the basis of leaf area of 100 cm^2.

Effects of nitrogen fertilization levels on honeydew production

To study the effect of nitrogen levels on honeydew production by 3rd–4th instar nymphs, plants were placed in a greenhouse in which whiteflies were continuously maintained and allowed to lay eggs for 48 h. Then, plants with eggs were placed in an insect proof room at 25 ± 2 °C, 60 ± 10% RH with a photoperiod of 16:8 (L:D) h. When eggs began to hatch, a fixed number of first instar nymphs were left on each leaf located at the10th position from the ground level and all other eggs and nymphs were removed. The maximum number of the nymphs tested was 60.

Water-sensitive papers (5.2×3.8 cm) (Novartis, Basel, Switzerland) were used to detect whitefly honeydew. Honeydew drops that

Table 1
Total nitrogen, total protein, total soluble sugar and total chlorophyll contents (mean ± SEM) of two cherry tomato cultivars, 'Pepe' and 'Koko', in three different plant strata and three different nitrogen concentrations.

Nitrogen concentration	Upper		Middle		Lower	
	Koko	Pepe	Koko	Pepe	Koko	Pepe
Total nitrogen content (%)						
High	4.9 ± 0.1a	4.6 ± 0.1a	3.4 ± 0.1	3.6 ± 0.1a	3.1 ± 0.1a	2.9 ± 0.1a
Medium	4.7 ± 0.1a	4.4 ± 0.2a	3.5 ± 0.1	3.4 ± 0.1a	2.8 ± 0.1a	2.6 ± 0.1b
Low	3.7 ± 0.1b	3.8 ± 0.2b	3.3 ± 0.1	3.1 ± 0.1b	2.4 ± 0.1b	2.5 ± 0.1b
Total protein content (ppm)						
High	117.5 ± 3.2aB	171.5 ± 0.3aA	70.5 ± 0.8aA	45.1 ± 2.8aB	28.1 ± 1.6a	30.9 ± 1.8a
Medium	96.9 ± 5.6bB	113.8 ± 9.2bA	52.3 ± 2.7bA	36.7 ± 2.6aB	18.9 ± 0.9b	15.4 ± 0.7b
Low	75.4 ± 4.3c	81.5 ± 8.2b	38.3 ± 4.2cA	22.3 ± 2.4bB	17.3 ± 0.2b	14.3 ± 4.0b
Total soluble sugar content (ppm)						
High	15.9 ± 2.0bB	25.1 ± 1.6bA	13.5 ± 0.1bB	20.5 ± 2.0bA	14.4 ± 1.4	16.0 ± 1.4b
Medium	18.5 ± 2.4bB	30.7 ± 1.7bA	11.2 ± 0.6bB	26.4 ± 2.0aA	12.9 ± 0.2B	20.0 ± 1.8aA
Low	23.4 ± 1.3aB	36.0 ± 1.3aA	14.6 ± 0.9aB	29.1 ± 2.0aA	14.2 ± 2.0B	25.0 ± 2.6aA
Total chlorophyll content (mg/ml)						
High	59.6 ± 1.8a	58.5 ± 2.8a	48.9 ± 1.2a	46.8 ± 0.8a	35.4 ± 0.2a	39.6 ± 1.3
Medium	55.5 ± 1.5b	57.3 ± 1.0a	45.4 ± 1.1b	46.0 ± 1.9a	33.8 ± 0.6b	38.2 ± 0.3
Low	51.7 ± 1.8b	55.5 ± 0.1a	41.1 ± 2.1b	41.0 ± 1.8b	32.3 ± 0.2b	37.8 ± 1.0

Means within a column followed by the same lower case letter and means within a row followed by the same upper case letter are not significantly different at $P = 0.05$ (Tukey's test). Cultivar comparison is performed in each plant stratum.

fell onto the papers appeared as easily seen and distinct blue spots. The paper was secured horizontally 5 cm beneath an infested leaf with a paper clip on the 10th node petioles of each of five plants chosen randomly in a greenhouse. After being exposed for about 1 h, the papers were collected and the honeydew production area (mm^2) was measured with a Matrox Inspector Image Analysis Software.

Statistical analyses

All the data of plant characteristics were analyzed using the general linear model (GLM) procedure (SAS Institute Inc, 1999). Data were analyzed by two-way analysis of variance (ANOVA). If significant differences were found, Tukey's HSD multiple comparisons test were performed to determine the differences.

In the choice test, there were three factors: cultivar (two levels: 'Koko' or 'Pepe'), nitrogen treatment (three levels: high, medium or low) and plant stratum (three levels: upper, middle or lower). All three factors were random, and the plant stratum factor was nested within the cultivar and nitrogen treatment factors. Therefore, to test for significant differences between mean egg numbers, and also to detect the cultivar × nitrogen level interaction of interest, a 3-way nested ANOVA was used, with plant stratum nested within cultivar and nitrogen level. Also, egg numbers found in the choice test were regressed with the corresponding plant chemical content values.

Results

Effects of nitrogen fertilization levels on cherry tomato plants

Symptoms of toxicity due to excess or deficiency of nitrogen were not observed in the tomato plants. Changes of plant chemical characteristics in relation to the nitrogen concentrations and leaf positions are summarized in Table 1. There were no significant differences in the total nitrogen content (TNC) between cultivars in the treatment of the same nitrogen ($t = 0.73$, $df = 52$, $P = 0.4763$). However, the level of nitrogen applied and the plant stratum significantly affected TNC in the leaves of both cultivars (Koko, nitrogen concentration: $F = 19.25$, $df = 2, 22$, $P < 0.001$; stratum: $F = 109.48$, $df = 2, 22$, $P < 0.001$; Pepe, nitrogen concentration: $F = 30.88$, $df = 2, 22$, $P < 0.001$; stratum: $F = 244.96$, $df = 2, 22$, $P < 0.001$) (Table 1). Within each nitrogen concentration, the leaves from the upper plant stratum always had significantly higher TNC than other strata. Within the same plant stratum, TNC of plants that received the higher nitrogen concentrations (high and medium) was significantly higher than that of low nitrogen concentration. The total protein content (TPC) in tomato leaves increased with increasing nitrogen concentration (Koko, nitrogen concentration: $F = 41.44$, $df = 2, 22$, $P < 0.001$; stratum: $F = 292.32$, $df = 2, 22$, $P < 0.001$; Pepe, nitrogen concentration: $F = 16.81$, $df = 2, 22$, $P < 0.001$; stratum: $F = 107.82$, $df = 2, 22$, $P < 0.001$). Higher TPC was always observed at the upper plant stratum, regardless of the nitrogen concentrations applied. When compared between the cultivars, no unique trends were observed, but, in general, 'Pepe' and 'Koko' had a higher TPC at the upper and middle stratum, respectively. Soluble sugar content (SSC) decreased as nitrogen concentration increased (Koko: $F = 6.79$, $df = 2, 22$, $P = 0.0051$; Pepe: $F = 71.07$, $df = 2, 22$, $P < 0.001$). The stratum effect was also significant in the SSC contents (Koko: $F = 25.02$, $df = 2, 22$, $P < 0.001$; Pepe: $F = 77.77$, $df = 2, 22$, $P < 0.001$). The highest SSC was observed in the upper leaves at all concentrations of nitrogen treatments. 'Pepe' contained higher SSC than 'Koko', if significant cultivar differences were observed (t-test, $P < 0.05$). Chlorophyll contents (CC) also increased as the nitrogen concentrations increased (Koko: $F = 36.43$, $df = 2, 22$, $P < 0.001$; Pepe: $F = 11.27$, $df = 2, 22$, $P = 0.004$) and decreased as the leaf position changed from the upper to the lower (Koko: $F = 13.17$, $df = 2, 22$, $P = 0.0064$; Pepe: $F = 6.87$,

Table 2
Leaf area (mean ± SEM, cm^2) of two cherry tomato cultivars, 'Pepe' and 'Koko', in three different plant strata and three different nitrogen concentrations.

Nitrogen concentration	Upper		Middle		Lower	
	Koko	Pepe	Koko	Pepe	Koko	Pepe
High	86.2 ± 5.5	94.2 ± 2.0	152.5 ± 12.0	150.6 ± 4.2	150.6 ± 4.2	142.1 ± 0.4
Medium	82.5 ± 9.0	96.1 ± 7.2	150.1 ± 4.1	145.7 ± 12.1	145.7 ± 12.7	147.8 ± 6.6
Low	96.2 ± 4.0	84.9 ± 5.4	146.6 ± 13.3	155.8 ± 0.7	165.8 ± 5.6	157.6 ± 11.4
Average[a]	90.0 ± 5.51		150.1 ± 7.73		151.6. ± 6.81	

[a] Average leaf areas of each plant stratum are calculated by pooling two cultivars and three nitrogen concentrations.

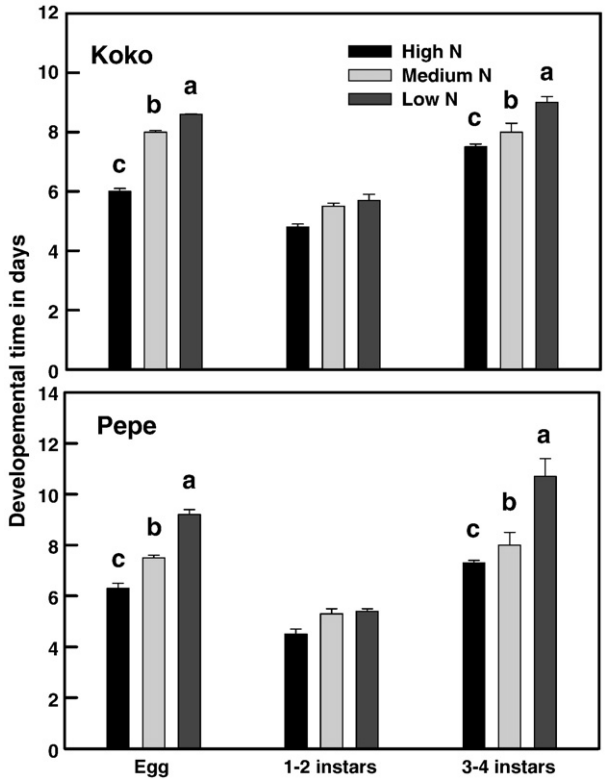

Fig. 1. Mean development time (±SEM, days) of eggs, 1–2 and 3–4 instars of *T. vaporariorum* reared on two cherry tomato cultivars, 'Koko' and 'Pepe', with different amounts (in ppm) of nitrogen treatment (high, 395; medium, 266; low, 199) at a fluctuating temperature condition (26.5 ± 2.5 °C). Treatments with the same letter are not significantly different at $P = 0.05$ (Tukey's test).

$df = 2, 22$, $P = 0.028$), but significant differences were not found between cultivars regardless of nitrogen concentration.

Neither nitrogen concentration (Koko: $F = 2.66$, $df = 2, 22$, $P = 0.09$; Pepe: $F = 0.53$, $df = 2, 22$, $P = 0.5963$) nor cultivar affected leaf expansion ($t = 0.04$, $df = 52$, $P = 0.9693$) (Table 2). Leaves located at the middle and lower strata had larger leaf areas than leaves at the upper. Since the two main factors did not influence the leaf area development, the data were pooled, and mean leaf area of each plant stratum was calculated. The mean leaf area for upper, middle and lower strata was 90.0, 150.1 and 151.6 cm^2, respectively (Table 2).

Effects on egg and nymph development times

Development time of whitefly was studied only at the upper plant stratum because leaf positions shifted from the upper to the lower stratum as the plants grew and because the highest numbers of eggs were found at the upper stratum. Egg development time was influenced significantly by the nitrogen concentration applied to two cultivars, decreasing development time as the nitrogen concentration increased (Koko: $F = 37.99$, $df = 2, 85$, $P = 0.0025$; Pepe: $F = 20.06$, $df = 2, 98$, $P = 0.0127$) (Fig. 1). The two nymphal stages (1st–2nd and 3rd–4th) exhibited similar development patterns according to nitrogen treatment concentrations, but significant nitrogen effect on the development time was observed only at the 3rd–4th stage (Koko: $F = 6.45$, $df = 2, 75$, $P = 0.0320$; Pepe: $F = 3.20$, $df = 2, 73$, $P = 0.0001$). In all stages, cultivar differences were not observed.

Effects of nitrogen levels on the cultivar preference of T. vaporariorum

In non-choice experiments, the concentration of nitrogen applied did not significantly affect preferences of whitefly (expressed as number of eggs deposited) (Koko: $F = 1.36$, $df = 2, 22$, $P = 0.2809$; Pepe: $F = 2.06$, $df = 2, 22$, $P = 0.1513$), while the plant stratum significantly affected preference (Koko: $F = 158.01$, $df = 2, 22$, $P < 0.001$; Pepe: $F = 76.79$, $df = 2, 22$, $P < 0.001$) (Table 3). The upper parts of 'Koko' received more egg depositions than those of 'Pepe' plants, but the egg numbers were not significantly different in the other two leaf positions ($F = 128.11$, $df = 2, 48$, $P < 0.001$).

The preference of whitefly in the choice experiments was different from that of the non-choice test. According to the results of the nested ANOVA, there were significant main effects of cultivar ($F = 5.76$, $df = 1, 42$, $P = 0.021$) and nitrogen concentration ($F = 27.70$, $df = 2, 42$, $P < 0.001$). The highest egg number was found in the upper position of the high nitrogen treated plants and the lowest egg number was found at the lower position of the low nitrogen treated plants. *T. vaporariorum* always preferred to oviposit on 'Koko' plants, if significant cultivar differences were detected (Table 3). Highly significant cultivar × nitrogen concentration interaction ($F = 3.30$, $df = 2, 42$, $P = 0.04$) indicated that these two factors were both involved in preference. Also, leaf stratum (nested within cultivar and nitrogen concentration) affected preference with a high significance ($F = 45.07$, $df = 6, 42$, $P < 0.001$).

When preference was compared to chemical contents of tomato leaves in the choice condition (Tables 1 and 3), an individual content of nitrogen, protein, and chlorophyll is significantly correlated with the preference, and the Pearson correlation coefficient was 0.88 ($P < 0.001$), 0.84 ($P < 0.001$) and 0.82 ($P < 0.001$), respectively. Sugar content was not related to host preference ($r = 0.09$; $P = 0.6679$) because the sugar content was higher in the low nitrogen treatment (Table 1).

Effects of nitrogen levels in tomato on honeydew production

Honeydew production (mm^2) was significantly higher at the low nitrogen concentration for both cultivars (Koko: $F = 10.85$, $df = 2, 12$,

Table 3
Mean ± SEM egg numbers[a] of *T. vaporariorum* on two cherry tomato cultivars, 'Pepe' and 'Koko', in three different plant strata and three different nitrogen concentrations in non-choice and choice experiments.

Nitrogen concentration	Upper		Middle		Lower	
	Koko	Pepe	Koko	Pepe	Koko	Pepe
Non-choice test						
High	16.2 ± 2.9A	9.6 ± 0.4B	7.5 ± 1.9	5.8 ± 0.7	2.8 ± 1.1	2.3 ± 0.8
Medium	15.5 ± 1.2A	12.8 ± 2.1B	5.8 ± 1.5	7.8 ± 1.9	3.3 ± 0.6	1.3 ± 0.7
Low	18.7 ± 1.1A	10.5 ± 1.2B	6.2 ± 2.4	5.7 ± 1.6	4.2 ± 1.1A	2.5 ± 0.4B
Choice test						
High	30.5 ± 2.1aA	17.7 ± 2.4aB	6.1 ± 1.3a	7.2 ± 2.0a	1.0 ± 0.2	1.9 ± 0.5a
Medium	12.1 ± 4.1b	14.6 ± 3.4b	1.7 ± 0.7b	3.2 ± 0.7b	1.1 ± 0.5	0.3 ± 0.2b
Low	11.9 ± 4.0bA	4.7 ± 2.6cB	3.4 ± 0.9bA	0.9 ± 0.7bB	0.7 ± 0.3	0.2 ± 0.2b

Means within a column followed by the same lower case letter and means within a row followed by the same upper case letter are not significantly different at $P = 0.05$ (Tukey's test). Cultivar comparison is performed in each plant stratum.

[a] Egg numbers are calculated based on 100 cm^2 tomato leaf.

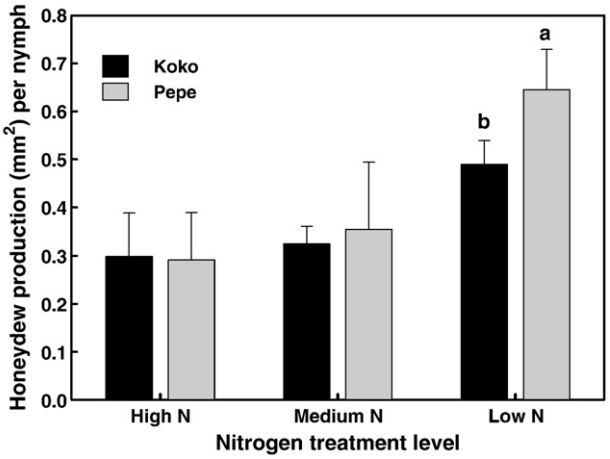

Fig. 2. Mean area (±SEM, mm^2) of honeydew drops produced per 3–4 instars of *T. vaporariorum* reared on two cherry tomato cultivars, 'Koko' and 'Pepe', with different amounts (in ppm) of nitrogen treatment (high, 395; medium, 266; low, 199). Treatments with the same letter are not significantly different at $P=0.05$ (Studentized t-test).

$P=0.001$; Pepe: $F=12.53$, $df=2, 12$, $P=0.001$) (Fig. 2). Cultivar differences were only observed at the low nitrogen concentration treatment: The nymphs produced more honeydew in 'Pepe' than in 'Koko' plants (high: $t=0.69$, $df=8$, $P=0.507$; medium: $t=1.09$, $df=8$, $P=0.760$; low: $t=2.69$, $df=8$, $P<0.0001$).

Discussion

Nitrogen is an important, and often limiting, nutrient for many herbivores (McNeil and Southwood, 1978; Mattson, 1980). It is well known that decreased nitrogen availability induces many morphological and physiological modifications in plants (Clarkson and Hanson, 1980). In our study, reducing nitrogen supply to the cherry tomato plants substantially decreased nitrogen concentration in the leaves. Regardless of nitrogen concentrations applied, the highest and lowest nitrogen content in the leaves was always observed at the upper and lower striatum, respectively (Table 1). The nitrogen levels in leaf tissue are positively correlated with rates of the greenhouse whitefly development and fecundity. This agrees with a report of Bentz and Larew (1992) who found that small (<3% as the maximal range of differences in the mean development times) but significant differences in egg development time related to nitrogen content of the host plant. Also, Blua et al. (1993) reported that increased nitrogen contents in cotton plants reduced the development time of the first instar *B. argentifolii*. It is probably a combination of total nitrogen content and the concentration of protein-bound essential amino acids that determines the nutritional value of leaf tissue for the greenhouse whitefly. Carbohydrates and the carbohydrate–amino acid ratio influence insect development (Simpson et al., 1995; Blackmer and Byrene, 1999). Our study shows that protein concentration in leaves is positively correlated with nitrogen availability. The protein content of leaf tissue might, therefore, also contribute to whitefly development on plants grown at high nitrogen availability.

Preference of *T. vaporariorum* for host plants reflects its suitability for the whitefly (van Lenteren and Noduls, 1990) and comparing fecundity is a suitable method to determine the degree of host preference in insect–host or host–predator interaction systems (Coll, 1996). Leaf age is one of the most important factors influencing host plant selection in greenhouse whiteflies. Female whiteflies spend significantly more time probing and feeding on young leaves and they stay longer (van Lenteren and Noduls, 1990). Our study provides strong evidence that leaf age (indicated by leaf position) is the major factor influencing host preference of whitefly in tomatoes. In conclusion, the plant stratum had a much greater effect on egg laying than the nitrogen concentration did, and this reflects the preference of whitefly females to oviposit on the upper leaves regardless of the nitrogen concentrations applied and the cherry tomato cultivars.

The concentration of nitrogen applied affected all chemical contents measured in our study. Plants receiving the higher nitrogen concentration had the higher nitrogen and protein contents, which agrees with previous findings (Keisling et al., 1995; Jauset et al., 1998; Bi et al., 2003). Interestingly, the sugar content decreased as the nitrogen concentration increased. However, Jauset et al. (1998) reports that the concentration of nitrogen applied did not affect the content of soluble sugars in all leaves of the tomato plant. The difference of tomato cultivars or cultural practices might contribute this discrepancy. Chemical content of tomato leaves affects host preferences of whiteflies (van Lenteren and Noduls, 1990). When comparing whitefly preference with chemical contents of tomato leaves in the choice condition, strong correlations with the contents of nitrogen, protein, and chlorophyll to preference are observed, but no correlation is found between preference and sugar content. However, the chemical content of tomato leaves alone cannot explain the preferences of whiteflies in our study. Multiple components should be simultaneously considered to explain the variation in herbivore response to plant nutrients (Busch and Phelan, 1999).

In our study, the greenhouse whitefly generated less honeydew on the highest nitrogen applied plants than on low or medium nitrogen treatment plants. If honeydew was produced in proportion their feeding rate, then the greenhouse whitefly compensated for low dietary nitrogen by feeding at a greater rate (Blua and Toscano, 1994). Feeding in compensation for low dietary nitrogen has been shown in other phloem feeding insects such as the pear psylla and the pea aphid (Pfeiffer and Burts, 1984; Prosser et al., 1992). The differences of honeydew production between cultivars were only observed at the low nitrogen treatment (Fig. 2). Chemical factors of the tomato leaves examined in this study cannot fully explain why the cultivar difference occurs only at the low nitrogen treatment, because TPC and TSC contents of the upper leaves of 'Pepe' are always higher than those of 'Koko' irrespective of nitrogen treatment levels (Table 1). In addition, the other two chemical contents (TNC and CC) are not significantly different between cultivars irrespective of nitrogen concentrations. Another possible explanation for this phenomenon is that low nitrogen treatment affects the morphological characteristics of two tomato cultivars differently, such as hairiness, leaf thickness and the length of epidermal cell boundaries at which penetration takes place, and subsequently, these produce different probing behavior of whiteflies after landing on the plants. Lei et al. (1998) reported that whiteflies generally displayed fewer but longer probes on highly acceptable cucumber than on less acceptable tomato. Jauset et al. (2000) demonstrated that nitrogen concentration affected the thickness of the leaflet lamina and of the abaxial epidermal cuticle of tomato plants; plants supplied with high nitrogen (308 ppm) had the thinnest leaf, compared to the low nitrogen treated plants (140 and 84 ppm).

The preference and honeydew production studies indicated that 'Koko' was a better host for *T. vaporariorum* than 'Pepe'. At present, there is a tendency in tomato production systems to provide a surplus of nitrogen fertilizers, which increases the nitrogen content of the plants (Minkenberg and Fredrix, 1989). As no significant differences in tomato yield were found within a wide range of nitrogen availability (Mason and Wilcox, 1982), these concentrations should be reduced to the minimum required. A potential risk by reducing nitrogen fertilization is that honeydew production is increasing with decreasing nitrogen concentrations, especially at the lowest nitrogen concentration applied in this study (Fig. 2). However, this risk can be minimized or compensated by population dynamics of whiteflies in relation to concentrations of nitrogen application. Several studies have been demonstrated that population size and growth rate of whiteflies are reduced as nitrogen concentrations applied to tomato plants are

decreased (Jauset et al., 1998; Bi et al., 2001; Jauset et al., 2000). Bi et al. (2001) reported that increased numbers of whiteflies occurs on cotton during population peaks with increasing amount applied nitrogen, and higher numbers result in increased levels of honeydew.

Our results provide evidence that *T. vaporariorum* select the host plants with a higher nitrogen content and that this factor causes an increase in egg laying and a decrease in honeydew production. Increasing our knowledge in these areas of whitefly-tomato plant interaction has much potential for practical application. In this study, cultivar 'Pepe' prove to be had an advantage over 'Koko' in terms of *T. vaporariorum* management. However, quantification of the economic advantages of cultivar 'Pepe' should be performed on tomato market basis. Cherry tomato growers in Korea receive economic benefits from reducing nitrogen fertilizers. Nitrogen fertilizer and application costs can be minimized while tomato yields maintains in maximum. Reduced population growth rate and population size of whiteflies on tomato plants fertilized with low nitrogen concentration, will enhance the application of integrated pest management programs in tomato greenhouses.

Acknowledgments

This research was funded by a grant from the Agricultural R&D Promotion Center (grant no. 301008032HD110). We thank Dr. Kwon Woo Park, Korea University, Division of Life Science and Biotechnology, for providing the cherry tomato cultivars and greenhouse space used for the experiments. Special thanks to Mr. Sang Don Lee for supplying the Rockwool materials and to Miss Mi Re Kim for her help in preparing the manuscript.

References

Arnon, D.I., 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant. Physiol. 24, 1–15.

Auclair, J.L, 1976. Feeding and nutrition of pea aphid, *Acyrthosiphon pisum* (Harris), with special reference to amino acids. Symp. Biol. Hung. 16, 29–34.

Bentz, J.A., Larew, H.G., 1992. Ovipositional preference and nymphal performance of *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae) on *Dendranthema grandiflora* under different fertilizer regimes. J. Econ. Entomol. 85, 514–517.

Bentz, J., Reeves, J., Barbosa, P., Francis, P., 1995. Within-plant variation in nitrogen and sugar content of Poinsettia and its effects on the oviposition pattern, survival, and development of *Bemisia argentifolii* (Homoptera: Aleyrodidae). Environ. Entomol. 24, 271–277.

Bi, J.L., Ballmer, G.R., Hendrix, D.L., Henneberry, T.J., Toscano, N.C., 2001. Effect of cotton nitrogen fertilization on *Bemisia argentifolii* populations and honeydew production. Entomol. Exp. Appl. 99, 25–36.

Bi, J.L., Toscano, N.C., Madore, M.A., 2003. Effect of urea fertilizer application on soluble protein and free amino acid content of cotton petioles in relation to silverleaf whitefly (*Bemisia argentifolii*) populations. J. Chem. Ecol. 29, 747–761.

Blackmer, J.L., Byrene, D.N., 1999. Changes in amino acids in *Cucumis melo* in relation to life-history traits and flight propensity of *Bemisia tabaci*. Entomol. Exp. Appl. 93, 29–40.

Blua, M.J., Toscano, N.C., 1994. *Bemisia argentifolii* (Homoptera: Aleyrodidae) development and honeydew production as a function of cotton nitrogen status. Environ. Entomol. 23, 316–321.

Blua, M.J., Toscano, N.C., Henneberry, T.J., 1993. Effects of cotton nitrogen status on sweetpotato whitefly development and honeydew production. In: Herber, D.J., Richter, D.A. (Eds.), Proceeding, Beltwide Cotton Conferences, Nashville, TN, 6-1-January. National Cotton Council, Memphis, TN, pp. 706–709.

Busch, J.W., Phelan, P.L., 1999. Mixture models of soybean growth and herbivore performance in response to nitrogen–sulfur–phosphorus nutrient interactions. Ecol. Entomol. 24, 132–145.

Clarkson, D.T., Hanson, J.B., 1980. The mineral nutrition of higher plants. Annu. Rev. Plant Physiol. 31, 239–298.

Coll, M., 1996. Feeding and ovipositing on plants by an omnivorous insect predator. Oecologia 105, 214–220.

Dorai, M., Papadopoulos, A.P., Gosselin, A., 2001. Influence of electric conductivity management on greenhouse tomato yield and fruit quality. Agronomie 21, 367–383.

Jauset, A.M., Sarasúa, M.J., Avilla, J., Albajes, R., 1998. The impact of nitrogen fertilization on feeding site selection and oviposition by *Trialeurodes vaporariorum*. Entomol. Exp. Appl. 86, 175–182.

Jauset, A.M., Sarasúa, M.J., Avilla, J., Albajes, R., 2000. Effect of nitrogen fertilization level applied to tomato on the greenhouse whitefly. Crop. Prot. 19, 255–261.

Jones, C.E., 1984. Animal feed. In: Wiliams, S. (Ed.), AOAC Official Analytic Chemists. Arlington, VA., pp. 155–156.

Jones, C.G., Hare, J.D., Compton, S.J., 1989. Measuring plant protein with the Bradford assay 1. Evaluation and standard method. J. Chem. Ecol. 15, 979–992.

Keisling, T.C., Mascagni, J.R., Maples, R.L., Thompson, K.C., 1995. Using cotton petiole nitrogen concentration for prediction of cotton nitrogen nutritional status on a clayey soil. J. Plant. Nutri. 18, 35–45.

Lei, H., Tjallingii, W.F., van Lenteren, J.C., 1998. Probing and feeding characteristics of the greenhouse whitefly in association with host-plant acceptance and whitefly strains. Entomol. Exp. Appl. 88, 73–80.

Mason, S.C., Wilcox, G.E., 1982. Nitrogen status evaluation of tomato plants. J. Am. Soc. Hortic. Sci. 107, 386–483.

Mattson, W.J., 1980. Herbivory in relation to plant nitrogen content. Ann. Rev. Ecol. Syst. 11, 119–161.

McClure, M.S., 1980. Foliar nitrogen: a basis for host suitability for elongate hemlock scale, *Fiorina externa* (Homoptera: Diaspididae). Ecology 61, 72–79.

McNeil, S., Southwood, T.R.S., 1978. The role of nitrogen in the development of insect–plant relationships. In: Harbone, J. (Ed.), Biochemical Aspects of Plant and Animal Co-evolutions. Academic, London, pp. 77–98.

Minkenberg, O.P.J.M., Fredrix, M.J.J., 1989. Preference and performance of an herbivorous fly *Liriomyza trifolii* (Deptera: Agromyzidae) on tomato plants differing in leaf nitrogen. Ann. Entomol. Soc. Am. 82, 350–354.

Mound, L.A., Halsey, S.M., 1978. Whitefly of the world. British Museum (Natural History). Wiley and Sons, Chichester, p. 340pp.

Ohta, K., Ito, N., Hosoki, T., Endo, K., Kajikawa, O., 1993. Influence of the nutrient solution on cracking of cherry tomato fruit grown hydroponically. J. Japan. Soc. Hort. Sci. 62, 407–412.

Pfeiffer, D.G., Burts, E.C., 1984. Effect of tree fertilization on protein and free amino acid content and feeding rate of Pear Psylla (Homoptera: Psyllidae). Environ. Entomol. 13, 1487–1490.

Prosser, W.A., Simpson, S.J., Douglas, A.E., 1992. How an aphid (*Acyrthosiphon pisum*) symbiosis responds to variation in dietary nitrogen. J. Insect. Physiol. 38, 301–307.

Rubeiz, I.G., Aslam, M., Chahine, H., Imad, A., 1995. Whitefly population in greenhouse cantaloupe as affected by poultry manure and inorganic nitrogen fertilizer application. Biol. Agric. Hortic. 12, 277–281.

SAS Institute Inc, 1999. SAS-STAT User Guide, version 6. SAS Institute, Cary, North Carolina.

Siddigi, M.Y., Kronzucker, H.J., Britto, D.T., Glass, A.D.M., 1998. Growth of a tomato crop at reduced nutrient concentrations as a strategy to limit eutrophication. J. Plant. Nutr. 21, 1879–1895.

Simpson, S.J., Abisgold, J.D., Douglas, A.E., 1995. Response of the pea aphid (*Acrythosiphon pisum*) to variation in dietary levels of sugar and amino acids: the significance of amino acid quality. J. Insect. Physiol. 41, 71–75.

van Emden, H.F., 1966. Studies on the relations of insect and host plant III. A comparison of the reproduction of *Brevicoryne brassicae* and *Myzus persicae* (Hemiptera: Aphididae) on Brussels sprout plants supplied with different rates of nitrogen and potassium. Entomol. Exp. Appl. 9, 444–460.

van Lenteren, J.C., Noduls, L.P.J.J., 1990. Whitefly–plant relationships: behavioural and ecological aspects. In: Gerling, D. (Ed.), Whiteflies: Their Bionomics, Pest Status and Management. Intercept Ltd, Andover, Hants, pp. 47–90.