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Push-pull Strategy for Control of Sweet-potato Whitefly, *Bemisia tabaci* (Hemiptera: Aleyrodidae) in a Tomato Greenhouse

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Push-pull 전략을 이용한 시설 토마토 담배가루이 방제효과

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ABSTRACT: This study was conducted to develop a technology for environmentally friendly control of sweet-potato whitefly, *Bemisia tabaci*, by controlling their behavior using a push-pull strategy in a tomato greenhouse. *B. tabaci* was attracted the most by yellow color, light source of 520 nm, whereas it avoided the complex light treatment of 450 + 660 nm. The two natural enemies of *B. tabaci*, *Cyrtopeltis tenuis* and *Orius laevigatus*, were attracted the most by 520 nm light source. *B. tabaci* was repelled by the volatile organic compounds ocimene and carvacrol and was the most attracted by methyl isonicotinate. When buckwheat was added into the tomato greenhouse, the density of *C. tenuis* was maintained at about 16 times higher than when untreated for 15 days. As a result of the combined treatment of push-pull strategy, the density per trap of *B. tabaci* was three times lower than when no treatment was applied, and the control of this pest increased with time and reached up to 68.7%.

Key words: *Bemisia tabaci*, Control, Natural enemy, Push-pull, Volatile compound

초 록: 본 연구는 시설토마토 재배지에서 push-pull 전략을 이용해 담배가루이와 천적곤충의 행동을 제어함으로써 담배가루이(*Bemisia tabaci*)를 친환경적으로 방제하기 위한 기술을 개발하고자 수행하였다. 담배가루이는 노란색에 유인율이 가장 높았으며, 520 nm의 광원에는 유인반응을 복합광원인 450 + 660 nm의 광원에는 회피반응을 보였다. 천적곤충인 담배장님노린재(*Cyrtopeltis tenuis*)와 미끌애꽃노린재(*Orius laevigatus*)는 모두 520 nm의 광원에 가장 높은 유인반응을 보였다. 휘발성 물질로는 ocimene과 carvacrol에 대해 기피반응을, methyl isonicotinate에는 유인반응을 보였다. 토마토 온실에 메밀을 투입하였을 경우 천적곤충인 담배장님노린재의 밀도는 무처리 대비 15일간 약 16배로 높게 유지되었다. Push-pull 세부전략들을 종합 처리한 결과, 처리 50일 경과 후 담배가루이의 트랩당 밀도는 무처리 대비 3배 이상 낮게 나타났으며, 이에 따른 시기별 방제효과는 시간이 경과함에 따라 증가하는 경향을 보인 가운데 최고 68.7%였다.

검색어: 담배가루이, 방제, 천적, Push-pull, 휘발성 물질

Sweet-potato whitefly, *Bemisia tabaci* (Gennadius), is the first insect pest found in Korea in 1998, which is known to affect more than 900 host species, including many vegetables and ornamental crops (Lee et al., 2000; Helmi, 2011). The most problematic biotypes among the more than 24 globally known biotypes are the B and Q biotype (Lee et al., 2005; Yang et al., 2009; Lee et al., 2012). Biotype Q is known to mediate more than 100 viruses including tomato yellow leaf curl virus (TYLCV) (Kim et al., 2008) and shows high resistance to neonicotinoid-based insecticides (Nauen et al., 2002; Lee et al., 2012). Therefore, it is necessary to identify diverse new strategies to control *B. tabaci*.

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The push-pull strategy was first used in 1987 in Australia to study repellent and attractive stimuli for the control of *Helicoverpa* sp. This approach has been developed as an integrated pest management tool that changes the distribution and density through behavioral control of pests or natural enemies (Cook et al., 2007; Khan and Pickett, 2008; Bhattacharyya, 2017). In the early days, companion crops (attractant or repellent plants) were mixed with crop cultivation, but in recent years, attempts have been made to utilize volatile compounds capable of attracting or avoiding insect pests (Cook et al., 2007; Bhattacharyya, 2017).

Apart from this, technologies for pest forecasting have been developed by using colors and light sources. If we use these forecasting techniques as a control strategy, we think that the push-pull strategy will be more successful. Colored traps are mainly used to monitor the density of insects. Kim and Lim (2011) devised a sticky trap with a yellow circular pattern on a black background as a way to capture more *B. tabaci* by using this visual cue. In recent years, along with colored traps, artificial light sources such as LED have also been widely used as a means of integrated pest management (Shimoda and Honda, 2013; Zheng et al., 2014). Most plants have functional compounds such as repellents, feeding deterrents, toxins, and growth regulators to defend against the attack of herbivorous insects (Maia and Moore, 2011). Plants that are damaged by insect pests are known to produce a variety of volatile organic compounds (VOCs) to protect them. Among these volatile substances, herbivore-induced plant volatiles (HIPVs) are closely related to each other, attracting other insects or natural enemies (Dicke and Baldwin, 2010; Xiao et al., 2012). Darshanee et al. (2017) have reported that *Trialeurodes vaporariorum* were strongly attracted to volatile compounds released from tomato leaves. Yang et al. (2010) have reported strong contact toxicity with essential oil extracted from *Thymus vulgaris* (insecticidal effect) and strong avoidance effect of the oil extracted from *Pogostemon cablin* (repellant effect) for *B. tabaci*. In order to enhance biological control of insect pest species, banker plants are widely used as a means to maintain insects that are natural enemies of these pests for a long period in the crop fields (Frank, 2010).

The purpose of this study was to select a companion plant, color, volatile matter, LED (light source), and banker plant that can regulate *B. tabaci* and attract natural enemies of this pest to establish a push-pull strategy in tomato greenhouses.

Materials and Methods

Insect rearing

Tomato (Solanum lycopersicum L.) was used as a host plant for rearing *B. tabaci* in the laboratory. One-month-old tomato seedlings were placed in a mesh cage (30 × 30 × 30 cm). The temperature was maintained at 25 ± 2℃, the relative humidity at 60-80%, and the light condition was maintained at 16 h of light and 8 h of darkness Occurrence pattern of *B. tabaci* in the greenhouse.

Occurrence pattern of *B. tabaci* in the greenhouse

To establish the optimal time for implementing the push-pull strategy, yellow sticky traps were placed at nine locations in a tomato (‘Superdotaerang’) greenhouse in Hwaseong area according to the method detailed in Song et al. (2014). The traps were collected daily to investigate the density of adult *B. tabaci*.

Selection of companion plants

Nine species including gypsophila (*Gypsophila elegans*), rosemary (*Rosmarinus officinalis*), marigold (Tagetes erecta L.), petunia (*Petunia hybrida* Vilm), spearmint (*Mentha arvensis* L.), lavender (*Lavandula angustifolia* L.), peppermint (Mentha piperita), geranium (*Pelargonium inquinans*), and sweet basil (*Ocimum basillicum*) were tested for their effectiveness as a companion plant in the tomato green house.

Each of these companion plants were placed in the tomato greenhouse in three replicates of three pots each. To ensure minimum interference from the other treatments, the distance between plants were maintained at 5 m. The density of *B. tabaci* was determined by replacing the yellow sticky traps on the top of the tomato plants every day and counting the trapped individuals.

Selection of attractant color

To determine which colors attracted and which repelled the pest species, we used red, black, white, green, blue, and yellow colored papers (21 × 15 cm each) sandwiched between two transparent sticky traps. The traps were placed at a distance of approximately 1 m parallel to the *B. tabaci* habitat. At each sampling location, a different colored trap was installed. The density of *B. tabaci* per trap was investigated by sequentially changing the position of the traps daily for seven days to minimize the effect of trap location.

Selection of volatile compound

Twelve volatile organic compounds (Table 2) were used in the tomato planted greenhouse (1200 m^2) to investigate their effect as either an attractant or repellent of *B. tabaci*. Commercially available insect pheromone lures were used. Each lure was treated by adding 30 μl of one of the twelve compounds, sealing it, and allowing it to be absorbed in a refrigerated condition (4℃) for 24 hours. A volatile compound-treated lure was attached to the center of the yellow sticky trap and placed on the top of the tomato plants. The distance between the traps was maintained at more than 5 m to reduce interference between treatments. The density of the trapped *B. tabaci* adults was examined after three days.

Selection of LED source

Based on literature, we tested 520, 660, and 730 nm LEDs (Fig. 1) and a composite light of 450 + 660 nm to select the light sources that can be packaged with companion plants. The test was carried out using a container (3 m × 3 m × 3 m) that prevented light from outside and in which the temperature could be maintained at 25 ± 2℃. Inside the container, one LED was placed on each wall and a tomato seedling was fixed with a colorless sticky trap, placed under each LED source. Then, approximately 350 adults were irradiated from the central part of the container and the number of *B. tabaci* adults captured per light source was investigated after 24 hours. All tests were conducted in three replicates.

Selection of banker plant

Based on literature and preliminary tests, we identified buckwheat (*Fagopyrum esculentum* Moench) as a potential banker plant to maintain natural enemies. Two pots (40.5 × 18.5 × 17.0 cm) planted with buckwheat (20 days after sowing) were placed per point at three points in the tomato planted greenhouse (1200 m^2). The distance between the points was maintained at 5 m or more to minimize the effect between treatments. The density of *B. tabaci* was investigated daily using a yellow sticky trap and six tomato plants around the banker plant were examined for the natural enemies.

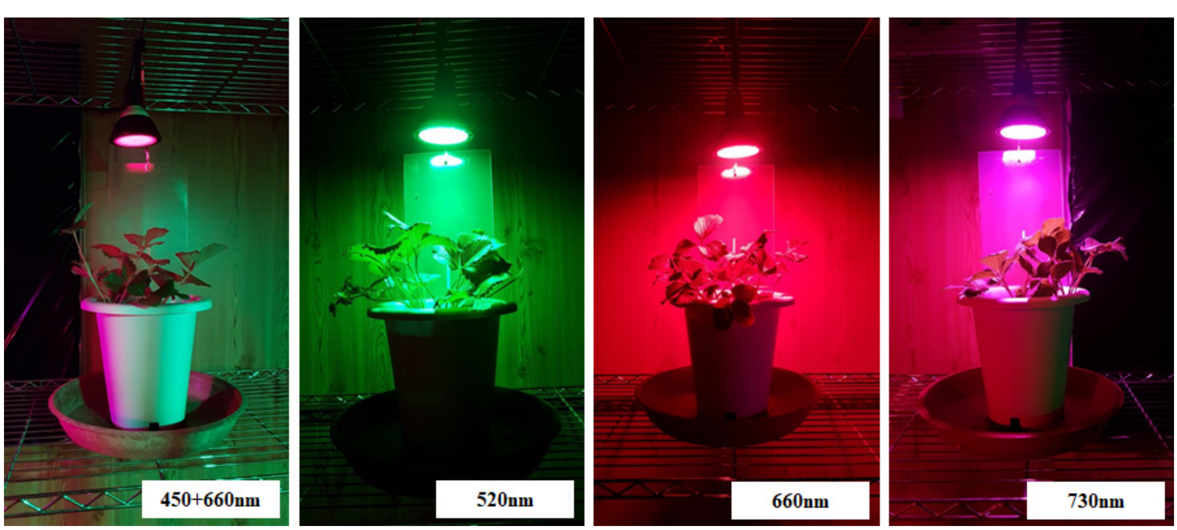

Fig. 1. Setup to test the attraction/repulsion of *Bemisia tabaci* and two natural enemies by LED source.

Integrated push-pull strategy effect test

The results from each of the above experiments were synthesized and tested for their combined effect in controlling *B. tabaci* in a tomato greenhouse (120 m^2). Sticky roll traps (20 cm × 100 m) were placed in two rows 15 cm above the tomato plants. Three carvacrol treated lures were hung above a tomato plant in the center of greenhouse as a repellent, and two methyl isonicotinate treated lures were placed at each greenhouse entrance areas as an attractant. Yellow sticky traps were set at two sites in the tomato greenhouse and the density of adult *B. tabaci* was investigated at weekly intervals. The detailed set up for this integrated push-pull strategy is shown in Table 1.

Statistical analysis

The relationship between volatile organic compounds and *B. tabaci* density was analyzed using SAS PROC ANOVA. The incidence rate of *B. tabaci* by LED source was analyzed using SAS PROC ANOVA after arcsine transformation (SAS Institute, 2013).

Results and Discussion

Occurrence pattern of *B. tabaci* in the greenhouse

Tomatoes in Gyeonggi area were cultivated twice a year. The *B. tabaci* density was significantly higher in the autumn season than in the spring season. In the spring season, *B. tabaci* increased greatly from early June, and in the autumn season, it increased from mid-September. Therefore, it can be surmised that the appropriate period for controlling *B. tabaci* using natural enemies is late May for the spring season and early September for the autumn season. Spatially, *B. tabaci* density gradually increased from the entrance to the inside of the greenhouse (Fig. 2). If *B. tabaci* can be efficiently attracted to a point near the greenhouse entrance, and if its natural enemy is also present at this location, not only can *B. tabaci* be detected quickly but the natural enemy can also be established efficiently. In addition, if a banker plant for the natural enemy is added at this location, it is possible to increase the effect of the natural enemy and minimize the pest density.

Selection of companion plants

Among the companion plants used in this test, geranium showed the most repelling effect for *B. tabaci*, lowering the density by 52% in comparison to untreated replicates. However, when spearmint, rosemary, and gypsophila were planted with tomato, *B. tabaci* density was, respectively, 265, 229, and 204% higher than when no companion plants were present (Fig. 3). Geranium may be used for the purpose of repelling *B. tabaci* using the plant itself or its extracts. Additionally, this species can be planted around the tomato greenhouse to inhibit *B. tabaci* infestation. On the other hand, plants attracting *B. tabaci* can be planted outside the tomato greenhouse along with treating the soil with systemic insecticides (Choi et al., 2016) to control *B. tabaci* around the tomato greenhouse. However, one of the most important aspects for consideration is whether the companion plant can play a lasting role. The companion plants selected in this study often showed other pest outbreaks and abnormal growth during

Table 1. Push-pull strategy for *Bemisia tabaci* control using natural enemy in tomato greenhouse

Technology introduced	Pull strategy		Push strategy
	Attraction of *B. tabaci*	Conserving N.E.	Repellent of *B. tabaci*
Light source (LED)	520 nm (outside the tomato community)		450 + 660 nm (inside the tomato community)
Volatile compound	Methyl isonicotinate (outside the tomato community)		Carvacrol (inside the tomato community)
companion plant	Egg plant (outside the tomato community)		-
Sticky trap	Yellow sticky roll trap (inside the tomato community)		-
Natural enemy (N.E.)	*Cyrtopeltis tenuis* (inside the tomato community)		
Banker plant	Buckwheat (inside the tomato community)		
Insect-proof net	-		Red colored net (outside the tomato community)

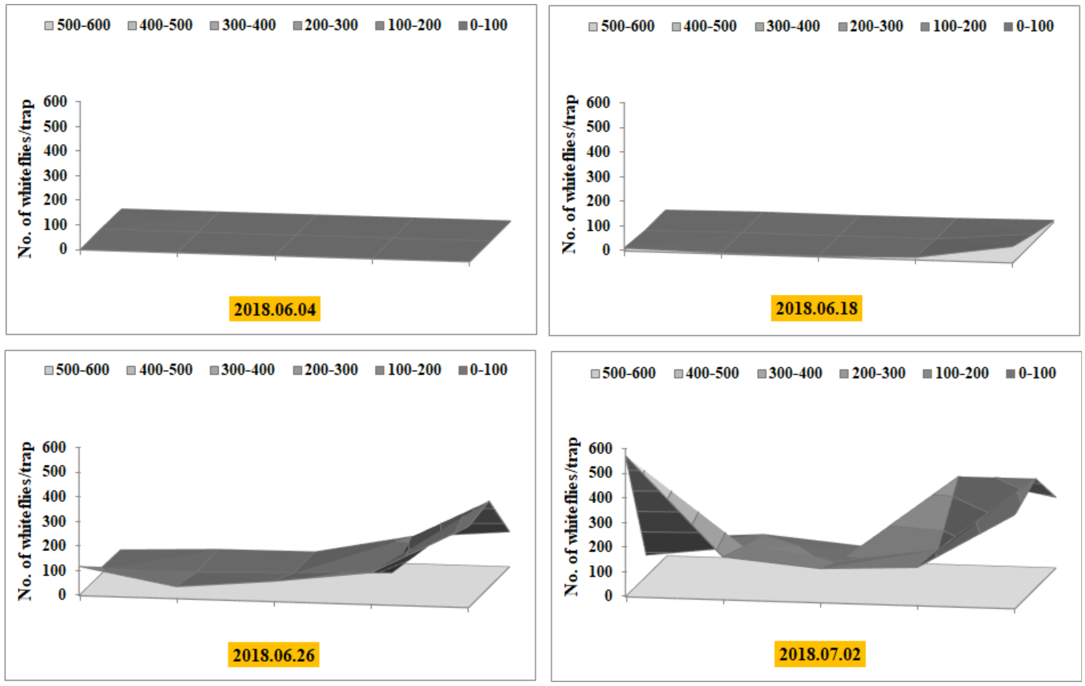

Fig. 2. Spatial and temporal patterns of occurrence of *Bemisia tabaci* in the tomato greenhouse.

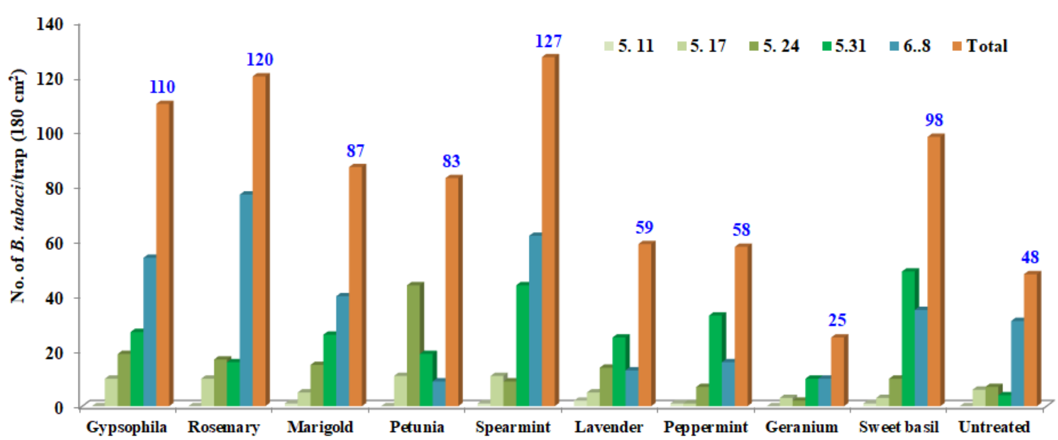

Fig. 3. Comparison of sweet-potato whitefly (*Bemisia tabaci*) densities across nine companion plant treatments in tomato greenhouse.

summer. Therefore, in this study, when developing and testing an integrated system using the various individual approaches studied here, eggplant seedlings that have been reported to strongly attract *B. tabaci* (Hasanuzzaman et al., 2017) were used as companion plants.

Selection of attractant color

The rate of attraction of *B. tabaci* was highest for yellow color at 62.4%. For white it was 1.6%, while it was lower than 0.8% for blue, red, and black was (Fig. 4). There are several studies that maximize the attraction of pests by using contrasting colors (Kim and Lim, 2011; Vernon and Gillespie, 1995). Vernon and Gillespie (1995) showed that western *Frankliniella occidentalis* was noticeably less attracted to yellow colored traps than those that were purple or yellow on blue backgrounds. Chu et al. (2000) reported that major sucking insects such as whitefly, thrips, and leaf hoppers preferred a color spectrum of 490 to 600 nm, and this spectrum was similar to the spectral reflectance curve on the back

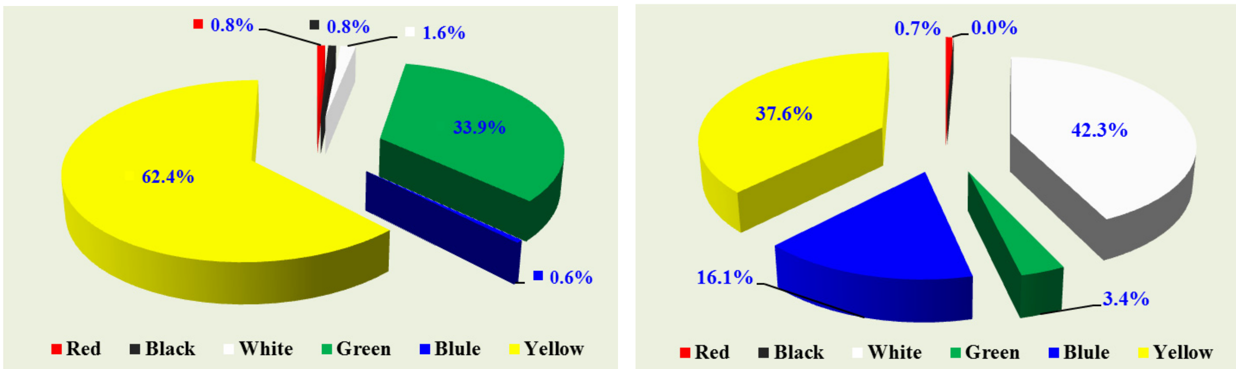

Fig. 4. Attraction rates of *Bemisia tabaci* (left) and *Frankliniella intonsa* (right) according to trap color.

surface of green leaves. Our results suggest that it is possible to maximally attract *B. tabaci* by using a yellow sticky trap with a black or red background, but further studies will be needed on this. Also, using red and black colored nets will be useful in blocking the influx of *B. tabaci* from outside.

Selection of volatile compound

Treatments where ocimene and carvacrol lures were used showed the lowest density of *B. tabaci* of 26% and 33%, respectively (Table 2). This suggests that these compounds acted as a *B. tabaci* repellant. On the other hand, the density of *B. tabaci* when methyl isonicotinate was used was 179% higher than when no VOC treated lure was used. The effect of volatile organic compounds in attracting insect pests as well as natural enemies has been studied (Koschier et al., 2002; Tan and Liu, 2014). The aim of this study was to improve the efficacy of natural enemies by using the push-pull strategy for controlling *B. tabaci* efficiently. Therefore, it may be desirable to install lures with methyl isonicotinate, which was highly effective in attracting *B. tabaci*, at the entrance of the tomato greenhouse where this pest first occurs. In addition, lures with carvacrol, which was the most efficient repellent, could be installed inside the tomato greenhouse.

Table 2. Comparison of *Bemisia tabaci* density according to treatment using 12 volatile organic compounds (VOCs) in tomato greenhouse

Volatile organic compound	No. of *B. tabaci* adult/trap/day				
	1 Rep.	2 Rep.	3 Rep.	Mean ± SD	Attraction rate (%)[b]
Methyl isonicotinate	83	79	78	80.0 ± 2.65a[a]	179
Dodecyl acetate	43	40	46	39.7 ± 3.51bc	89
Ethyl nicotinate	42	36	44	40.7 ± 4.16bc	91
Ocimene	15	11	9	11.7 ± 3.06i	26
cis-3-Hexenyl acetate	26	22	32	26.7 ± 5.03ef	60
Methyl salicylate	40	36	34	36.7 ± 3.06cd	82
cis-Jasmone	32	28	36	32.0 ± 4.00de	72
Ethyl isonicotinate	22	19	11	17.3 ± 5.69ghi	39
Carvacrol	15	11	18	14.7 ± 3.51hi	33
(1S)-(-)-Verbenone	20	17	20	19.0 ± 1.73gh	43
Methyl jasmonate	24	19	18	20.3 ± 3.21fgh	46
Methyl anthranilate	26	22	21	23.0 ± 2.65fg	51
Untreated	38	46	50	44.7 ± 6.11b	100

[a]Means followed by the same letter within a column are not significantly different at $\alpha = 0.05$, Duncan's multiple range test.
[b]Attraction rate (%) = No. of *B. tabaci* per substance (No. of *B. tabaci* untreated) × 100.

Selection of LED source

We selected an LED source that could be packaged with the banker plant for the attraction and early detection of *B. tabaci* in the tomato greenhouse. The 520 nm light source was the most effective in attracting *B. tabaci*, at 66.5%, while the lowest attraction rate was 3.7% for a combined light source of 450 + 660 nm (Table 3). Similar to the results from our study, Jahan et al. (2014) reported that *B. tabaci* adults were highly attracted to green light source (526 nm) regardless of the biotype, and they were least attracted to the blue light source. Meanwhile, Zheng et al. (2014) reported that the attraction of whitefly (*Aleurodicus dispersus*) adults to purple (405 nm) LED traps was higher than to blue (460 nm), green (520 nm), and yellow (570 nm) ones. LED source of 405 nm wavelength was not tested in this study and further studies on its effect on *B. tabaci* will be needed. The intensity of radiation differed with the light source. However, the intensity of radiation and the attraction of *B. tabaci* adults did not seem to have a positive correlation (Table 3). The effect of LED source on the natural enemies of *B. tabaci* is shown in Table 4. Two natural enemies, *Cyrtopeltis tenuis* and *Orius laevigatus*, were highly attracted to the 520 nm light source. Thus, the light source of 520 nm, which not only attracted *B. tabaci* but also two of its natural enemies at the same time, could be installed at the entrance of the tomato greenhouse along with planting the selected banker plant, at a time when *B. tabaci* occurrence begins.

Selection of banker plant

The first study in the use of a banker plant involved growing tomato plants that were pre-inoculated with *Encarsia formosa* along with the crop to control greenhouse whitefly, *T. vaporariorum*, in tomato greenhouse (Stacey, 1977). Of the studies undertaken on banker plants, 92% focus on the control of aphids, while less than 15% of the studies focus on the control of whitefly and thrips (Frank, 2010).

Figs. 5, 6 show the results of the density of *B. tabaci* and its natural enemy, *Cyrtopeltis tenuis*, when buckwheat was grown along with the tomato in the greenhouse. A high density of *C. tenuis* was maintained with the buckwheat treatment when compared to treatments where buckwheat was not used. Therefore, planting of buckwheat with *C. tenuis* in tomato greenhouse will be effective in increasing the control of *B. tabaci* and the additional input of *C. tenuis* can be reduced.

Presence of buckwheat and *C. tenuis* resulted in a 50% reduction in the density of *B. tabaci* in tomato greenhouse (Fig. 6). It is believed that *C. tenuis*, which was in the buckwheat,

Table 3. Comparison of *Bemisia tabaci* density according to LED sources

Light source (LED)	Intensity of radiation (Lux)	No. of adult/trap/day (%)			Attraction rate[a] (%)
		1 Rep.	2 Rep.	3 Rep.	
450 + 660 nm	5,154	1 (1.6)	2 (9.1)	1 (0.4)	3.7 ± 4.7c[b]
520 nm	1,497	45 (71.4)	11 (50.0)	200 (78.1)	66.5 ± 14.7a
660 nm	9,119	10 (15.9)	5 (22.7)	47 (18.4)	19.0 ± 3.5b
730 nm	87	7 (11.1)	4 (18.2)	8 (3.1)	10.8 ± 7.5bc

[a]Attraction rate (%) = No. of *B. tabaci* adult pet trap/Total no. of *B. tabaci* adult × 100.
[b]Means followed by the same letter within a column are not significantly different at α = 0.05, Duncan's multiple range test.

Table 4. Comparison of density of two natural enemies, *Cyrtopeltis tenuis* and *Orius laevigatus*, according to LED sources

Light source (LED)	No. of *Cyrtopeltis tenuis* (%)			No. of *Orius laevigatus* (%)			Total No. of NE	Attraction rate[a] (%)
	NI	NA	%	NI	NA	%		
450 + 660 nm	500	23	4.6	250	25	10.0	48	22.3
520 nm	500	118	23.6	250	45	18.0	163	75.8
660 nm	500	1	0.2	250	0	0.0	1	0.5
730 nm	500	3	0.6	250	0	0.0	3	1.4

[a]NI: No. of inoculated, NA: No. of attracted, NE: natural enemy.

moved to the tomato plants and effectively reduced the density of *B. tabaci*. On the other hand, another natural enemy, *O. laevigatus*, remained too low in density and eventually disappeared within seven days (Fig. 7). Therefore, it will be necessary to identify suitable banker plants to maintain *O. laevigatus*.

Integrated push-pull strategy effect

To effectively control *B. tabaci* in a tomato greenhouse, it is meaningful to integrate our results from testing the various push-pull approaches. The changes in the density of *B. tabaci* adults under an integrated push-pull strategy were investigated

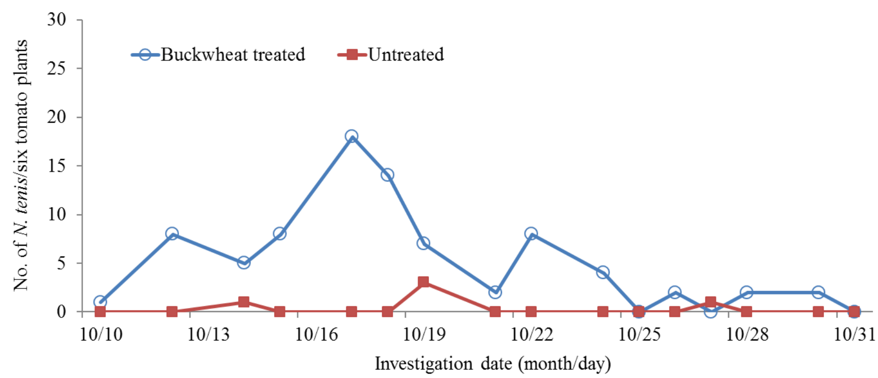

Fig. 5. Density changes of *Cyrtopeltis tenuis*, a natural enemy of *Bemisia tabaci*, when treated with buckwheat as a banker plant in a tomato greenhouse.

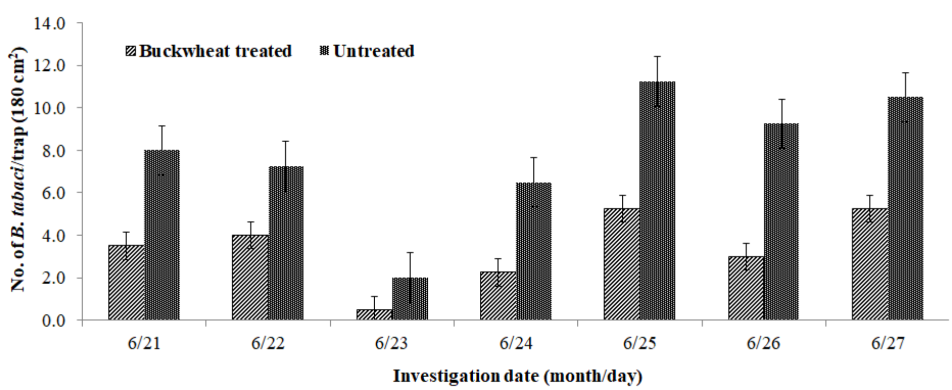

Fig. 6. Density changes of sweet-potato whitefly, *Bemisia tabaci*, when buckwheat and *Cyrtopeltis tenuis* were both present in the tomato greenhouse.

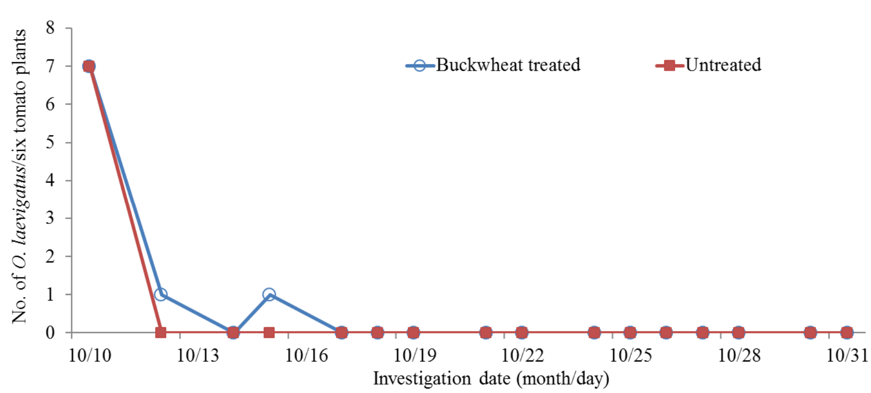

Fig. 7. Density changes of *Orius laevigatus*, a natural enemy of *Bemisia tabaci*, after including buckwheat in the tomato greenhouse.

in the tomato greenhouse (Fig. 8). After 50 days of treatment, the density of *B. tabaci* adults was 250.8 adults per trap, which was three times lower than that when no treatment was applied. Control of the pest infestation increased with time after treatment and showed a maximum value of 68.7%. Until now, many studies on pest control have focused on the reduction of pest density, but it is meaningful that the study also verifies an increase in the yield, the ultimate goal of pest control. Table 5 compares the tomato yield when the integrated push-pull strategy for controlling *B. tabaci* control was applied with yield obtained when no treatments were used. In the case of non-treatment, the quantity of tomato produced per 990 m^2 was 3648 kg excluding 20.8% of non-commodities, while that produced after the integrated strategy treatment was 5230 kg, which was 143.4% higher, and the non-commodities rate decreased by about 5%. This result cannot be regarded as an effect exclusively through the control of *B. tabaci*. However, this strategy can be used as a means to further expand biological control because it has the advantage of maximizing the control effect, sustainability, and minimizing the adverse effects on the environment in the absence of the use of insecticides. Also, efforts must be made to create conducive environmental conditions before applying the push-pull strategy developed in this study to commercial greenhouse, which may incur costs. However, if there are long-term investments supported by further studies to reduce the cost incurred, this could become the best push-pull strategy to control *B. tabaci* in tomato greenhouses in Korea.

Acknowledgements

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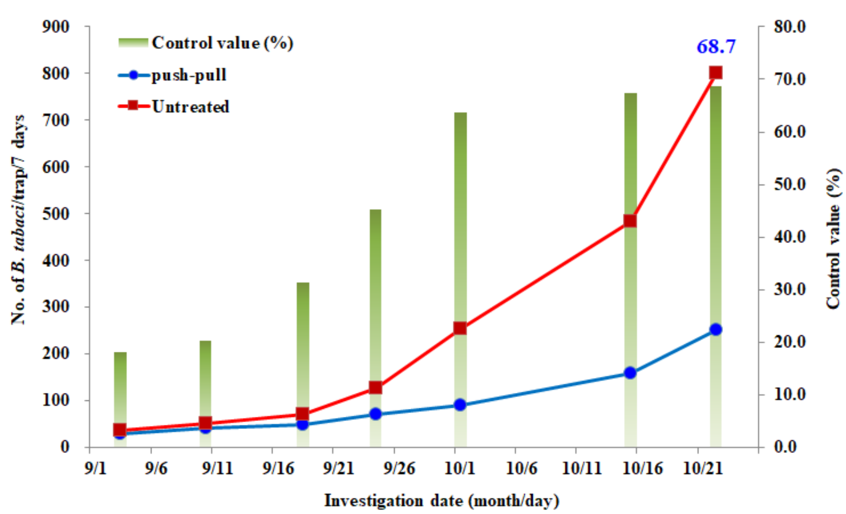

Fig. 8. *Bemisia tabaci* density and changes in its control because of the use of push-pull techniques in a tomato greenhouse.

Table 5. Comparison of tomato yield due to push-pull techniques in greenhouse (kg/990 m^2)

Push-pull techniques treated			Untreated		
Commodity	Non-commodity	Sum	Commodity	Non-commodity	Sum
5,230	924	6,154	3,648	957	4,605

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