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RESEARCH

# 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. The overhead sprinkling of plants with PDJ did not suppress the initial colonization of plants by released adult thrips. However, it significantly reduced subsequent feeding damage on leaves and the reproduction of larval offspring. Rates of 1 and 2 L/m$^2$ of 0.8 mM PDJ equally suppressed *F. occidentalis* and feeding damage without phytotoxic effects, although 3 L/m$^2$ reduced plant growth. Frequencies of 2 and 3 applications of PDJ at different intervals had similar inhibitory effects on *F. occidentalis*, suggesting the possibility of low-frequency, labor-saving applications in chrysanthemums. Weekly applications suppressed the occurrence of TSWV transmitted by viruliferous adult thrips dispersed from inoculum sources 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 offers a valuable option for controlling *F. occidentalis* and orthotospovirus diseases transmitted by it in chrysanthemum production greenhouses.

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**Keywords** Chrysanthemum · Prohydrojasmon · *Frankliniella occidentalis* · Tomato spotted wilt virus · *Orthotospovirus* · Thrips transmission

## 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 causes considerable aesthetic feeding damage to ornamental and fruiting crops and 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 the stable production of crops worldwide (Gilbertson et al., 2015; Tsuda & Sano, 2014).

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 $68 \times 10^9$ JPY ($520 \times 10^6$ USD) in 2020. *F. 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) (genus *Orthotospovirus*) (Matsuura et al., 2007). However, many pesticides are inadequate to control *F. occidentalis* in chrysanthemums since the thrips aggregate in tightly enclosed feeding spaces such as apical buds, where they are obscured from pesticides. 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 promoting 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 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 plant biomass. Thus, PDJ has the potential to protect various crops from herbivore attacks.

Our aims here were to assess whether PDJ application to chrysanthemums could suppress infestation by *F. occidentalis* and to identify the optimal conditions under which it can be applied in commercial chrysanthemum production. We also evaluated its efficacy in reducing primary infection by TSWV by suppressing thrips feeding.

## Materials and methods

### Chrysanthemum cultivation, PDJ application, thrips population, and virus isolate

'Jimba', one of Japan's most popular standard-type chrysanthemum cultivars, was used in all experiments. Rootless cuttings were planted, and plantlets were rooted and 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 (34°25′02″N, 132°42′00″E, 222 m a.s.l.). 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 erected ~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. Fertigation was automatically applied (75 mg N/L, 40 mg P/L, 85 mg K/L; OKF-1, OAT Agrio Co., Ltd.) via a 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 tomatoes (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 virus 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 a 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 in the glasshouse.

## Thrips monitoring

We investigated 20 (24 in the virus 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 (Tokyo, Japan).

## Effects of PDJ application on thrips infestation and chrysanthemum growth

We first evaluated the efficacy of a high application rate of PDJ at 3 L/m$^2$ at suppressing *F. occidentalis*. Chrysanthemum plantlets (17 days after planting of rootless cuttings) 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 applied to plantlets in the nursery 3 days before transplanting and then in the beds 1, 6, and 12 days after transplanting (DAT) (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 adult thrips per plant. Five days after release (12 DAT), lufenuron (reference agent) was sprayed at 0.25 L/m$^2$. Thrips infestation and feeding damage were investigated in the interior 20 plants in each plot until 10 days after the final application of PDJ (22 DAT). The heights of the same 20 plants in each plot were measured at 22 DAT.

## Effect of rate of PDJ application on thrips infestation

Plantlets (16 days after planting of rootless cuttings) 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 1 and 2 L/m$^2$, lufenuron at 0.3 L/m$^2$, and an untreated control. PDJ was applied to plantlets in the nursery 2 days before transplanting and then in the beds 2, 7, and 12 DAT (total of 4 times). One day after the second PDJ application in beds (8 DAT), ~300 adult thrips were released in each plot as above, equivalent to ~5.4 adult thrips per plant. Lufenuron was sprayed at 0.3 L/m$^2$ 12 DAT. Thrips infestation and feeding damage were investigated in the interior 20 plants in each plot until 7 days after the final application of PDJ (19 DAT).

## Effect of frequency of PDJ application on thrips infestation

Plantlets (17 days after planting of rootless cuttings) 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 applied to plantlets in the nursery 2 days before transplanting and then in the beds at either 6 and 14

DAT (2-application plots) or at 4, 8, and 14 DAT (3-application plots). At 8 DAT, ~400 adult thrips were released in each plot as above, equivalent to ~7.1 adult thrips per plant. Lufenuron was sprayed at 0.25 L/m$^2$ at 11 DAT. Thrips infestation and feeding damage were investigated on the interior 20 plants in each plot until 7 days after the final application of PDJ (21 DAT).

### Effect of PDJ application on the occurrence of TSWV

Chrysanthemum plantlets (8- or 9-leaf-stage rooted cuttings) 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. Since it was difficult to precisely adjust the number of dispersing (viruliferous) adult thrips to each plot from inoculum-source plants, we improved the accuracy by increasing the area of each plot and the number of replicates and using only water treatment for comparison. Thus, 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 (or water) was applied 1, 8, 15, and 22 days after pruning (DAP).

*Datura stramonium* plants were grown in 15-cm-diameter plastic pots in an isolated glasshouse. To obtain TSWV-infected *Datura* plants, we ground leaf tissue from systemically infected *N. rustica* plants in 0.1 M phosphate buffer, pH 7.0, and rubbed the inoculum on carborundum-dusted leaves of *D. stramonium* plants at the 5-leaf stage on 20 January 2022. One hundred adult thrips were released onto each of 16 *D. stramonium* plants with mottling symptoms at the 6- to 7-leaf stage. We confirmed that these mottled plants were infected with TSWV by testing a small part of the middle leaves with an ImmunoStrip assay (Agdia Inc., Elkhart, IN, USA). The plants were enclosed in a 0.3-mm mesh net to prevent thrips escape. These symptomatic *D. stramonium* plants, in which larval offspring had hatched, were introduced at the 11- to 12-leaf stage onto the beds as TSWV inoculum sources: they were placed on both long sides of each plot 4 days after the first PDJ application (5 DAP) on 14 February 2022. Thus, four *D. stramonium* plants were placed along each bed (12 plants in the greenhouse). Thereafter emerged adults, many of them were assumed to be viruliferous, dispersed under natural conditions. Thrips infestation and feeding damage were investigated on the interior 24 chrysanthemum plants in each plot until 31 days after introducing the *D. stramonium* plants (36 DAP). The incidence of TSWV symptoms in chrysanthemums was assessed by eye 29 and 36 DAP, with attention to pronounced foliar symptoms such as leaf chlorosis, necrosis, and deformation (Matteoni & Allen, 1989) on 48 plants in the middle part of each plot (including both sides of 4 rows). Leaves with suspicious symptoms were detached and tested with an ImmunoStrip assay 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 (36 DAP): 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 was performed using monoclonal antibody to the nucleocapsid (N) protein of TSWV (Tsuda et al., 1994) in 96-well plates (C96 Maxisorp immune plate, Nunc, Roskilde, Denmark). Both anti-TSWV N protein IgG and alkaline phosphatase (AP)-conjugate were diluted 1:500 from 1 mg/mL stocks. After 2 h incubation with AP-substrate (*P*-nitrophenyl phosphate) at room temperature, the samples' optical density (OD) at 405 nm was rated. 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

### Effects of PDJ application on thrips infestation and chrysanthemum growth

The number of adult *F. occidentalis* on plants at 8 DAT was not significantly different between the 3 L/m$^2$ PDJ plots and the untreated control ($P = 0.114$), indicating no repellent effect of PDJ against adults (Fig. 1a). The number of thrips (>98% were larval offspring) at 22 DAT was significantly lower in the PDJ plots than in 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

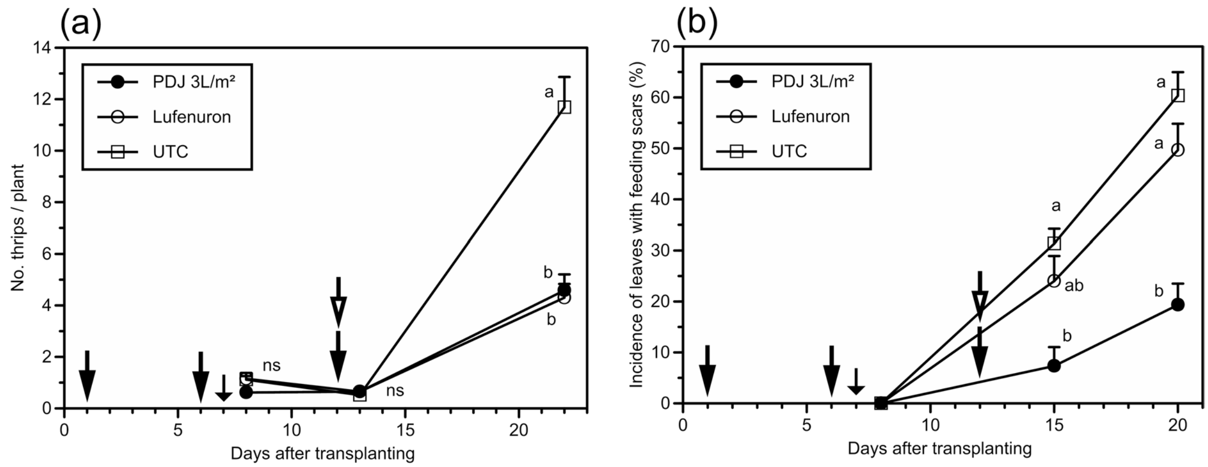


**Fig. 1** Effect of prohydrojasmon (PDJ) application on thrips infestation. (**a**) Number of *Frankliniella occidentalis* (larvae + adults) per chrysanthemum plant and (**b**) incidence of leaves with feeding scars in each treatment. Small arrow, release time of *F. occidentalis* adults; solid arrows, 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 before transplanting

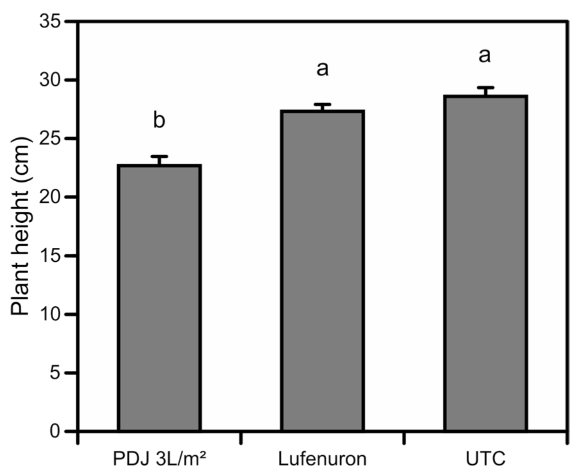


**Fig. 2** Effect of prohydrojasmon (PDJ) application on chrysanthemum growth. Plant height in each treatment measured at 22 days after transplanting (DAT). Values are means ± SE. Means with the same letter are not significantly different ($n = 3$, $P < 0.05$; Tukey's test)

was significantly lower in the PDJ plots than in control at 15 ($P < 0.05$) and 20 ($P < 0.01$) DAT, and also lower than in the lufenuron plots at 20 DAT ($P < 0.01$; Fig. 1b). Plant height at 10 days after the final application (22 DAT) was significantly lower in the PDJ plots than in 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 at 9 DAT were not significantly different between 1 or 2 L/m$^2$ PDJ and the control ($P = 0.945$, $P = 0.703$, respectively; Fig. 3a). The numbers of thrips (>96% were larval offspring) at 19 DAT were also not significantly different between the PDJ plots and the control ($P = 0.197$, 1 L/m$^2$; $P = 0.119$, 2 L/m$^2$), and were similar to those in the lufenuron plots ($P = 0.318$, $P = 0.485$, respectively; Fig. 3a). On the other hand, the incidence of leaves with feeding scars at 14 DAT tended to be lower in the PDJ plots, especially at 2 L/m$^2$ (at ~25% of the control incidence; $P = 0.054$; Fig. 3b), and those at 19 DAT were significantly lower at both 1 and 2 L/m$^2$ PDJ and in the lufenuron plots than in control ($P < 0.05$). Plant height was not significantly different between the PDJ plots at either rate and the control (data not shown).

## Effect of frequency of PDJ application on thrips infestation

The numbers of adult *F. occidentalis* on plants at 11 DAT were not significantly different between 2 L/m$^2$ PDJ and the control with either 2 ($P = 0.941$) or 3 ($P = 0.996$) applications (Fig. 4a). The number of thrips (>94% were larval offspring) at 21 DAT was

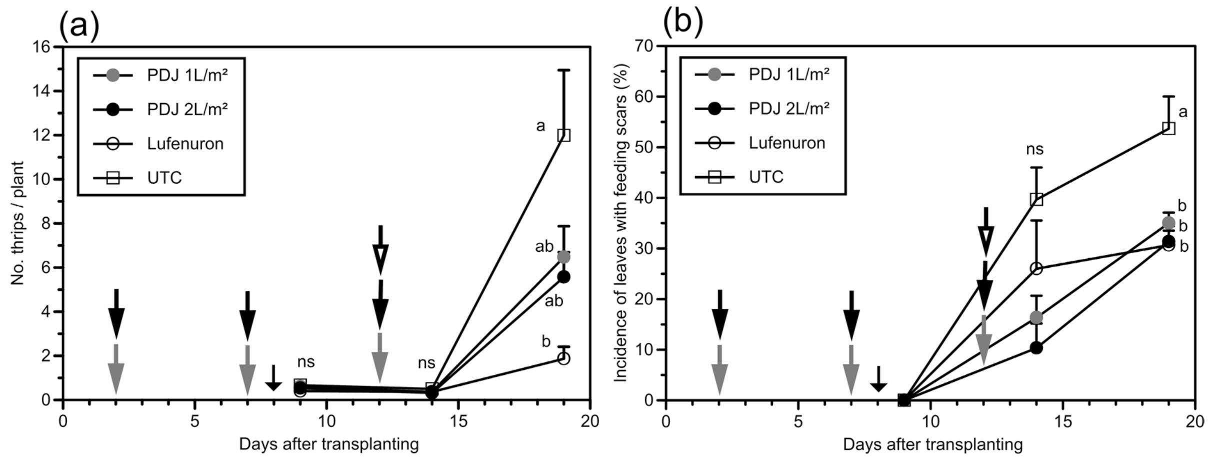


**Fig. 3** Effect of rate of prohydrojasmon (PDJ) application on thrips infestation. (**a**) Number of *Frankliniella occidentalis* (larvae + adults) per chrysanthemum plant and (**b**) incidence of leaves with feeding scars in each treatment. Small arrow, release time of *F. occidentalis* adults; solid arrows, PDJ applications at (gray) 1 L/m$^2$ and (black) 2 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 before transplanting

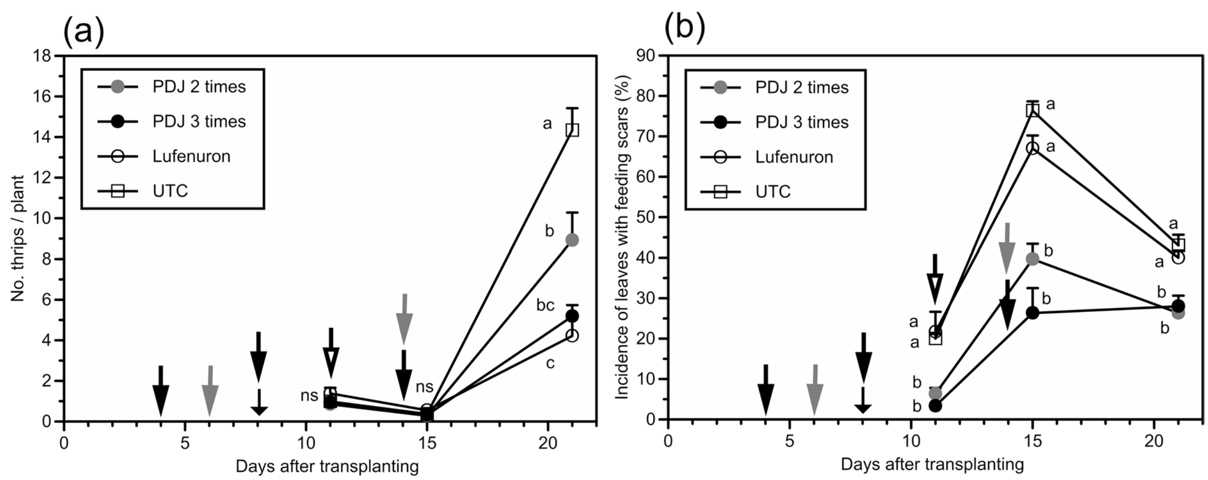


**Fig. 4** Effect of prohydrojasmon (PDJ) application frequency on thrips infestation. (**a**) Number of *Frankliniella occidentalis* (larvae + adults) per chrysanthemum plant and (**b**) incidence of leaves with feeding scars in each treatment. Small arrow, release time of *F. occidentalis* adults; solid arrows, PDJ applications (gray) 2 times, (black) 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 before transplanting

significantly lower in PDJ plots than in control at both 2 ($P<0.05$) and 3 ($P<0.01$) applications, and that in the 3-application plots was similar to that in the lufenuron plots ($P=0.906$; Fig. 4a). The incidence of leaves with feeding scars was significantly lower in PDJ plots than in the control and lufenuron plots at all time points. In particular, the incidence of feeding damage at 15 DAT in the 3-application plots was decreased by about 65% of the control ($P<0.001$; Fig. 4b). Although that at 21 DAT was significantly lower in the PDJ plots than in control, the inhibitory effect was only about 35% of the control ($P<0.01$; 2 and 3 applications; Fig. 4b). Plant height was not significantly different between the PDJ plots and the control throughout the experiment at either application frequency (data not shown).

Effect of PDJ application on the occurrence of TSWV

The number of adult thrips on chrysanthemum plants that emerged and dispersed from TSWV-infected *D. stramonium* plants (inoculum sources) started to increase about 10 days after the introduction of *D. stramonium* (15 days after pruning [DAP]; Fig. 5a). The numbers of thrips (majority emerged adults >83%) were not significantly different between the PDJ and control plots before 36 DAP, except at 22 DAP (Fig. 5a), suggesting that PDJ application does not repel migrating (viruliferous) adult thrips. The number of thrips (majority larval offspring >81%) at 36 DAP was significantly lower in PDJ plots than in control ($P<0.05$) (Fig. 5a). The incidence of leaves with feeding scars at 22, 29 and 36 DAP was lower in the PDJ plots than in control ($P<0.05$; Fig. 5b). The incidence of plants that expressed TSWV symptoms at 29 DAP was remarkably suppressed in the PDJ plots relative to the control ($P<0.01$; Fig. 6a). Although the occurrence of symptomatic plants subsequently increased in the PDJ plots, the final incidence of TSWV-infected plants at 36 DAP, both symptomatic and ELISA-positive symptomless plants, was lower in the PDJ plots than in control ($P<0.01$; Fig. 6b). About 90% of TSWV-infected plants expressed TSWV symptoms in both the PDJ and control plots at 36 DAP, indicating that the symptoms appeared equally in both treatments (Fig. 6a, b).

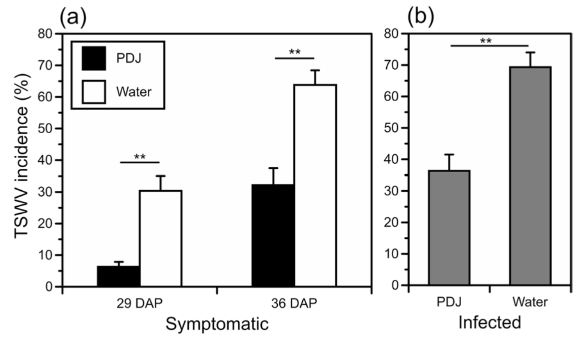


**Fig. 6** Effect of prohydrojasmon (PDJ) application on the occurrence of tomato spotted wilt virus (TSWV). (**a**) Incidence of TSWV-symptomatic chrysanthemum plants in each treatment at 29 and 36 days after pruning (DAP) and (**b**) incidence of TSWV-infected (symptomatic + asymptomatic) chrysanthemum plants at 36 DAP. Values are means ± SE. **Significantly different ($n=4$, $P<0.01$; *t*-test)

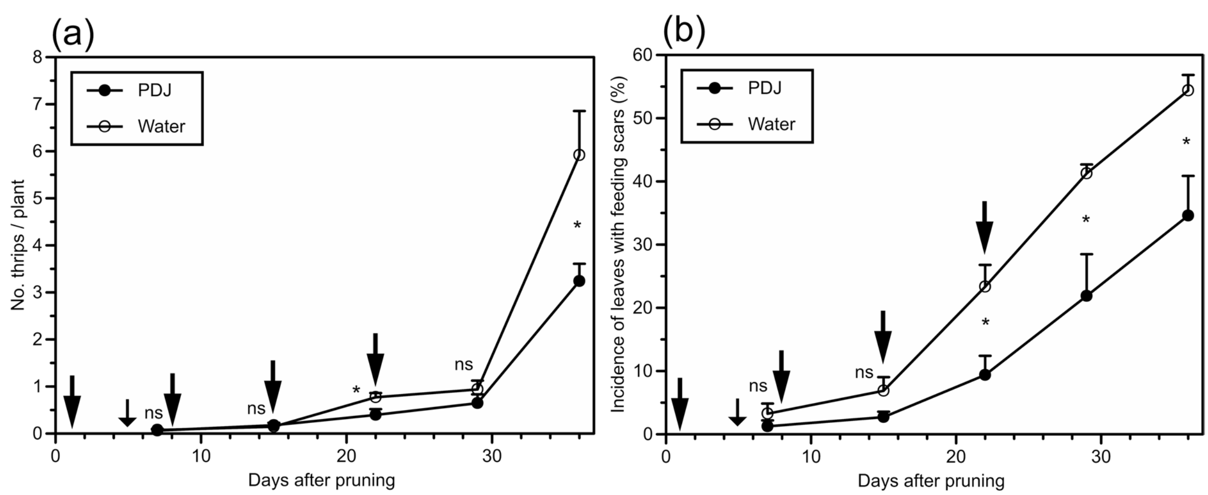


**Fig. 5** Effect of prohydrojasmon (PDJ) application on (viruliferous) thrips infestation. (**a**) Number of *Frankliniella occidentalis* (larvae + adults) per chrysanthemum plant and (**b**) incidence of leaves with feeding scars in each treatment. Small arrow, time of introduction of tomato spotted wilt virus (TSWV) inoculum-source plants infested by larval thrips; solid arrows, PDJ applications. Values are means ± SE. *Significantly different ($n=4$, $P<0.05$; *t*-test)

## Discussion

It is crucial to prevent thrips infestation early during crop growth with 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 (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 thrips 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 assumed that PDJ treatment of particular crops might repel migrating adult thrips and interfere with their settlement. However, overhead sprinkling of PDJ from a showerhead nozzle did not suppress initial colonization of chrysanthemums by 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 sprayed on chrysanthemums may not repel migrating adults, 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, cannot 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, despite 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 been confirmed in *Arabidopsis* plants treated with JA (Abe et al., 2008). Thus, we assume that the application of PDJ to chrysanthemums substantially suppresses the feeding behavior of *F. occidentalis*. Chen et al. (2020) found that JA application to apical leaves of chrysanthemums reduced *F. occidentalis* damage in newly developed leaves. And 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 compounds contribute to the enhanced resistance to thrips. It would be interesting to ascertain whether similar metabolic alterations occur in PDJ-treated chrysanthemums. Application of PDJ reduced the number 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 2 and 3 applications of PDJ with different intervals. This suggests that PDJ has a long residual effect (at least one week) on chrysanthemums and could control thrips with low-frequency, labor-saving applications.

A high rate of PDJ application (3 L/m$^2$, 0.8 mM) significantly suppressed chrysanthemum growth, although this phenomenon does not necessarily reduce the market value of cut flowers. 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 below-ground parts of Japanese radish (Yoshida et al., 2021). Thus, PDJ treatment likely reduces 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 used rates had no phytotoxic effects on the flowering date, cut flower length, cut flower weight, or flower formation in various chrysanthemum cultivars, including standard-type and spray-type, of different flower colors, in the open field and greenhouse experiments (unpublished data). These results suggest that rates of $\leq$2 L/m$^2$ of 0.8 mM PDJ would be acceptable for practical use on chrysanthemums.

Weekly application of PDJ significantly suppressed TSWV infection by dispersed (viruliferous) *F. occidentalis* adults. Considering the developmental temperature of *F. occidentalis* (McDonald et al., 1998; Ishida et al., 2003), we presume that all TSWV-infected chrysanthemum plants were infected through TSWV transmission by the emerged viruliferous adults that originated from the introduced larval thrips (offspring of initially released adults). We verified that the disease incidence and severity in PDJ-treated chrysanthemum plants that were mechanically sap-inoculated with TSWV were 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 infection or multiplication in PDJ-treated plants. The degree of TSWV suppression by PDJ application was consistent with that of feeding damage (Figs. 5b and 6). The mean infection access period for TSWV transmission by *F. occidentalis* is 1–2 h, but the optimum can be >40 h, depending on plant species (Wijkamp et al., 1996). Such a long infection access period may explain the consistency of suppression levels between TSWV infection and feeding damage. Due to experimental scale constraints, we did not establish insecticide plots for comparison in the viral suppression experiment. It seems unlikely that the TSWV-suppressive effect of PDJ treatment would be inferior to that of lufenuron spraying because a string of experiments revealed that the suppressive effect of PDJ sprinkling on thrips feeding damage was equal to or greater than that of lufenuron spraying. However, further studies are necessary to verify the effect of PDJ treatments on suppressing viral infection compared to synthetic larvicides, including lufenuron.

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 (*LOX*) and the transcription factor *MYC2*, but also those related to the biosynthesis of secondary metabolites, such as glucosinolate. A mechanism based on a plant-induced defense system poses a low risk of developing resistance in thrips. In view of its unique mechanism based on disrupting thrips behavior, PDJ may offer a valuable option for the control of *F. occidentalis* and orthotospovirus diseases transmitted by thrips in chrysanthemum grown in greenhouses.

In conclusion, application of 0.8 mM PDJ at 1–2 L/m$^2$ to chrysanthemum plants in the nursery followed by 2 or 3 weekly overhead applications in the early growth stage could suppress feeding damage and reproduction of larval offspring of *F. occidentalis* without phytotoxic effects on plants. Application could also inhibit primary infection of TSWV transmitted by migrating adult thrips. Based on 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 crop cultivation as a component of integrated pest management.

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**Declarations**

**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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