Use of prohydrojasmon to suppress *Frankliniella occidentalis* and tomato spotted wilt virus in chrysanthemums

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Abstract

We evaluated the suppressive effect of prohydrojasmon (PDJ) on *Frankliniella occidentalis* and tomato spotted wilt virus (TSWV) in chrysanthemums under semi-commercial conditions. Overhead sprinkling of plants with PDJ did not suppress initial colonization by released adult thrips. However, it significantly reduced subsequent feeding damage on leaves and reproduction of larval offspring. Rates of 1 and 2 L/m^2 0.8 mM PDJ equally suppressed *F. occidentalis* and feeding damage without phytotoxic effect on chrysanthemums, although 3 L/m^2 reduced plant growth. The PDJ had a residual effect of at least 8 days. Weekly applications suppressed the occurrence of TSWV transmitted by viruliferous adults dispersed from infection-source plants to a degree consistent with that of feeding damage, so PDJ might inhibit primary infection via disruption of feeding behavior. All our findings suggest that PDJ may offer a powerful option for the control of *F. occidentalis* and *Orthotospovirus* diseases transmitted by it in chrysanthemum greenhouses.

Introduction

Thrips are important horticultural pests globally because of the damage they inflict by feeding and their ability to transmit plant viruses (Reitz et al., 2011). *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae), the western flower thrips, is one of the most destructive pests of vegetables and ornamental crops worldwide because of its polyphagous nature (Reitz, 2009). It is difficult to manage because it tends to occupy enclosed and concealed tiny spaces in plants and has developed resistance to various insecticides worldwide (Jensen, 2000; Herron & James, 2005; Gao et al., 2012). It not only causes considerable aesthetic feeding damage to ornamental and fruiting crops, but also transmits *Orthotospovirus* viruses such as tomato spotted wilt virus (TSWV) to various crops and cut flowers (Rotenberg et al., 2015). Thus, this so-called supervector is a serious menace to stable production of crops around the world.

Chrysanthemum (*Chrysanthemum morifolium* Ramat.; Asteraceae) is one of the most economically important flower crops worldwide. The flower stalks are harvested from fields across Japan over an area totaling more than 4300 ha, and had an economic value of 68×10^9 JPY in 2020. *Frankliniella occidentalis* not only causes deterioration of the cut flower quality due to feeding damage, but also causes severe yield losses by efficiently transmitting TSWV (Matsuura et al., 2002) and chrysanthemum stem necrosis virus (CSNV) (Matsuura et al., 2007). However, many pesticides are inadequate to control it in chrysanthemums, since it concentrates in tightly enclosed feeding spaces such as apical buds, where pesticides fail to reach. In addition, the Japanese government has formulated measures that include aiming at a 50% reduction in the use of chemical pesticides, including neonicotinoid insecticides, by promotion of integrated pest management and new alternatives.

Jasmonic acid (JA), a plant hormone, is involved in the induction of direct defenses against herbivores in infested plants (Smith et al., 2009). Using an Arabidopsis–thrips system, Abe et al. (2008, 2009) revealed that *F. occidentalis* infestation induced the JA defense-signaling pathway, and exogenous application of

JA to plants reduced thrips damage and enhanced plant resistance. Thus, JA plays an important role in tolerance to thrips feeding.

Prohydrojasmon (PDJ), an analog of JA, was first registered as a plant growth regulator and is used for promoting apple and grape coloring (Koshiyama et al., 2006; Atay, 2015). Subsequently, Uefune et al. (2014) reported that PDJ treatment of lima bean plants reduced the performance of *Tetranychus urticae* (two-spotted spider mite). Spraying of PDJ onto tomato plants suppressed thrips infestation under greenhouse conditions without adverse effects on fruit yield and quality (Matsuura et al., 2020). Yoshida et al. (2021) reported that foliar application of PDJ to Japanese radish plants induced direct defenses against several insect pest species, including thrips, under open field conditions, although it also reduced their biomass. Thus, PDJ has the potential to protect various crops from herbivore attacks.

Our aims here were to verify whether PDJ application to chrysanthemums could suppress infestation by *F. occidentalis* and to clarify optimal conditions under which this agent can be applied in commercial chrysanthemum production. We also evaluated its efficacy at reducing primary infection of TSWV by the suppression of feeding on chrysanthemum plants.

Materials And Methods

Chrysanthemum cultivation, PDJ application, thrips population, and virus

Chrysanthemum cultivar ‘Jimba’, which is one of the most popular standard-type cultivars in Japan, was used in all experiments. Cuttings were rooted and plantlets were raised in 200-cell plug trays filled with vermiculite in a nursery glasshouse under misting. The experiments were conducted in a 90-m^2 (6 m × 15 m; height, 3.6 m) plastic film (polyolefin) greenhouse at the Hiroshima Prefectural Technology Research Institute (location: 34° 25' 02" N, 132° 42' 00" E, altitude 222 m). Three raised beds (Super Drain Bed, JA Zennoh, Tokyo, Japan; 1.1 m × 12 m; depth, 0.4 m) containing granite soil and composted bark were constructed ~ 0.9 m apart in the greenhouse. Insect-proof screens (0.4-mm mesh size) were installed on all openings to prevent other insect pests from entering. Plantlets raised for 16–17 days in the nursery were transplanted into the beds in 4 rows (60 plants per row), with 20 cm between plants. Irrigation was automatically applied as a fertigation solution of water and nutrients (75 mg N/L, 40 mg P/L, 85 mg K/L; OKF-1, OAT Agrio Co., Ltd.) via a Dosatron fertilizer injector (Dosatron International, Inc., Bordeaux, France). The greenhouse air temperature was maintained between 16 and 28°C by a heating system (NEPON Inc. KA-321, Tokyo, Japan) and ventilation fans. Chrysanthemums were grown under a natural photoperiod plus 4-h night-interruption lighting (22:00 to 02:00) supplied by incandescent lamps.

We used a 1:250 (0.8 mM) commercial PDJ formulation (5% soluble liquid; Jasmomate-Ekizai, MMAG Co., Ltd., Tokyo, Japan) in all experiments. Preliminary unpublished experiments showed that, unlike on solanaceous plants such as tomato (Matsuura et al., 2020), the suppressive effect of foliar application of PDJ by atomizing spray on thrips on chrysanthemum plants is low. Therefore, we applied the PDJ

solution by overhead sprinkling from a watering can with a showerhead nozzle. The solution that runs off the plants is assumed to be applied simultaneously to the roots. It was also sprinkled onto the plantlets at 2 L per plug tray 2 or 3 days before transplanting except in the viral transmission experiment. Lufenuron (5% emulsifiable concentrate; Match, Syngenta Co., Tokyo, Japan), an insect growth regulator (IRAC Group 15), was used as a reference. It was diluted 1:1000 and was sprayed to runoff from knapsack-type power sprayer.

A colony of *F. occidentalis*, originally collected in an open field of chrysanthemums in Hiroshima Prefecture in 2010, was maintained on broad beans in cages in a growth chamber at 23°C.

TSWV isolate HC-1 (accession no. LC712334), derived from a chrysanthemum production area in Hiroshima Prefecture in 2010, was multiplied in *Nicotiana rustica* plants by sap inoculation.

Thrips investigation

We investigated 20 (24 in the viral transmission experiment) interior plants (surrounded by plants on the borders and both sides of 4 rows) in each plot. We counted the adult and larval thrips carefully by eye with the aid of a 4× magnifier on the apical buds and upper 5 leaves of interior plants in each plot. In the final investigations, thrips on middle to upper developed leaves were counted by eye, and then the thrips infesting apical buds were extracted by stirring in 70% ethanol and counted under a stereomicroscope. We counted the number of leaves with feeding scars among the 5 topmost developed leaves in each plot. A scar was defined as clearly visible silvering or a keloid on the leaf surface. Plant heights were measured to assess effects of PDJ on plant growth. Data were tested by ANOVA, followed by comparison by Tukey's HSD test, in IBM SPSS v. 20 software.

Effects of PDJ application on thrips infestation and chrysanthemum growth

We first evaluated the efficacy of a large application of PDJ at 3 L/m^2 at suppressing *F. occidentalis*. Chrysanthemum plantlets were transplanted into the beds on 12 November 2020. The experiment was designed as a 3 × 3 Latin square with randomization. Each treatment plot (1.1 m × 3 m) consisted of 56 plants. As PDJ can prevent infestation of tomato plants by thrips (Matsuura et al., 2020), we applied it before thrips release. The treatments consisted of 3 L/m^2 PDJ, 0.25 L/m^2 lufenuron, and an untreated control. PDJ was first applied to plantlets in the nursery 3 days before transplanting. It was next applied in the beds 1, 6, and 12 days after transplanting (total of 4 times). One day after the second PDJ application in the beds, ~ 300 adult thrips were released from a glass tube placed 1.6 m above the plant canopy in the center of each plot, equivalent to ≈ 5.4 per plant. Five days after release (DAR), lufenuron (reference agent) was sprayed at 0.25 L/m^2. Thrips infestation was investigated in the interior 20 plants in each plot until 10 days after the final application of PDJ (15 DAR). The height of the same 20 plants in each plot was measured at the final investigation (22 days after transplanting).

Effect of rate of PDJ on thrips infestation

Plantlets were transplanted into the beds on 15 May 2021. The experiment was arranged in a completely randomized design with 3 replications. Each treatment plot (1.1 m × 3 m) consisted of 56 plants. The treatments consisted of PDJ at 2 and 1 L/m^2, lufenuron at 0.3 L/m^2, and an untreated control. PDJ was first applied to plantlets in the nursery 2 days before transplanting. It was next applied in the beds 2, 7, and 12 days after transplanting (total of 4 times). One day after the second PDJ application in beds, ~ 300 adult thrips were released in each plot as above, equivalent to ≈ 5.4 per plant. Four DAR, lufenuron was sprayed at 0.3 L/m^2. Thrips infestation and feeding damage were investigated in the interior 20 plants in each plot until 7 days after the final application of PDJ (11 DAR).

Effect of frequency of PDJ application on thrips infestation

Plantlets were transplanted into the beds on 21 October 2021. The experiment was arranged in a completely randomized design with 3 replications. Each treatment plot (1.1 m × 3 m) consisted of 56 plants. The treatments consisted of PDJ at 2 L/m^2, 0.25 L/m^2 lufenuron, and an untreated control. PDJ was first applied to plantlets in the nursery 2 days before transplanting. It was next applied in the beds 4, 8, and 14 days after transplanting (total of 4 times) or 6 and 14 days after transplanting (total of 3 times). Eight days after transplanting, ~ 400 adult thrips were released in each plot as above, equivalent to ≈ 7.1 per plant. Three DAR, lufenuron was sprayed at 0.25 L/m^2. Thrips infestation was investigated on the interior 20 plants in each plot until 7 days after the final application of PDJ (13 DAR).

Effect of PDJ application on occurrence of TSWV

Chrysanthemum plantlets were transplanted into the beds on 3 December 2021. We cut the main stem at 5 cm above the soil surface and pruned each branch to a single stem 3–5 cm long on 9 February 2022. The treatments consisted of PDJ (2 L/m^2) and a water-treated control. The experiment was arranged in a completely randomized design with 4 replications. Each treatment plot (1.1 m × 4 m) consisted of 80 plants. PDJ was applied in the beds 1, 8, 15, and 22 days after pruning (total of 4 times).

Datura stramonium plants were grown in 15-cm-diameter plastic pots in an isolated glasshouse. To obtain TSWV-infected plants, leaf tissue from systemically infected *N. rustica* plants was ground in 0.1 M phosphate buffer, pH 7.0. Inocula were rubbed on carborundum-dusted leaves of *D. stramonium* plants at the fifth-leaf stage on 20 January. One hundred adult thrips were released onto each of 16 plants with mottling symptoms at the sixth- to seventh-leaf stage. The plants were enclosed by a 0.3-mm mesh net to prevent thrips escape. These plants, in which larval offspring had hatched, were introduced at the 11th to 12th leaf stage onto the beds as TSWV infection sources: they were placed on either long side of each plot 4 days after the first PDJ application on 14 February. Thus, four *D. stramonium* plants were placed along each bed (12 plants in greenhouse). Thereafter, emerged (viruliferous) adults dispersed under natural conditions. Thrips infestation and feeding damage were investigated on the interior 24 chrysanthemum plants in each plot until 31 days after the introduction of the *D. stramonium* plants (36 days after pruning). The incidence of TSWV in chrysanthemums was assessed by eye, with attention to pronounced foliar symptoms such as leaf chlorosis, necrosis, and deformation (Matteoni & Allen, 1989) on the 48 plants in the middle part of each plot (including both sides of 4 rows), 24 and 31 days after the

introduction of the source plants. Leaves with suspicious symptoms were detached and tested with an ImmunoStrip assay (Agdia Inc., Elkhart, IN, USA) to detect TSWV. To confirm latent infections, leaves of symptomless chrysanthemum plants were tested by double antibody sandwich – enzyme-linked immunosorbent assay (DAS-ELISA) at the final investigation: in brief, 3 leaves were detached from the upper, middle, and lower parts of symptomless plants, since this virus does not spread systemically in chrysanthemums (Matsuura et al., 2004). The leaves were mixed and ground in a 10× volume of 0.02 M phosphate-buffered saline with 0.05% Tween 20 (pH 7.4). DAS-ELISA to detect TSWV was performed using monoclonal antibody to the nucleocapsid (N) protein of this virus (Tsuda et al., 1994) in 96-well plates (Nunc C96 Maxisorp immune plate, Roskilde, Denmark). Both anti-TSWV N protein IgG and alkaline phosphatase (AP)-conjugate were diluted 1:500 from 1 mg/mL stocks. The optical density (OD) of the samples at 405 nm was rated after 2 h incubation with AP-substrate (*P*-nitrophenyl phosphate) at room temperature. The cutoff value was defined as 2× the mean value of homogenates of different healthy (negative) samples (mean ± SD = 0.080 ± 0.003, $n = 10$).

Results

Effect of PDJ application on thrips infestation and chrysanthemum growth

The number of adult *F. occidentalis* on chrysanthemum plants in PDJ plots 1 DAR was not significantly different from the untreated control ($P = 0.114$), indicating no repellent effect of PDJ against adults (Fig. 1a). The number of thrips (> 98% were larval offspring) 15 DAR in PDJ plots was significantly lower than the control ($P < 0.01$), and was comparable to that in lufenuron (reference agent) plots ($P = 0.969$) (Fig. 1a). The incidence of leaves with feeding scars in PDJ plots was significantly lower than the control at 8 ($P < 0.05$) and 13 ($P < 0.01$) DAR, and also lower than lufenuron plots at 13 ($P < 0.01$) DAR (Fig. 1b). Plant height in PDJ plots 10 days after the final application (22 days after transplanting) was significantly lower than the control ($P < 0.01$), indicating that 3 L/m^2 PDJ reduced chrysanthemum growth (Fig. 2).

Effect of rate of PDJ application on thrips infestation

The numbers of adult *F. occidentalis* on plants in PDJ plots 1 DAR were not significantly different from the control ($P = 0.703$, 2 L/m^2; $P = 0.945$, 1 L/m^2; Fig. 3a). The numbers of thrips (> 96% were larval offspring) in PDJ plots 11 DAR were also not significantly different from the control ($P = 0.119$, 2 L/m^2; $P = 0.197$, 1 L/m^2), and were similar to those in the lufenuron plots ($P = 0.485$, 2 L/m^2; $P = 0.318$, 1 L/m^2; Fig. 3a). On the other hand, the incidence of leaves with feeding scars 6 DAR tended to be lower in PDJ plots, especially at 2 L/m^2 (at ~ 25% of the control incidence; $P = 0.054$; Fig. 3b); and those 11 DAR at both PDJ rates and in lufenuron plots were significantly lower than the control ($P < 0.05$). Plant height in the PDJ plots was not significantly different from the control (data not shown).

Effect of frequency of PDJ application on thrips infestation

The number of adult *F. occidentalis* on plants in PDJ plots 3 DAR was not significantly different from the control with either 4 ($P = 0.996$) or 3 ($P = 0.941$) applications (Fig. 4a). The number of thrips (> 94% were larval offspring) in PDJ plots 13 DAR was significantly lower than the control at both 4 ($P < 0.01$) and 3 ($P < 0.05$) applications (Fig. 4a). That in the 4-application plots was similar to that in the lufenuron plots ($P = 0.906$; Fig. 4a). The incidence of leaves with feeding scars in PDJ plots was significantly lower than in the control and lufenuron plots at all times. In particular, the incidence of feeding damage 7 DAR in the 4-application plots was suppressed to about 35% of the control ($P < 0.001$; Fig. 4b). Although that in PDJ plots 13 DAR was significantly lower than the control, the inhibitory effect decreased to about 60–65% of the control ($P < 0.01$, 4 and 3 applications; Fig. 4b). Plant height and the number of leaves in PDJ plots throughout the experiment were not significantly different from the control at either application frequency (data not shown).

Effect of PDJ application on occurrence of TSWV

The number of adults on chrysanthemum plants that emerged and dispersed from TSWV-infected *D. stramonium* plants (infection source) started to increase about 10 days after the introduction (DAI) of *D. stramonium* (Fig. 5a). The numbers of thrips (> 83% were emerged adults) were not significantly different between PDJ and control plots until 24 DAI of *D. stramonium* except at 17 DAI (Fig. 5a), suggesting that PDJ application might not repel migrating viruliferous adults. The number of thrips (> 81% were larval offspring) was significantly lower in PDJ plots than in the control 31 DAI ($P < 0.05$) (Fig. 5a). The incidence of leaves with feeding scars remained lower in PDJ plots than in the control 17, 24 and 31 DAI ($P < 0.05$; Fig. 5b). The incidence of chrysanthemum plants which expressed TSWV symptoms was remarkably suppressed in PDJ plots relative to the control 24 DAI ($P < 0.01$; Fig. 6a). Although the occurrence of symptomatic plants subsequently increased in PDJ plots, the final incidence of TSWV-infected plants, including ELISA-positive symptomless plants, in PDJ plots was lower than in the control 31 DAI ($P < 0.01$; Fig. 6b). About 90% of TSWV-infected plants expressed TSWV symptoms in both PDJ and control plots 31 DAI, indicating that the symptoms appeared equally in both treatments (Fig. 6).

Discussion

It is crucial to prevent thrips infestation early during crop growth in any strategy to control feeding damage and viral diseases. The characteristic feature of PDJ, an analog of JA, is that it controls herbivores by disrupting their behavior, as opposed to conventional synthetic insecticides, which just kill pests (Uefune et al., 2014; Matsuura et al., 2020; Yoshida et al., 2021). Here, we evaluated the suppressive effect of PDJ application on infestation by *F. occidentalis* and TSWV primary infection transmitted by it in the early growth stage of chrysanthemums under semi-commercial conditions.

Spraying of tomato plants with PDJ significantly repelled released *F. occidentalis* adults and inhibited subsequent reproduction of larval offspring (Matsuura et al., 2020). Moreover, the incidence of thrips on

PDJ-treated Japanese radish plants was always lower than that in water-treated control plants (Yoshida et al., 2021). Thus, we assume that PDJ treatment of particular crops repels migrating adult thrips and interferes with their settlement. Here, however, overhead sprinkling of PDJ from a showerhead nozzle did not suppress initial colonization of released *F. occidentalis* adults under any experimental conditions, even at 3 L/m^2, which applied more PDJ than atomizing spray. These results suggest that PDJ has minimal repellent effect against migrating adults in chrysanthemums, unlike on tomato (Solanaceae) or radish (Brassicaceae). Unlike in tomatoes, *F. occidentalis* adults commonly concentrate in enclosed apical buds in chrysanthemums. It is possible that the apical buds, with undeveloped leaves, are unable to induce a defense response via PDJ signaling pathways. On the other hand, PDJ significantly reduced thrips feeding damage in new leaves developed after release in almost all experiments, in spite of its insufficient inhibition of settlement by released adults. PDJ-sprayed tomato plants had few feeding scars, even though a few thrips were wandering on the leaves; this suggests that spraying tomato plants with PDJ may disrupt the thrips' feeding behavior (Matsuura et al., 2020). This phenomenon has also been confirmed in *Arabidopsis* plants treated with JA (Abe et al., 2008). Thus, we assume that application of PDJ to chrysanthemums substantially suppresses feeding behaviors of *F. occidentalis*. Chen et al. (2020) found that JA application to apical leaves of chrysanthemums reduced *F. occidentalis* damage in newly developed leaves. They also found that JA enhanced levels of phenolic compounds such as chlorogenic acid and caffeoylquinic acid, which are associated with chrysanthemum resistance to thrips (Leiss et al., 2009), suggesting that these phenolic compounds contribute to the higher resistance to thrips after JA application. It would be interesting to ascertain whether similar metabolic alterations occur in PDJ-treated chrysanthemums. Application of PDJ reduced the amount of larval offspring in almost all experimental conditions to a similar extent as lufenuron (the reference agent). This may be due to decreased fitness of *F. occidentalis* adults associated with deterred feeding behavior on PDJ-treated chrysanthemums. There was no significant difference in efficacy between 4 and 3 applications, so the residual activity of PDJ against *F. occidentalis* on chrysanthemum is at least 8 days.

A high rate of PDJ application (3 L/m^2, 0.8 mM) significantly suppressed the growth of chrysanthemums. Five consecutive sprays of 0.4 mM PDJ transiently inhibited the growth of tomato (Matsuura et al., 2020). PDJ significantly inhibited the growth of roots of komatsuna (*Brassica rapa* var. *periviridis*) and eggplant (Azis et al., 2020). Similarly, it reduced the weights of both above- and belowground parts of Japanese radish (Yoshida et al., 2021). Thus, PDJ treatment seems likely to reduce the growth of various crops. However, rates of $\leq$ 2 L/m^2 did not inhibit chrysanthemum growth and were effective at suppressing thrips infestations. Furthermore, we have confirmed that these rates had no phytotoxic effects on various chrysanthemum cultivars, including standard-type and spray-type, in open field and greenhouse experiments (unpublished data). These results suggest that rates of $\leq$ 2 L/m^2 0.8 mM PDJ would be acceptable for practical use on chrysanthemums.

Thus, weekly application of PDJ significantly suppressed TSWV infection by dispersed viruliferous *F. occidentalis* adults in spite of the high infection pressure. Considering the developmental temperature of *F. occidentalis* (McDonald et al., 1998; Ishida et al., 2003), we presume that all TSWV-diseased

chrysanthemum plants were infected through transmission by the emerged viruliferous adults originated from the introduced larval thrips (offspring of initial released adults). We verified that the disease incidence of PDJ-treated chrysanthemum plants which were mechanically sap-inoculated with TSWV was not different from that in untreated control plants (unpublished data), indicating that suppression of TSWV was associated with the inhibition of feeding behavior of the thrips, not inhibition of virus multiplication in PDJ-treated plants. The degree of TSWV suppression by PDJ application was consistent with that of feeding damage (Figs. 5b, 6). The mean infection access period for TSWV transmission by *F. occidentalis* is 1–2 h, and the optimum period can be > 40 h, depending on the inoculated plants (Wijkamp et al., 1996). Such a long infection access period may explain the consistency of suppression levels between TSWV infection and feeding damage.

Thrips lacerate plant cells and imbibe cellular fluids through their stylets (Hunter & Ullman, 1989). Such cell-content feeding induces the JA-regulated defense-signaling pathway (Walling, 2000). JA-regulated plant defense against herbivore attack is defined by complex physiological phenomena through the expression of various genes and the synthesis of secondary plant metabolites (Walling, 2000; Pieterse et al., 2012; Okada et al., 2015). It seems likely that PDJ, as a JA analog, has a similar mode of action. Yoshida et al. (2021), in fact, revealed that PDJ treatment of Japanese radish plants induced the expression of various genes, not only those regulated by the JA signaling pathway, such as lipoxygenase gene (*LOX*), but also genes related to the biosynthesis of secondary metabolites, such as glucosinolate. A mechanism based on a plant-induced defense system probably poses a low risk of the development of resistance in thrips. In view of its unique mechanism based on disrupting thrips behavior, PDJ may offer a powerful option for the control of *F. occidentalis* and *Orthotospovirus* diseases transmitted by it in chrysanthemum grown in open fields or greenhouses.

In conclusion, 3 or 4 weekly overhead applications of 0.8 mM PDJ to chrysanthemum plants at 1–2 L/m^2 in the early growth stage could suppress feeding damage and reproduction of larval offspring of *F. occidentalis* without phytotoxic effect. They could also inhibit the primary infection of TSWV transmitted by migrating adult thrips. On the basis of these results, we have applied for registration of PDJ as a thrips control agent on chrysanthemum in Japan for practical use. Further studies are needed to improve the control effects of PDJ against *F. occidentalis* and *Orthotospovirus* through its systemic use combined with insecticides or natural enemies in cultivation based on integrated pest management.

Declarations

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Authors' contributions

S.M. and T.S. conceived and planned the research. S.M. and U.T. conducted the experiments. S.M. wrote the manuscript. All authors read and approved the manuscript.

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Ethics approval

This article does not contain any studies undertaken on humans or sentient animals.

Conflict of interest

The authors declare no conflicts of interest.

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Figures

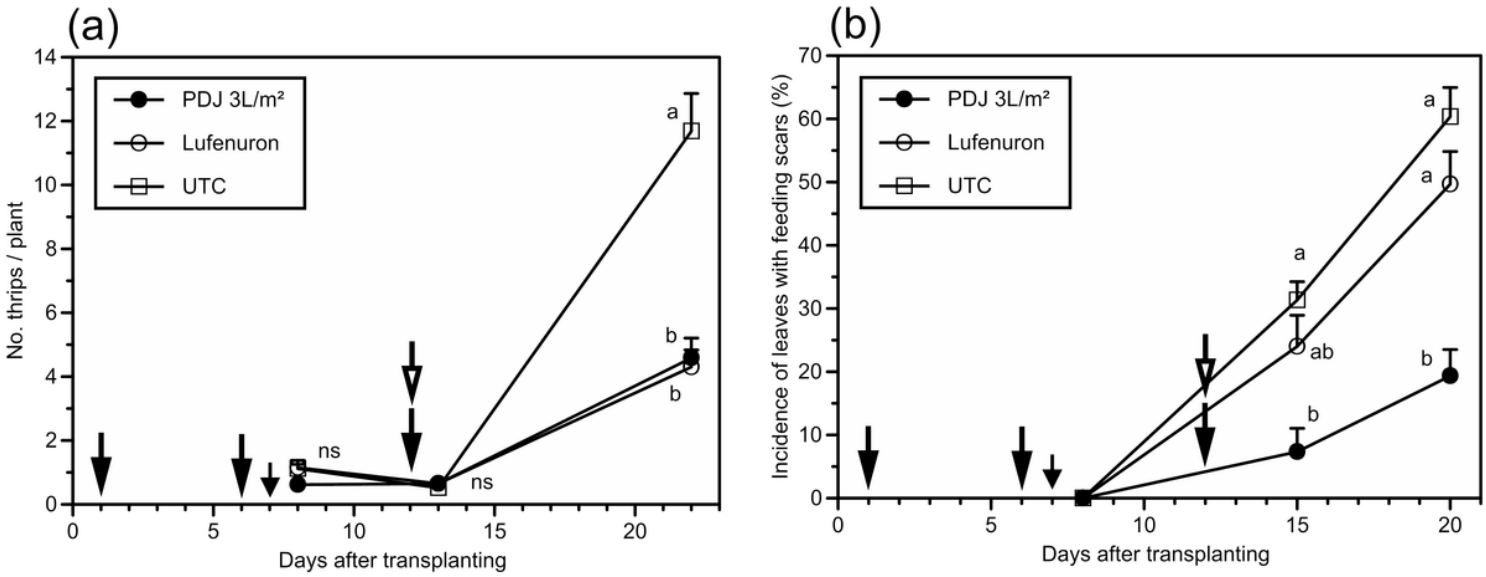

Figure 1

(a) Numbers of *Frankliniella occidentalis* (larvae + adults) and (b) incidence of leaves with feeding scars per chrysanthemum plant in each treatment. Small arrow, time of release of *F. occidentalis* adults; solid arrows, PDJ applications; open arrow, lufenuron spray. UTC = untreated control. Values are means ± SE. Means with the same letter are not significantly different ($n = 3$, $P < 0.05$; Tukey's test). PDJ was applied first in the nursery.

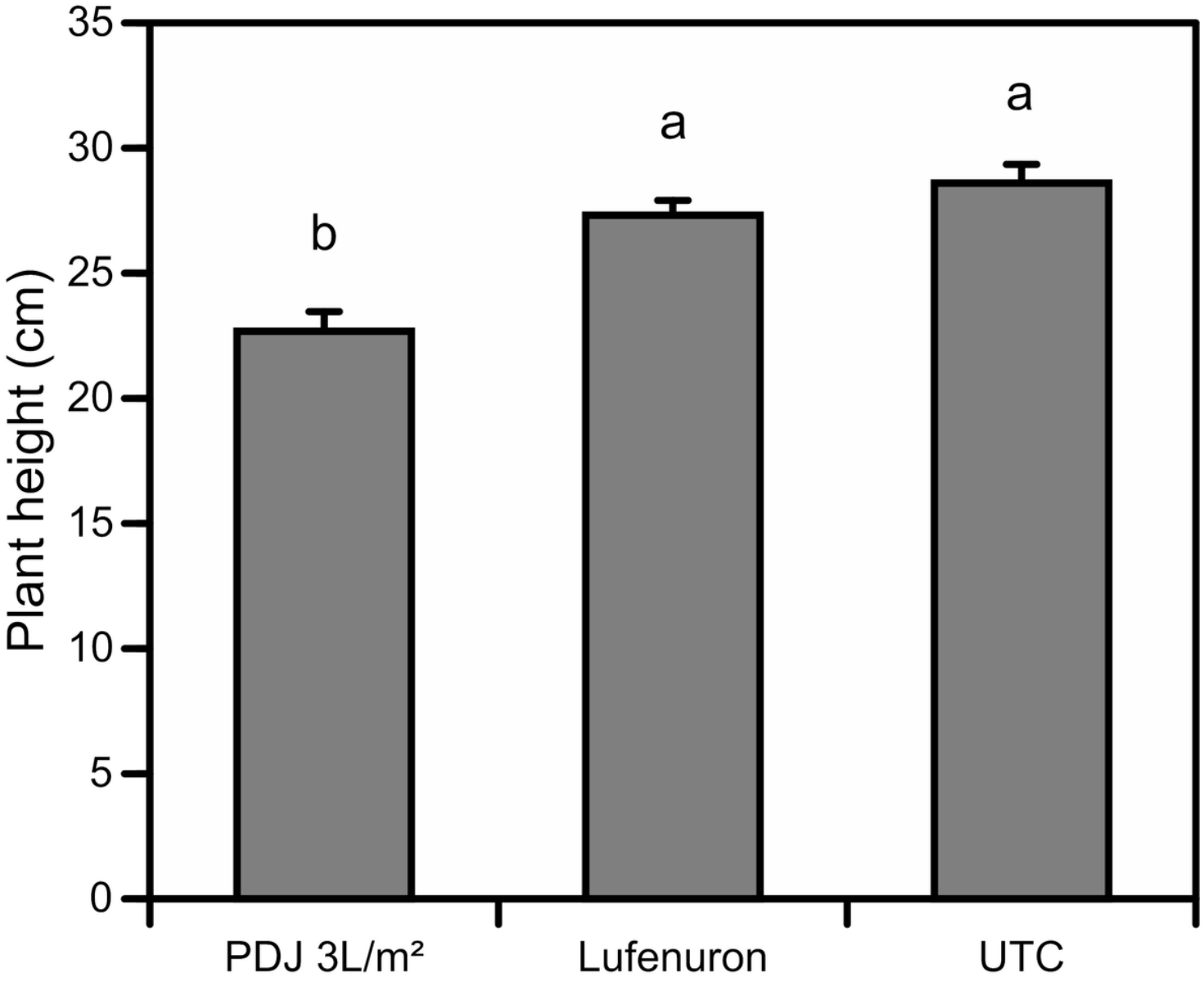

Figure 2

Plant height in each treatment 22 days after transplanting. Values are means ± SE. Means with the same letter are not significantly different ($n = 3$, $P < 0.05$; Tukey's test).

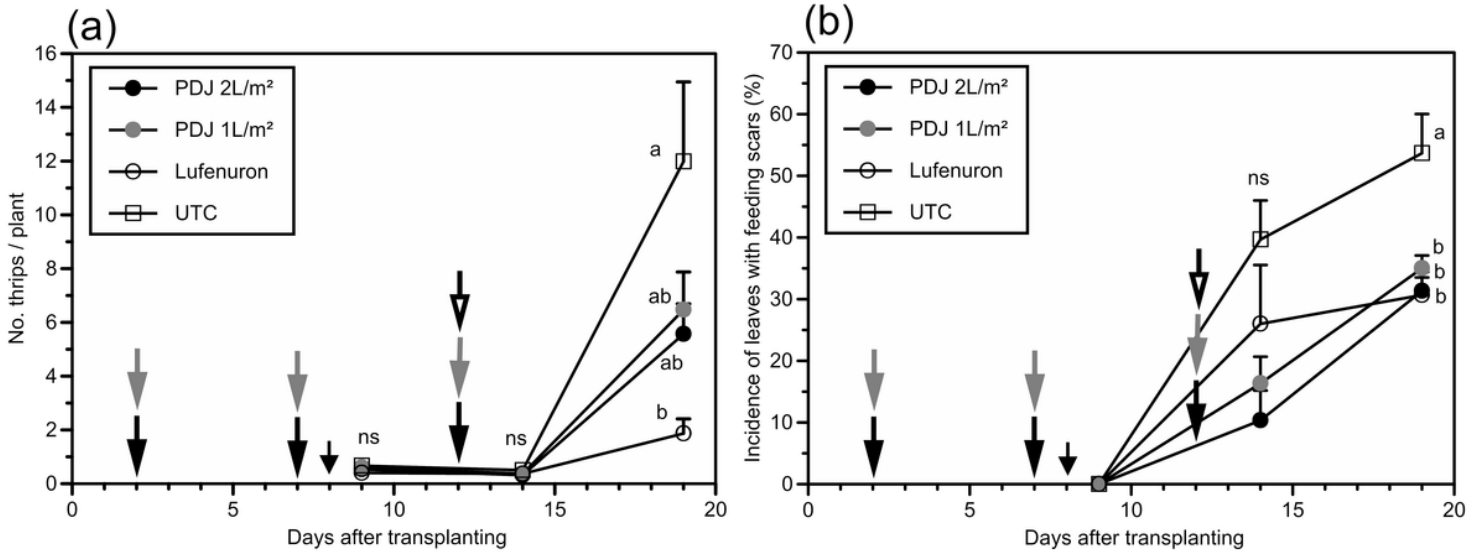

Figure 3

(a) Numbers of *Frankliniella occidentalis* (larvae + adults) and (b) incidence of leaves with feeding scars per chrysanthemum plant in each treatment. Small arrow, time of release of *F. occidentalis* adults; solid arrows, PDJ applications at (black) 2 L/m^2 and (gray) 1 L/m^2; open arrow, lufenuron spray. Values are means ± SE. Means with the same letter are not significantly different (n = 3, P < 0.05; Tukey's test). PDJ was applied first in the nursery.

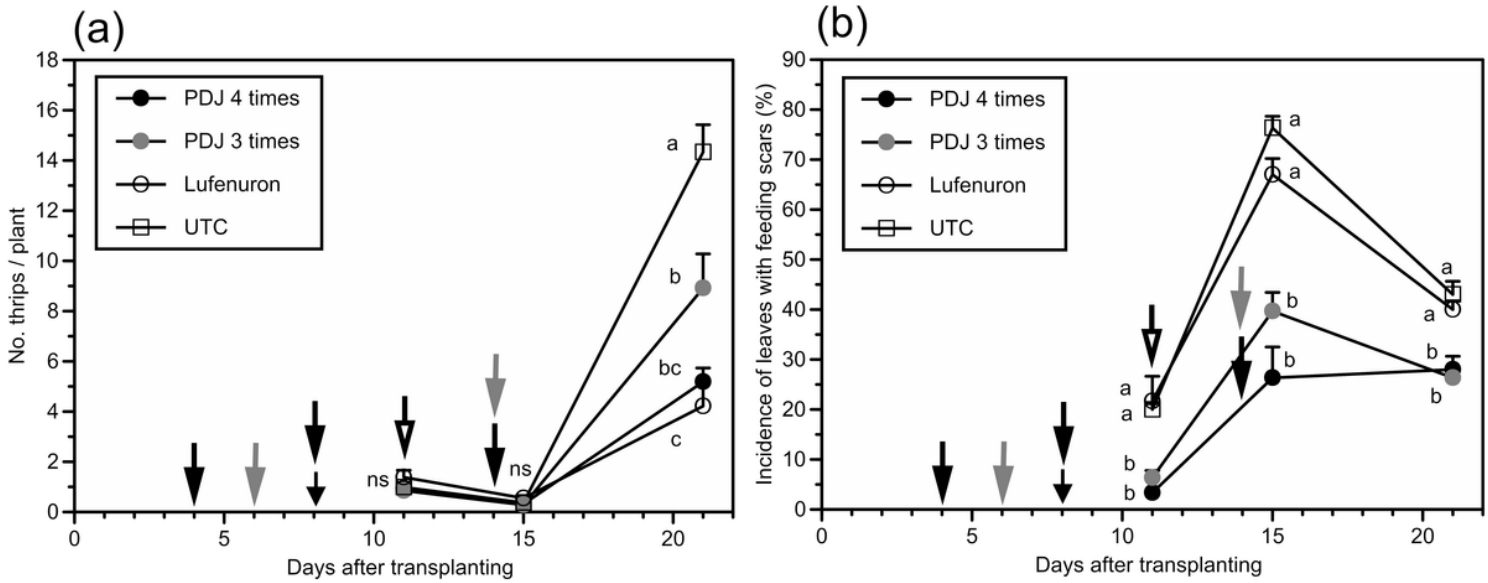

Figure 4

(a) Numbers of *Frankliniella occidentalis* (larvae + adults) and (b) incidence of leaves with feeding scars per chrysanthemum plant in each treatment. Small arrow, time of release of *F. occidentalis* adults; solid arrows, PDJ applications (black) 4 times, (gray) 3 times; open arrow, lufenuron spray. Values are means ± SE. Means with the same letter are not significantly different (n = 3, P < 0.05; Tukey's test). PDJ was applied first in the nursery.

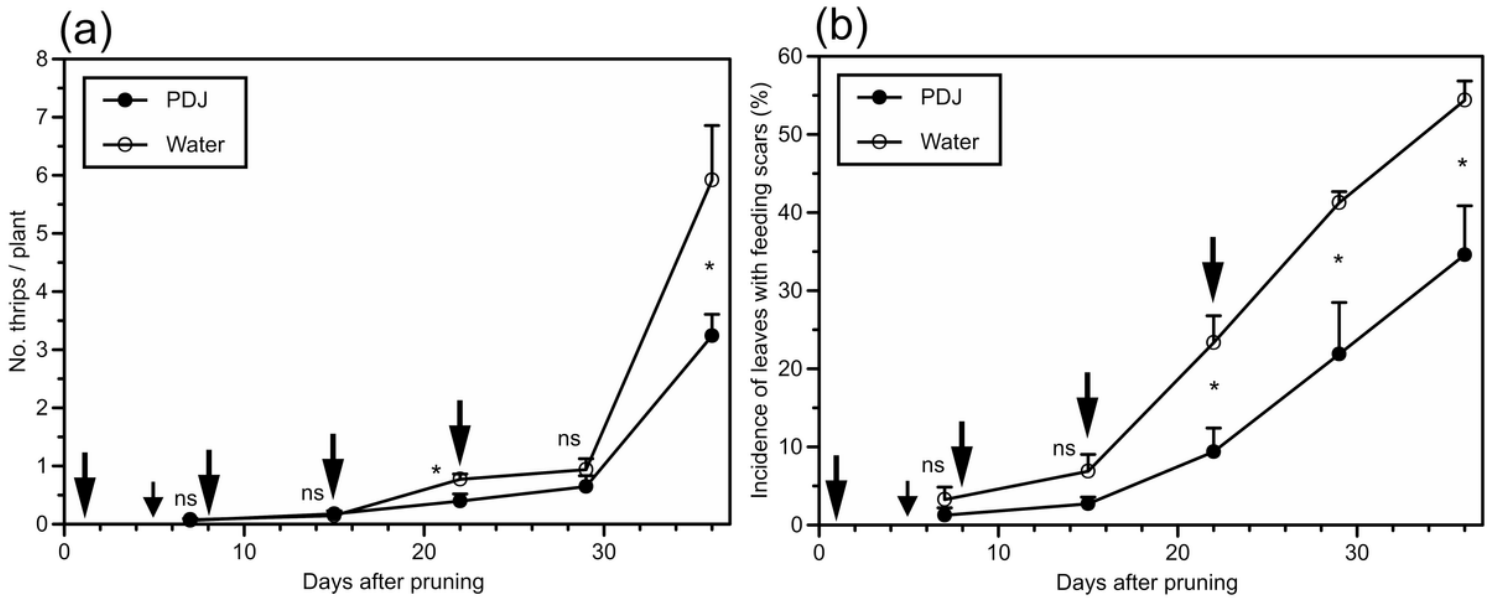

Figure 5

(a) Numbers of *Frankliniella occidentalis* (larvae + adults) and (b) incidence of leaves with feeding scars per chrysanthemum plant in each treatment. Small arrow, time of introduction of TSWV infection-source plants infested by larval thrips; black arrows, PDJ applications. Values are means ± SE. *Significantly different (n = 4, P < 0.05, paired t-test).

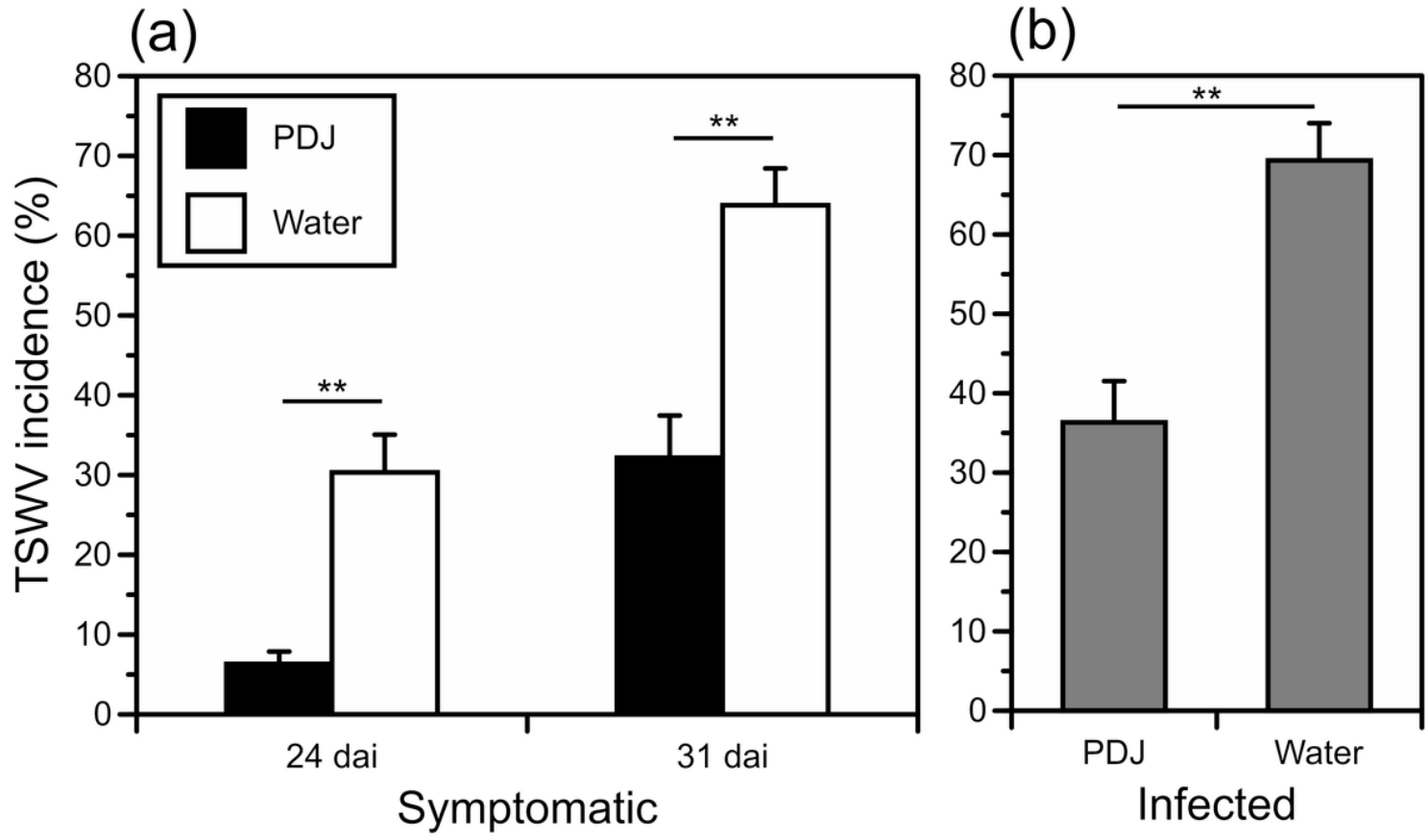

Figure 6

(a) Incidence of TSWV-symptomatic chrysanthemum plants in each treatment 24 and 31 days after introduction (“dai”) of TSWV infection-source plants. (b) Incidence of TSWV-infected (symptomatic + asymptomatic) chrysanthemum plants 31 days after introduction. Values are means ± SE. **Significantly different (n = 4, P < 0.01, paired t-test).