Trichome mimics: sprayable plant-based adhesives for crop protection against thrips

Bierman, T.V.

Chapter 3

Adhesive droplets made from plant-derived oils for control of western flower thrips

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This chapter was based on a publication:

Bierman TV, Vrieling K, Van Zwieten R, Kodger TE, Macel M, Bezemer TM (2024) Adhesive droplets made from plant-derived oils for control of western flower thrips. J Pest Sci 97:2175–2186. Doi: 10.1007/s10340-024-01755-4

ABSTRACT

Arthropod pests cause significant problems in agricultural crops all around the world. As chemical pesticide use becomes less desired, there is a need for alternative methods of pest control. Inspired by the natural adhesiveness of arthropod trapping plants, we examined the effectiveness of adhesive droplets made from oxidised and cross-linked plant-derived oils for control of western flower thrips. Two filter paper droplet adhesiveness assays and three detached chrysanthemum leaf assays were carried out to test efficacy against thrips. Suspensions containing adhesive droplets and other constituents were applied to filter papers and leaves via spraying or dipping. On filter papers, droplets made from oxidised rice germ oil (RGO) of different sizes caught 40–93% of thrips. Droplets made of a mixture of sunflower, olive, and linseed oil (MIX) caught up to 94% of thrips. Likewise, adhesive droplet-treated filter papers showed higher thrips mortality than untreated or control solution-treated filter papers. On chrysanthemum leaves, thrips were caught by both RGO (up to 40%) and MIX droplets (up to 20%) and thrips damage and reproduction were reduced. On MIX-treated leaves, thrips mortality was also increased. Within treatments, droplets of different size classes occurred and larger droplets were more effective at catching thrips in general. Droplets were also robust to rinsing with water, which is of importance for their application in horticulture. In conclusion, adhesive droplets made from edible plant oils show potential for use in control of western flower thrips.

Key words - *Frankliniella occidentalis*, Pest management, Plant oil, Adhesive droplets, Biobased

INTRODUCTION

Arthropod pests are responsible for annual losses of 20–40% of global crop production and costs for pest control exceed 200 billion US dollar per year (Deutsch et al. 2018; Pimentel 2009). Control of arthropod pests is commonly done via chemical pesticides, which are considered unsustainable as many of them are associated with negative effects on human health, biodiversity, and the environment (van der Werf 1996; Asghar et al. 2016; Ndakidemi et al. 2016). In addition, arthropods are becoming increasingly resistant against pesticides (Roush and Tabashnik 1990). As global pesticide regulations become increasingly strict and pesticide use becomes undesired, alternative approaches to control arthropod pests are urgently needed.

Plants defend themselves against herbivores using a range of chemical and mechanical approaches, such as the production of toxic and repellent metabolites, thorns, and trichomes (Fürstenberg-Hägg et al. 2013). One form of plant defence is the secretion of adhesive mucilage e.g., from glandular trichomes (Levin 1973; LoPresti et al. 2015). For example, the glandular trichomes of carnivorous plants, wild tomato, tarflower, and tarweed act as traps for insect herbivores, which leads to reduced damage to the plant (Cuevas et al. 2023; Alcalá et al. 2010; Simmons et al. 2004; Bar and Shtein 2019; Krimmel and Pearse 2016; Chautá et al. 2022). Other work has shown that plants without adhesive trichomes are more susceptible to herbivores than plants possessing such trichomes (Jaime et al. 2013; Goldberg et al. 2021). While the effectiveness of stickiness as a natural plant defence is well established, the external application of sticky substances to crops as a method for pest control in agriculture has not been extensively explored.

Natural materials, including plant-derived oils, such as castor oil, mustard oil, and linseed oil, have been shown to possess inherent adhesive properties which can be further improved or altered through oxidation or epoxidation (Maassen et al. 2016). During these chemical processes, fatty acid containing triglycerides (the main constituents of many plant-derived oils) may cross-

link to form longer polymers and networks with improved cohesion and viscoelasticity, which facilitates adhesion (Betz and Kölsch 2004; Li and Sun 2015). Epoxidised oils are already used successfully as sticky substances for insect monitoring and trapping (Singh and Sood 2020; Singh et al. 2022). A new way of crop protection is to use oxidised plant oils to produce adhesive droplets, which are then sprayed on the plant. So far, such an approach has only involved the use of nanoscopic adhesive droplets as carriers for pesticides and essential oils that act against arthropod pests e.g., via deterrence (Athanassiou et al. 2018; Lai et al. 2006; Campos et al. 2018). By encapsulating the active compounds within plant-derived lipids (e.g., triglycerides), their volatility can be reduced and UV-resistance increased, which may lead to longer retention times. Hence, when combined with a greater dispersal capacity, effectiveness of these active compounds may be improved (Katopodi and Detsi 2021; Tortorici et al. 2022). Whether spraying plants with adhesive droplets made from plant-derived oils with similar dimensions to those of glandular trichomes can also be used as a pest control method to immobilise and kill pest insects, which oils are most suitable for this purpose, and what size of droplets are needed, are questions that are still poorly understood.

The mucilage of glandular trichomes is diverse, ranging from aqueous, low-cost sugar-based glues (e.g., *Drosera* sp.) that are easily rinsed away or largely desiccate to triterpenoid rich resins (e.g, *Roridula* sp.) that function even in dry climates (Voigt et al. 2015). The adhesive strength of either of these mucilages depends for a large part on capillary and viscosity forces (Gorb 2008; Betz and Kölsch 2004), which occur after wetting upon contact. Studies on carnivorous plants suggest that the success of glandular trichomes to capture arthropods depends on contact area and prey size. Small arthropods may be easily trapped by a single trichome, unable to detach due to the capillary tension of the mucilage, while larger preys often escape unless they contact multiple trichomes or, in other words, a sufficient surface area of adhesive mucilage (Voigt and Gorb 2008; Gibson and Waller 2009). Hence, we expect that droplet size may affect the ability of adhesive droplets to catch

arthropods. Since the size of the adhesive droplets can be manipulated, this provides the opportunity to optimise capture rates or to make them specific against particular sizes of arthropods. The size of adhesive droplets may also influence other properties, such as how well the droplet adheres to a leaf surface (Grillo et al. 2021; Cao et al. 2022) or how long droplets stay adhesive, which are important for use in horticulture. Therefore, important questions are if droplet size influences insect capture ability and if droplets of different sizes can withstand rinsing off with water and remain effective over time.

To evaluate the potential of adhesive particles made from plant-derived oils for pest control, we used an important agricultural pest, *Frankliniella occidentalis* (Pergande, Thysanoptera: Thripidae), commonly called western flower thrips, and an economically important ornamental plant: chrysanthemum (*Chrysanthemum* x *morifolium*, Ramat). *Frankliniella occidentalis* is one of the most destructive herbivorous insect pests of a wide variety of ornamentals and food crops around the world (Mouden et al. 2017). Thrips damage to plants may be direct by feeding, visible as spots of empty cells (often referred to as 'silver damage'), or may be indirect via the transmission of plant viruses that can devastate entire yields, such as tospoviruses (Steenbergen et al. 2018). Due to their thigmotactic behaviour and rapid lifecycle, thrips may avoid pesticides and quickly build up populations, making them a difficult pest to manage (Reitz 2009). We tested whether adhesive particles made from different plant-derived oils could catch thrips and reduce thrips survival, reproduction, and damage to chrysanthemum leaves. Additionally, we examined the resistance of particles to rinsing with water which could be an important aspect for future application. The current study provides the proof of concept for a new pest control method using adhesive particles made from natural materials.

MATERIALS AND METHODS

Insects

In 2008, a culture of *Frankliniella occidentalis* was established from individuals collected in a commercial greenhouse in the Westland area in the Netherlands. In the laboratory, the thrips were reared on chrysanthemum (Chrysanthemum × morifolium, Ramat; cv. Baltica Yellow), obtained from a commercial greenhouse (Naaldwijk, the Netherlands). Rearing took place in two transparent plastic cages with a removable thrips-mesh covered opening, in a climate room at 60% RH, 25 °C, and 16:8 light:dark photoperiod under fluorescent TL-light. Each cage contained five vases with cut flowers. Every week, the vase with the oldest flowers was replaced. Before use, flowers were carefully inspected for contamination with thrips or other arthropods and only clean flowers were used. To ensure purity of the *F. occidentalis* culture, the species identity of a number of thrips was determined regularly using a microscope (at least once every two months).

Plants

Rooted chrysanthemum (*Chrysanthemum* × *morifolium*; cv. Baltica White) cuttings were obtained from Deliflor B.V. (Maasdijk, The Netherlands). Rooted cuttings were transplanted to 10 × 10 × 11 cm plastic pots filled with a mixture of potting soil, 10 g/L vermiculite, and 1.5 g/L osmocote pellets. Plants were then grown in a climate room at 70% RH, 20 °C, 16:8 light:dark photoperiod, under fluorescent TL-light (15,340 lm m^{-2}). A second batch of unrooted chrysanthemum cuttings (obtained from Deliflor B.V.) were treated with rooting powder (Chryzotek beige 0.4%), planted in peat blocks (4 × 4 × 3 cm) and covered with a transparent plastic sheet for 14 days to form roots. The rooted cuttings were then potted and grown as described above.

Bioassay climate room conditions

All bioassay experiments were carried out in a walk-in climate room at 60% RH, 25 °C, 16:8 h light-dark photoperiod, 80 cm below fluorescent TL-lights.

Production of plant oil-based adhesive droplets

Five different suspensions containing adhesive droplets made from plant-derived oils were produced using different oxidation temperatures, grind settings, and from different plant-derived oils. Rice germ oil (RGO) adhesive droplets were made by filling a glass beaker with 600 ml rice germ oil (Makana®, Offenbach an der Queich, Germany). The beaker was placed in an oil bath and heated at a constant temperature of 120 °C. The RGO was aerated with pressurised air at a rate of 5−6 l min^{-1} via a capillary tube. An overhead mixer (IKA T10, IKA Werke GmbH and Co. KG, Staufen im Breisgau, Germany) was used to stir the rice germ oil at 80 rpm. Oxidisation was stopped after 4.5 days. At this time, the torque value was 10 N-cm higher than when the oil first reached a temperature of 120 °C. The oxidised oil was left to cool to room temperature. Then, 6.25 g oxidised oil was combined with a solution containing 1 wt% of a block copolymer surfactant: Pluronic® F-108 (PEG-PPG-PEG, No. 542342, Sigma Aldrich, Missouri, U.S.A), 2 wt% alginic acid, and 97 wt% tap water. The solution was added to the oxidised oil up to a volume of 500 ml. The oxidised oil plus solution were then ground for 10 min using a commercial kitchen mixer to create a suspension with oil chunks. The suspension with oil chunks was then added to a Magic Lab laboratory milling machine (UO79206, IKA) and ground further at a rotation speed of 15,000 rpm for 15 min per 250 ml sample. This method was repeated three times with different gap sizes of the cone mill to create three different suspensions with droplets of different sizes: R180 (gap size of 0.159 mm), R360 (0.318 mm) and R720 (0.636 mm). All final suspensions contained 1.25 wt% adhesive droplets, 0.99 wt% F-108, 1.98 wt% alginic acid and 95.78 wt% tap water.

A second batch of adhesive droplets was made in the same way as the R360 sample, but with several adjustments: oxidation temperature was set at 90 °C, oxidation time was 11.5 days, and final delta torque was 6.2 N-cm. After grinding, the suspension consisted of 2 wt% alginic acid, 0.25 wt% F-108, 77.75 wt% water and 20 wt% adhesive droplets. A 1% $CaCl_2$ solution was

added to the Magic Lab in a ratio of 2:9 $CaCl_2$ solution to suspension with ground oil droplets. The gap size of the cone mill was set to maximum, and the sample was mixed at 10000 rpm for another 15−20 min. During mixing, the alginic acid and $CaCl_2$ physically cross-linked to form a reversible gel. The final suspension contained 81.6 wt% tap water, 16.4 wt% RGO360-90 oil droplets, 0.2 wt% F-108, of 1.6 wt% alginic acid and 0.2 wt% $CaCl_2$. This product was labelled as R360-90. A control solution containing 0.2 wt% F-108, 1.6 wt% alginic acid, 0.2 wt% $CaCl_2$ and water was also made.

A final two batches of adhesive droplets were made from a mixture of 325 ml sunflower oil, 270 ml olive oil, 7.2 ml linseed oil (a formulation resembling the triglyceride composition of rice germ oil). Oxidation temperature was 90 °C, oxidation time was 7 days and final delta torque was 7 N-cm. Gap size of the cone mill was 0.318 mm. The final suspension contained 81.6 wt% tap water, 16.4 wt% MIX oil droplets, 0.2 wt% F-108, of 1.6 wt% alginic acid and 0.2 wt% $CaCl_2$. This product was labelled as MIX. A control solution containing only F-108, alginic acid, $CaCl_2$ and water, in the same concentrations as the MIX suspension, was also made. Before use in experiments, the MIX and R360-90 suspensions and control solution were diluted (1:3) with tap water. Five separate assays were then done to test the adhesive droplet suspensions.

Experiment 1: Filter paper thrips adhesion assay with R180, R360 and R720 droplets

The ability of suspensions with RGO adhesive droplets to catch thrips, their effects on thrips mortality, and resistance of droplets to rinsing off with water were tested using a filter paper adhesion assay. Ten replicates were tested for three sizes of adhesive droplets: R180, R360, and R720, and two conditions: rinsed or not rinsed with water. The assay was performed as follows: a pneumatic paint spraying gun was used to spray 90 mm diameter filter paper discs with 4 ml adhesive droplet suspension under 1.4 bar pressure at 25 cm distance. After spraying 20 filter paper discs with each suspension, the filter papers were dried at room temperature for one day. Ten filter papers per

adhesive droplet suspension were then rinsed for ten seconds under a gentle stream of tap water (1.6 L per min.). After rinsing, the filter papers were left to dry for six h in the laboratory. Ten unsprayed, unrinsed filter papers were added as a control treatment for thrips survival. Each filter paper was placed in a Petri-dish lid (90 mm diameter). A 2 ml Eppendorf-tube lid with tap water and covered with stretched parafilm was placed on the centre of each filter paper as a water source for thrips. The Petri-dish base was placed like a dome over the filter paper. Ten adult female thrips were added using an aspirator along the outer edge of the filter paper by slightly lifting the Petri-dish base. After confirming the presence of ten thrips, Petri-dishes were sealed with parafilm and placed randomly (14 columns, 5 rows) in the bioassay climate room. The number of thrips stuck in droplets and number of alive and dead thrips (including those in droplets) were counted after 17, 23, 38, and 45 h. Additionally, the length of all caught thrips and of 100 thrips from the culture was measured to investigate if the size of caught thrips was different from the average size of those in the rearing. At the end of the experiment, photographs were taken through a 570 nm LP filter under blue UV light that provided excitation between 440–460 nm (NIGHTSEA^{tm} Royal blue, NIGHTSEA, Lexington, U.S.A.). Droplet coverage and droplet area in mm^2 were estimated from these pictures for all droplets above 0.01 mm^2 using the image processing package ImageJ v 2.9.0 (for more details see "Supplementary information").

Experiment 2: Filter paper thrips adhesion assay with R360-90 and MIX droplets

In a second bioassay, suspensions with adhesive droplets R360-90 and MIX were investigated for their ability to catch thrips and their effects on thrips survival. A solution without adhesive droplets consisting of 0.2% F-108, 1.6 wt% alginic acid, 0.2 wt% $CaCl_2$ and tap water was included as a control treatment. Ten replicates were included per treatment. The second adhesion bioassay was performed as described for the first bioassay with the following changes: suspensions were sprayed on 90 mm filter paper discs using a commercial sprayer (Birchmeier Spray-Matic 2S, DCM Nederland B.V.,

Nieuw-Vennep, the Netherlands), modified with a connector piece to fit a Teejet 2502-SS nozzle (Teejet Technologies Northwest Europe, Schorndorf, Germany). Spraying was done at 3 bar pressure with 30 ml suspension per replicate at 25 cm distance. There was no rinsing step as MIX droplets had already been observed in preliminary trials to still be present after rinsing off with water. Drying time at ambient conditions before addition of thrips was 2.5 h. The number of thrips stuck in droplets and the number of living and dead thrips were counted after 17 h and the experiment was terminated as almost all thrips were stuck in droplets or had already died. Thrips length was measured and droplet coverage and size were estimated in the same way as in experiment 1, but photographs were taken with a 500 nm filter and analysed using different brightness, contrast, and threshold parameters (see "Supplementary information").

Experiment 3: leaf assay with R180, R360, R720 droplets

A detached leaf assay was performed to test the ability of adhesive droplets R180, R360 and R720 to catch thrips and their effects on thrips survival, thrips damage, and reproduction on chrysanthemum leaves. The effect of rinsing with tap water on droplet coverage of sprayed leaves was also examined. There were ten replicates for each treatment. The oldest leaves were taken from side branches originating from the middle part of the shoot of 117-day-old chrysanthemum plants. These leaves were sprayed in a similar way as in the first Petri-dish adhesion assay with 4 ml of the respective adhesive droplet suspension or tap water as a control. Leaves were left to dry for one hour in the lab with moist filter paper covering their stems. Because only few droplets were visible on the leaf surface, the leaves were then also pressed three times with the adaxial side on 90 mm filter paper discs that had been sprayed in advance with 4 ml of respective adhesive droplet suspension. In this way, droplets were transferred from these filter papers to the surface of the leaves and droplet density could be increased. Leaves were again left on moist paper towel to dry. Ten leaves of each treatment (including the control) were then rinsed-off with tap water for ten seconds. All leaves were then placed with the entire abaxial side into a layer of 25 ml lukewarm 1%

agar in a 90 mm Petri-dish and left to dry for 3.5 h. Ten female adult thrips were added along the inner side of each Petri-dish, which was then closed with a lid that had a 45 mm diameter hole in the centre covered with thrips-proof mesh (200 µm). Petri-dishes were sealed with parafilm and placed in a randomised way in the climate room. Experiments lasted for five days (because larvae were observed after 3 days at 25 °C in preliminary assays). After five days, the number of thrips stuck in droplets, the number of live and dead thrips, number of larvae, and thrips feeding damage (area of silver damage in mm^2) at the adaxial leaf side were recorded by eye (Visschers et al. 2018). Droplet coverage was estimated visually using a binocular microscope (10× magnification).

Experiment 4: leaf assay with MIX droplets via dipping

A detached leaf assay was performed to test the ability of MIX adhesive droplets to catch thrips and the effects of MIX droplets on thrips survival, thrips damage, and thrips reproduction on chrysanthemum leaves. A dipping method was used because it had been observed that some droplets had agglomerated, possibly after 72 h of storage or during transport. The second leaf from the bottom was taken of sixty 45-day old plants of the first batch (ca. 49 cm high, with ca. 26 unfolded leaves per plant). These 60 leaves were randomly divided into three groups with twenty replicates each. Treatments were applied by dipping the adaxial side of detached leaves into 10 ml of MIX droplet suspension, control solution without adhesive droplets, or water. While leaves were drying, 60 petri-dishes (90 mm diameter) were filled with 25 ml 0.8% agar. Leaves were inserted into the solidified agar with the petiole under a slight angle so that the leaf was almost horizontal, but the abaxial side remained accessible for thrips. The rest of the procedure was as described for experiment 3. Damage was measured after 5 days on both sides of the leaf and the ratio of adaxial to total damage was calculated. Droplet coverage and size were estimated from photographs of leaves that were made and analysed as in experiment 1 and 2 and it was noted which droplets had caught thrips.

Experiment 5: leaf assay with MIX droplets via spraying

In a subsequent assay, it was tested if MIX droplets were sprayable on plants before agglomeration. Six, 25-day-old chrysanthemum plants of the second batch (ca. 25 cm high, ca. 14 unfolded leaves) were sprayed with 200 ml MIX sample (1:3 dilution from stock), control solution, or water, using the Birchmeier 2S sprayer with Teejet 2502-SS nozzle. Plants were left to dry for 30 min, then sprayed again (to increase coverage) with another 200 ml of adhesive droplet suspension, control solution, or water. The second, third and fourth leaf from the bottom of each plant were taken and placed into 0.8% agar in a Petri-dish. For each treatment there were now a total of eighteen replicates. The rest of the assay and further measurements were done as in experiment 4.

Data analyses and statistics

IBM SPSS® v. 27 software was used for data analysis. *P* values smaller than 0.05 were considered significant. Assumptions for normality and homogeneity of variance were tested using Shapiro−Wilk test and Levene's test respectively. In case the assumption for normality was violated, data were transformed or tests that do not require normally distributed data were used.

Droplet distributions: For experiment 1 and 2, droplet size distributions were binned by droplet area (0.5 mm^2 bins and a final bin of > 5 mm^2 for experiment 1, 1 mm^2 bins and a final bin of > 10 mm^2 for experiment 2) and then averaged over replicates prior to analysis. Comparison between droplet size distributions of different grind settings was then done using Kruskal–Wallis tests, for rinsed and unrinsed filter papers separately. Additionally, the percentage area of filter papers covered with droplets was compared between treatments using two-way ANOVA. Factors tested were rinsing (rinsed or not rinsed) and grind setting (R180, R360, R720). The relationship between droplet size and the proportion of droplets that caught thrips for each size category was determined using linear regression. The length of thrips caught in droplets and of thrips from the rearing was compared using one way ANOVA.

Catch rate and mortality: The number of thrips caught in experiment 1, 2, and 3, and thrips mortality in all experiments were analysed using GLMs with Poisson distribution and log-link function. For experiment 1 and 3, two factors were tested: rinsing (rinsed or not rinsed) and grind setting (R180, R360, R720). For experiment 2, 4 and 5, one factor: treatment was tested. There were two exceptions: (1) mortality in experiment 1 was analysed with one factor and using only unrinsed data as there was no rinsed control treatment, (2) in experiment 3, data of rinsed and unrinsed leaves were pooled together for each grind setting before analysis as there was no effect of rinsing on catch rate in experiment 1, and no significant difference found in coverage between rinsed and unrinsed leaves.

For experiment 3, 4 and 5, total leaf damage was analysed using one-way ANOVAs. For experiment 3, total leaf damage was first ln(x+1) transformed to meet normality assumptions. The number of newborn larvae in experiment 3, 4, and 5 and the ratio of adaxial to total leaf damage in experiment 4 and 5 were analysed using Kruskal–Wallis test as the assumptions for normality were violated. For all experiments, multiple comparisons after GLM (with Poisson distribution) and Kruskal–Wallis tests were done using Bonferroni-corrected Dunn's tests. Significant ANOVAs were followed by a Tukey Post hoc test.

RESULTS

Experiment 1 and 2: Filter paper thrips adhesion assays

Droplet size distributions were left skewed in all treatments of experiment 1 and 2 (Fig. 3.1a). In experiment 1, the mean (±SE) number of droplets above 0.01 mm^2 on filter papers was 1030 ±55 for R180, 433 ±20 for R360, and 344 ±19 for R720. Mean (±SE) droplet area was 0.19 ±0.00, 0.38 ±0.01, and 0.39 ±0.01 mm^2 with maximum areas of 4.88, 6.77, and 10.22 mm^2 for R180, R360, and R720 droplets respectively. R180, R360 and R720 droplet distributions were all different on unrinsed filter papers (Kruskal–Wallis, $H = 116.5$, df = 2, $p < 0.01$), but were not shown to be different for R360 and R720 on rinsed filter papers (Kruskal–Wallis, $H = 91.9$, df = 2, $p < 0.01$). In

general, R180 droplets were the smallest, while differences between R360 and R720 droplet distributions on unrinsed filter papers were mainly due to differences in the larger droplet categories, with R720 having more droplets above 2 mm^2 (Fig. 3.1a, b). Mean (±SE) adhesive droplet coverage on filter papers was similar (2.0 ±0.2% to 2.8 ±0.2%) in all treatments (ANOVA, $F_{2, 54} = 0.85$, $p = 0.36$; Table S3.1). In experiment 2, the mean (±SE) number of droplets on filter papers above 0.01 mm^2 was on average 693 ±57 for R360-90 and 472 ±61 for MIX. Mean (±SE) droplet area was 1.12 ±0.03 mm^2 with a maximum of 39.11 mm^2 for R360-90 and 0.83 ±0.04 mm^2 with a maximum of 40.14 mm^2 for MIX droplets. Droplet size distributions were different, with MIX sample filter papers having less and relatively more small droplets than R360-90 filter papers (Mann–Whitney, $U = 10.7$, $p < 0.01$). Mean (±SE) droplet coverage was also higher on R360-90 (12.3 ±1.7%) than on MIX (6.2 ±0.7%) filter papers (ANOVA, $F_{1, 18} = 10.5$, $p < 0.01$; Table S3.2).

The area of thrips catching droplets ranged from 0.09 mm^2 to 7.67 mm^2 in experiment 1, with average areas of 0.37, 1.74 and 2.24 mm^2 for R180, R360, and R720 treatments, respectively. In experiment 2, the area of droplets that caught thrips ranged from 1.06 to 34.7 mm^2, with average areas of 9.98 ±0.9 mm^2 for R360-90 droplets, and ranged from 1.00 to 37.89 mm^2, with an average area of 10.88 ±1.02 mm^2 for MIX droplets. In both experiment 1 and 2, the proportion of droplets that had caught thrips per total droplets in a size category increased as the droplet size category increased (linear regression: R360: $F_{1, 52} = 107.4$, $p < 0.01$, $R^2 = 0.67$; R720: $F_{1, 70} = 86.0$, $p < 0.01$, $R^2 = 0.55$; Table S3.3; R360-90: $F_{1, 57} = 29.6$, $p < 0.01$, $R^2 = 0.35$; MIX: $F_{1, 47} = 17.9$, $p < 0.01$, $R^2 = 0.28$; Fig. 3.1c; Table S3.4). An increase in the number of thrips catching droplets per total area of droplets, as droplet size category increased, was only visible for the R360 and R720 treatments of experiment 1 and not visible for the MIX and R360-90 treatments of experiment 2 (linear regression, R360: $F_{1, 52} = 38.3$, $p < 0.01$, $R^2 = 0.42$; R720: $F_{1, 70} = 7.46$, $p < 0.01$, $R^2 = 0.10$; Fig. S3.1; Table S3.5, S3.6). The length of adult female thrips from the rearing did not differ from the length of thrips caught in droplets in experiment 1 (ANOVA, $F_{3, 280} = 0.18$, $p = 0.91$), or experiment 2 (ANOVA, $F_{2, 289} = 0.2$, $p = 0.98$).

In experiment 1, neither rinsing with water (GLM, $\chi^2 = 0.161$, df = 1, $p = 0.69$), nor the interaction between grind setting and rinsing (GLM, $\chi^2 = 0.578$, df = 2, $p = 0.75$) was shown to have a significant effect on the number of thrips caught. After 45 h, the mean (±SE) % of thrips caught was highest (42 ±4%) in R720, intermediate (26 ±3%) in R360 and lowest (3 ±1%) in R180 droplets (GLM, $\chi^2 = 81.2$, df = 2, $p < 0.01$; Fig. 3.2a). Thrips mortality was higher on R720 filter papers than on untreated control filter papers (GLM, $\chi^2 = 17.65$, df = 3, $p < 0.01$; Fig. 3.2b). In experiment 2, after 17 h, R360-90 droplets caught on average (±SE) 94 ±2% and MIX droplets caught on average (±SE) 93 ±4% of the introduced thrips, a non-significant difference (GLM, $\chi^2 = 0.005$, df = 1, $p = 0.94$; Fig. 3.2c). Thrips mortality was higher on R360-90 sprayed filter papers and MIX sprayed filter papers than on solution control sprayed filter papers (GLM, $\chi^2 = 43.62$, df = 2, $p < 0.001$; Fig. 3.2d).

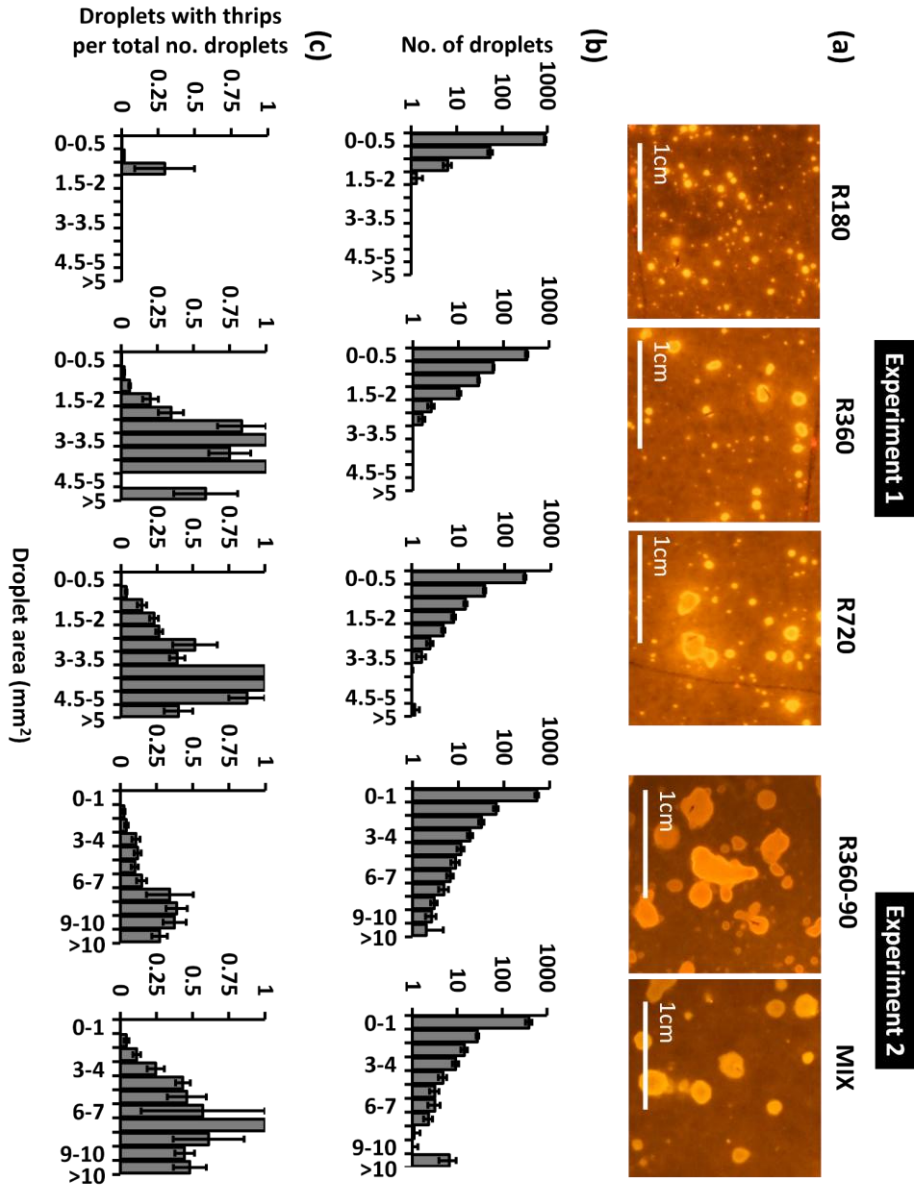

Fig. 3.1 Representative pictures of UV fluorescent adhesive droplets on filter papers (a). The mean (±SE) number of droplets on filter papers per size category (b). The mean (±SE) proportion of thrips catching droplets out of the total droplets in each size category after 45 h in experiment 1 and 17 h in experiment 2 (c). Suspensions containing rice germ oil adhesive droplets were made using different grind settings

(R180, R360, R720) in experiment 1, and made using different oil types (R360-90, MIX) in experiment 2.

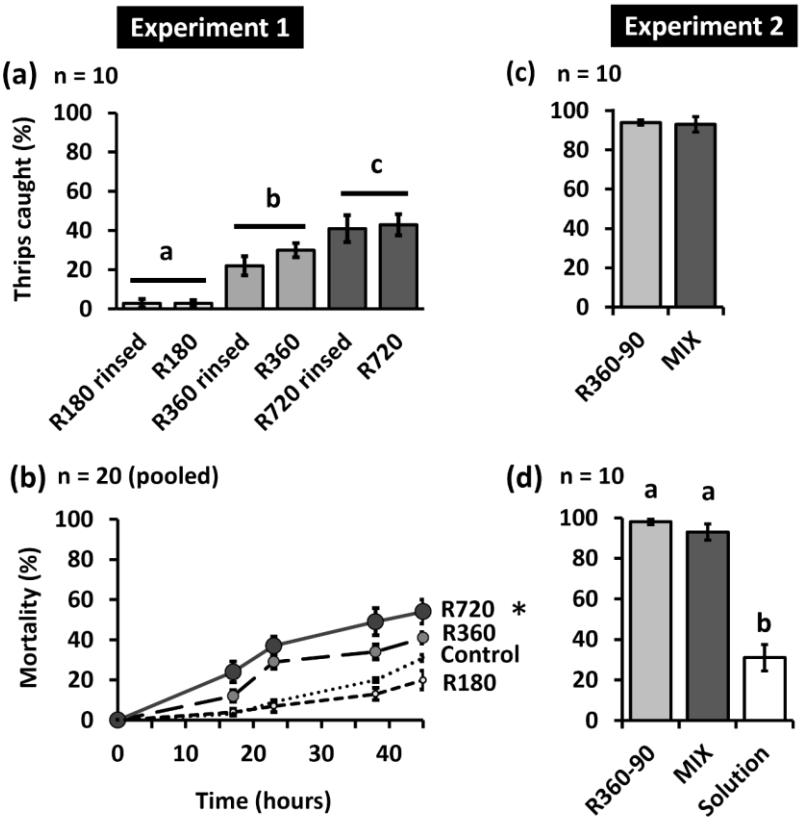

Fig. 3.2 The mean (±SE) percentage of thrips caught by droplets after 45 h in experiment 1. Filter papers were rinsed after spraying with tap water or not (a). Data of unrinsed and rinsed filter papers was pooled as rinsing had no effect on catch rate. The mean (±SE) percentage thrips mortality over time in experiment 1 (b). The mean (±SE) percentage of thrips caught by droplets after 17 h in experiment 2 (c). The mean (±SE) percentage thrips mortality after 17 h in experiment 2 (d). Bars with identical letters do not significantly differ based on Dunn's test. The asterisk in Fig. b indicates a significant difference of R720 from the unsprayed control based on Bonferroni-corrected Dunn's tests (p <0.05). Suspensions with rice germ oil-based adhesive droplets were made using different grind settings (R180, R360, R720) in experiment 1 and made using different oil types (R360-90, MIX) with additional components in experiment 2.

Experiment 3, 4 and 5: Leaf assays

In experiment 3, the mean (±SE) percentage of leaf area covered with droplets was lower for the R180 sprayed leaves (11.5 ±0.32%) than for the R360 (12.6 ±0.31%) and R720 (12.2 ±0.24%) sprayed leaves (ANOVA, $F_{2, 54} = 11.45$, $p < 0.01$; Table S3.7) and was not affected by rinsing (ANOVA, $F_{1, 54} = 0.17$, $p = 0.68$; Table S3.2), nor was there an interaction between spray treatment and rinsing (ANOVA, $F_{1, 54} = 0.06$, $p = 0.95$). More thrips were caught on R720 and R360 than on R180 sprayed leaves (GLM, $\chi^2 = 117.3$, df = 2, $p < 0.01$; Fig. 3.3a). Mortality of adult thrips was close to 100% in all treatments and did not differ between them (GLM, $\chi^2 = 0.27$, df = 3, $p = 0.97$; Fig. 3.3b). Thrips damage was lower in the R720 and R360 treatments than in the water control (ANOVA, $F_{3, 76} = 7.6$, $p < 0.01$; Fig. 3.3c). Fewer thrips larvae were observed in the R720 and R360 treatments than in the water control (Kruskal–Wallis, $H = 20.4$, df = 3, $p < 0.01$; Fig. 3.3d).

In experiment 4, the mean (±SE) droplet coverage was 18.1 ±0.9% (Table S3.8). The mean (±SE) percentage of thrips stuck in droplets (as seen in Fig. 3.3e) was 18.0 ±2.2%. Thrips mortality was higher in the control solution and MIX suspension treatments compared to the water control (GLM, $\chi^2 = 6.56$, df = 2, $p = 0.038$; Fig. 3.3f). Leaf damage was lower in the MIX treatment than in the control solution and water treatments (ANOVA, $F_{2, 57} = 4.12$, $p = 0.02$; Fig. 3.3g). No difference in the ratio of adaxial to total thrips damage was found between treatments (Kruskal–Wallis, $H = 0.06$, df = 2, $p = 0.97$). On average, fewer larvae were observed in the MIX treatment compared to the water control (Kruskal–Wallis, $H = 6.69$, df = 2, $p = 0.035$; Fig. 3.3h).

In experiment 5, mean (±SE) coverage with adhesive droplets was 11.0 ±1.1% (Table S3.9). The mean (±SE) percentage of thrips caught in MIX droplets was 21.7 ±3.6%. Mortality was highest in the MIX treatment, intermediate in the solution treatment, and lowest in the water control (GLM, $\chi^2 = 24.4$, df = 2, $p < 0.001$; Fig. 3.3j). Leaf damage was lower in the MIX and solution treatments than in the water treatment (ANOVA, $F_{2, 51} = 11.8$, $p < 0.001$; Fig. 3.3k).

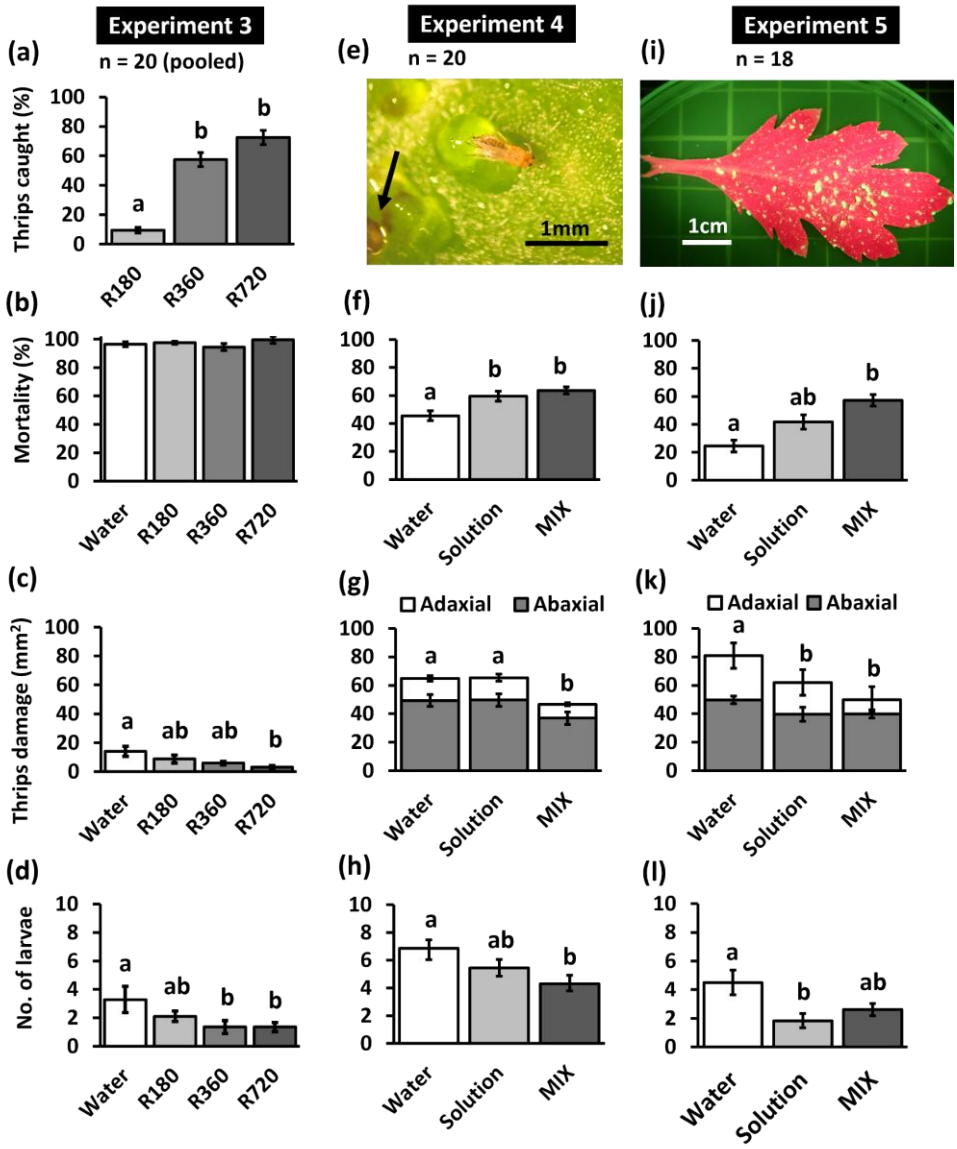

Fig. 3.3 Data of rinsed and unrinsed leaves of experiment 3 was pooled before analysis. The mean (±SE) number of thrips caught on leaves with adhesive droplets in experiment 3 (a). The water treatment is absent in figure a because droplets had evaporated by the time of measurement. The mean (±SE) % thrips mortality in Petri-dishes with leaves of experiment 3 (b), 4 (f), and 5 (j). The mean (±SE) mm^2 adaxial thrips damage per treatment in experiment

3 (c). The mean (±SE) mm^2 adaxial and abaxial thrips damage in experiment 4 (g) and 5 (k). The mean (±SE) number of thrips larvae in experiment 3 (d), 4 (h), and 5 (l). Close up photograph of MIX treated chrysanthemum leaf with a thrips caught in an adhesive droplet (e), a black arrow indicates necrosis damage where droplets contact the leaf as was also observed in experiment 4 and 5. Photograph of chrysanthemum leaf of experiment 5 with MIX droplets that show green fluorescence under a 500nm filter and blue UV light (i). Bars with identical letters do not significantly differ based on Dunn's or Tukey tests at $p < 0.05$. Tested adhesive droplets of different sizes were made from rice germ oil (R180, R360, R720, and R360-90) or made from a mixture of sunflower, olive, and linseed oil (MIX). All measurements were taken after 5 days.

Also in experiment 5, the mean (±SE) ratio of adaxial to total thrips damage was lower in the MIX treatment (0.23 ±0.04) than in the water (0.40 ±0.04) and solution (0.37 ±0.05) treatments (Kruskal–Wallis, $H = 10.4$, df = 2, $p = 0.006$). Fewer larvae were observed in the solution treatment than in the water control (Kruskal–Wallis, $H = 7.9$, df = 2, $p = 0.01$; Fig. 3.3l).

DISCUSSION

Inspired by naturally adhesive plants, this study investigated the potential of adhesive droplets made from plant-derived oils for control of a small arthropod pest. Results from the filter paper and leaf assays showed that suspensions with adhesive droplets made from rice germ oil and those made of a mixture of sunflower, olive and linseed oil were both capable of catching *Frankliniella occidentalis* females and reducing thrips survival, reproduction, and damage to chrysanthemum leaves. In addition, droplets were still present after rinsing with water, which indicates a potential for increased longevity and usage in both indoor and outdoor conditions.

In carnivorous plants with glandular trichomes, larger leaf area or adhesive surface are related to larger prey capture ability (Gibson 1991; Gibson and Waller 2009). In our study, thrips caught in adhesive droplets were immobilised in a similar way as on sticky plants (Nelson et al. 2019a; Simmons et al. 2004). Overall, using larger grind gap sizes produced suspensions that contained fewer small droplets and more droplets of larger sizes. When applied, suspensions containing larger droplets caught more thrips and resulted in higher thrips mortality. Within droplet distributions resulting from each grind setting, an increase in efficiency with droplet size was also observed. As droplet size increased, larger droplets up to 4.5 mm^2 in experiment 1 and up to 8 mm^2 in experiment 2 caught more thrips than smaller droplets relative to the total number of droplets per size category or (only in experiment 1) relative to the total surface area that droplets occupied on the filter papers. For application in agriculture, identification of the optimum size of droplets will be important for cost effectiveness and to be able to adapt the droplets to target pests of different sizes.

In addition to herbivorous arthropods, predators, pollinators and other groups of arthropods may be affected by the adhesive droplets. While some arthropod predators are adapted to adhesive plants and may coexist, providing the plant with a form of indirect defence in return for scavenging carrion (Krimmel and Pearse 2013; LoPresti et al. 2015, 2018a), other predators may experience negative effects of adhesive trichomes and surfaces (Kennedy 2003). Like the natural situation, carrion trapped in oil-based adhesive droplets may provide a food source for predators and help to increase their populations. Negative effects of adhesive droplets on non-target arthropods may at the same time be partially or entirely mitigated by changing the type of oil used, the size of the droplets and their adhesiveness. Further studies are required however before conclusions on the impact of adhesive droplets on non-target organisms can be made and the droplets may safely be used with integrated pest management methods that utilise beneficial arthropods.

Besides trapping insects, the plant oil-based droplets and other compounds used in the suspensions may have acted as a repellent, toxins or plant defence inducers. Several of the oils used in this study are known to have repellent or fumigant effects on arthropods, such as linseed oil (Rajput et al. 2017), sunflower oil (Lachance and Grange 2014) and rice bran oil (Shaaya et al. 1997). Volatiles released from the rice germ oil droplets or other compounds are unlikely to have acted as a strong fumigant toxin in our experiments as in the R180 treated filter papers, where not many thrips were caught, thrips mortality was similar to that on untreated filter papers over the course of 45 h (Fig. 3.2b). However, if one would compare the mortality at 17 h in the untreated control of experiment 1 against the solution treatment of experiment 2, an argument could be made that the solution containing F-108, alginic acid and $CaCl_2$ does have a negative effect on thrips survival. Based on the overall results of the leaf assays, it seems evident that suspensions with droplets may have thrips repellent or oviposition deterring properties or may have induced plant defences. In general, treatments with suspensions containing adhesive droplets showed the strongest effects while the control solution also showed an effect in some instances. In experiment 3, effects on thrips survival were not clearly visible as overall high thrips mortality was observed. While previous work has indicated that thrips may drown when contacting water droplets (Terry et al. 2014), survival of thrips can also be reduced due to lack of humidity (Shipp and Gillespie 1993). Since water droplets were no longer visible prior to addition of thrips, it is unlikely that thrips had drowned. Without access to a more humid environment on the abaxial leaf side, it is possible that thrips desiccated over the course of five days.

Next to having direct effects on arthropods, the application of essential oils (Kesraoui et al. 2022) and alginic acid (Saberi Riseh et al. 2022) on plants may lead to the induction of plant resistance pathways, such as the jasmonic acid and salicylic acid pathways. Since thrips also induce the jasmonic acid and salicylic acid defensive pathways through feeding (Zheng et al. 2019), induction of these plant defences by the oils themselves or alginic acid may

have contributed to the lower observed damage and reproduction. Additionally, application of surfactants such as F-108 may have direct harmful effects on insects (Affeld et al. 2004), and 10 mM $CaCl_2$ application has already been shown to be effective against *Frankliniella occidentalis* (Zeng et al. 2020). To what extent the adhesive oil droplets and individual compounds of the solution have repellent effects on thrips and whether adhesive droplet spraying induces local and constitutive defences remains to be investigated.

In conclusion, spraying adhesive droplets made from natural oils on plants holds potential as a pest control method that may be applicable in various crop systems, especially those where homogeneous coverage can be achieved and appearance of foliage is of lesser importance e.g., tomato or cucumber. In chrysanthemum and other cut flower crops, application may be done during early stages when foliar density is low and on parts of the plant that will be removed during harvest. However, before adhesive droplets can reliably be used in practice, market receptibility should be assessed and further research regarding application of adhesive droplets in IPM is necessary. For example, it may be tested if the observed agglomeration of the droplets could be prevented to extend product shelf life, or could be resolved, e.g., by using equipment that actively separates droplets rather than forcing them into a combined volume while spraying. Effectiveness on several pests, natural enemies, other non-target organisms, and retention time of adhesive droplets should also be assessed further in full plant studies, as well as effects on photosynthesis, plant growth and phytotoxicity, the latter of which occurred to some extent in our study and is more often observed when using natural oils on plants (Werrie et al. 2020). Additionally, other ways to improve the droplets may also be explored. Just like nanodroplets, larger adhesive droplets could act as carriers of arthropod deterrent or attractive compounds, or plant growth and defence promotors. Overall, we have found that adhesive droplets made from plant-derived oils provide an interesting direction of study in the search for sustainable alternative control method for arthropod pests.

Acknowledgements
We thank Prof. Peter Klinkhamer for his contributions to the project and for acting as project leader at early stages of the research. We thank Karin van Veen-van Wijk for assistance in maintaining the thrips colony.

Funding
This work was funded by the Netherlands Organization for Scientific Research (NWA-ORC grant number: NWA.1160.18.071).

Author contributions
All authors took part in conceptualization of the work. TK and RZ created the adhesive droplet suspensions at Wageningen University. TB performed the bioassays at Leiden University. TB, KV and TMB created the figures and wrote the manuscript. MM provided extensive feedback prior to and during experiments and on the manuscript. All authors read the manuscript and provided comments. All authors approved the final version of the manuscript.

Competing interests
The authors have no financial or non-financial interest to disclose.

Data availability
The datasets of this study are available via Dryad: “Adhesive droplets made from plant-derived oils for control of western flower thrips” Doi: 10.5061/dryad.j3tx95xnr

SUPPLEMENTARY - INFORMATION

Counting and area estimation of adhesive droplets

A Canon 1300D and a Nikon Z6 II camera were used to make photos of all filter papers with adhesive droplets and of leaves with MIX adhesive droplets. Photos were made under blue ultraviolet light (Nightsea^{tm} Royal blue, excitation between 440-460 nm) using a 570 nm LP filter in experiment 1 and a 500 nm LP filter in all other experiments. Fiji-ImageJ software v. 2.9.0 was used to count the number of droplets and to estimate droplet size by pixel area. Workflow was similar for each image. At the start of analysis, scale (pixel/mm) was set based on the diameter of the Petri-dish or a scale indicator in the photo. The image was converted to 8 bit. Background outside the filter paper area or leaf was removed. Brightness and contrast were adjusted, and the threshold function was used to isolate the droplets from the background. The best parameters for these functions were determined based on visual comparison with the original image. There was one exception: in the second Petri-dish adhesion assay with MIX and RGO360-90 droplets, the remove background function with rolling ball radius 100 was used instead of adjusting brightness and contrast before thresholding. For each experiment, the selection via thresholding was then again compared via overlay with the original image. The image was then converted to binary. Overlapping droplet areas were separated by hand with a single pixel wide straight line. The analyze droplet function was used with threshold: 0.1 mm^2 - infinity, circularity: 0.00-1.00. Droplet area measurements were used to calculate % coverage, investigate size distribution, and the mean droplet diameter that thrips would get stuck in. For experiment 1, brightness and contrast values were 82 and 273 respectively and the threshold value was set to select values from 120 up to 255. For experiment 2, contrast values were 82 and 273 and threshold value was set to select values from 20 up to 255. For experiment 4, brightness and contrast values were 0 and 255, and threshold value was set to select values from 152 up to 255. For experiment 5, brightness and contrast values were 164 and 345 respectively and threshold value was set to select values from 18 up to 255.

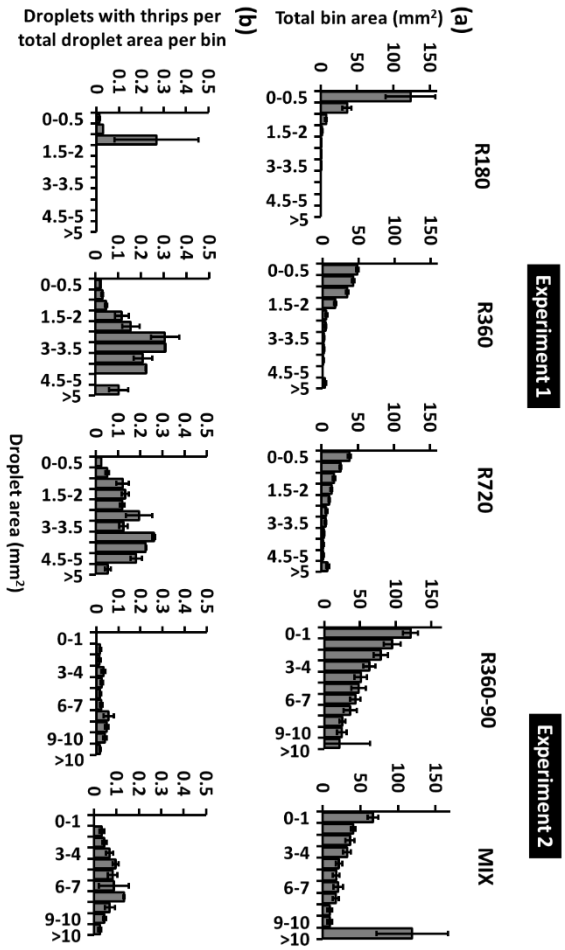

Fig. S3.1 The mean (± SE) total droplet area (mm^2) per size category of droplets on filter papers (a). The mean (± SE) number of thrips catching droplets per total area of droplets in each size category (b). Different sizes of rice germ oil derived droplets were made using different grind settings (R180, R360, R720) in experiment 1, and made of different oil types (R360-90, MIX) in experiment 2.

Table S3.1 Experiment 1, no. of droplets per cm^2, filter paper area covered by droplets (%), droplet area (mm^2) and area of droplets that caught thrips (mm^2). All values are means ± SE. Filter papers were sprayed with rice germ oil derived adhesive droplets of three different sizes (R180, R360, R720). For each size, half of the filter papers were rinsed under tap water. Adult thrips were then added to investigate if the droplets could trap them. The rightmost column gives an indication of the size of droplets that were successful in catching thrips.

Treatment	n	Droplets (no. cm^{-2})	Area covered by droplets (%)	Droplet area (mm^2)	Thrips catching droplet area (mm^2)
R180	10	14.5 ± 0.9	2.5 ± 0.3	0.17 ± 0.01	0.25 ± 0.05
R180-rinsed	10	15.6 ± 0.8	2.8 ± 0.2	0.18 ± 0.01	0.44 ± 0.10
R-360	10	5.9 ± 0.3	2.4 ± 0.2	0.42 ± 0.03	1.74 ± 0.20
R360-rinsed	10	7.6 ± 0.3	2.7 ± 0.1	0.36 ± 0.02	1.72 ± 0.24
R720	10	5.5 ± 0.4	2.1 ± 0.1	0.39 ± 0.01	2.21 ± 0.20
R720-rinsed	10	5.3 ± 0.8	2.0 ± 0.2	0.39 ± 0.02	2.24 ± 0.16

Table S3.2 Experiment 2, no. of droplets per cm^2, filter paper area covered by droplets (%), droplet area (mm^2) and area of droplets that caught thrips (mm^2). All values are means ± SE. Filter papers were sprayed with droplets made from rice germ oil (RGO360-90) or a mixture of olive-, sunflower-, and linseed oil (MIX). Adult thrips were added to the filter papers to investigate if the droplets could trap them. The rightmost column gives an indication of the size of droplets that were successful in catching thrips.

Treatment	n	Droplets (no. cm^{-2})	Area covered by droplets (%)	Droplet area (mm^2)	Thrips catching droplet area (mm^2)
R360-90	10	10.9 ± 0.9	12.3 ± 1.7	1.12 ± 0.03	8.76 ± 0.76
MIX	10	7.4 ± 1.0	6.2 ± 0.7	0.83 ± 0.04	9.03 ± 0.96

Table S3.3 Experiment 1, statistics of linear regressions on the ratio of droplets that caught thrips per total droplets.

Treatment	N	R^2	*F*	*df*	*p*
R360	53	0.67	107.4	1, 52	< 0.01
R720	71	0.55	86.03	1, 70	< 0.01

Table S3.4 Experiment 2, statistics of linear regressions on the ratio of droplets that caught thrips per total droplets.

Treatment	N	R^2	*F*	*df*	*p*
R360-90	57	0.35	29.55	1, 56	< 0.01
MIX	47	0.28	17.94	1, 46	< 0.01

Table S3.5 Experiment 1, statistics of linear regressions on the ratio of droplets that caught thrips per total droplet area per droplet size category.

Treatment	N	R^2	*F*	*F df*	*p*
R360	53	0.42	38.25	1, 52	< 0.01
R720	71	0.096	7.46	1, 70	< 0.01

Table S3.6 Experiment 2, statistics of linear regressions on the ratio of droplets that caught thrips per total droplet area per droplet size category.

Treatment	N	R^2	*F*	*F df*	*p*
R360-90	57	0.02	1.06	1, 56	0.31
MIX	47	0.01	0.51	1, 46	0.48

Table S3.7 Experiment 3, no. of droplets per cm^2 on the adaxial side of detached chrysanthemum leaves and leaf area covered by droplets (%). All values are means ± SE. Leaves were sprayed with rice germ oil derived adhesive droplets of three different sizes (R180, R360, R720). For each size, half of the leaves were rinsed under tap water.

Treatment	n	Droplets (no. cm^{-2})	Area covered by droplets (%)
R180	10	63.1 ± 0.3	11.3 ± 0.3
R180-rinsed	10	65.8 ± 0.6	11.7 ± 0.6
R-360	10	39.9 ± 0.5	12.4 ± 0.5
R360-rinsed	10	39.6 ± 0.4	12.8 ± 0.4
R720	10	34.8 ± 0.3	12.2 ± 0.3
R720-rinsed	10	35.1 ± 0.4	12.3 ± 0.4

Table S3.8 Experiment 4, no. of droplets per cm^2 on the adaxial side of detached chrysanthemum leaves, leaf area covered by droplets (%), the area of a droplet (mm^2), and area of droplets that caught thrips (mm^2). All values are means ± SE. Adult thrips were added to the leaves to investigate if the droplets could trap them. The rightmost column gives an indication of the size of droplets that were successful in catching thrips.

Treatment	n	Droplets (no. cm^{-2})	Area covered by droplets (%)	Droplet area (mm^2)	Thrips catching droplet area (mm^2)
MIX (dipped)	20	23.6 ± 1.5	18.1 ± 0.9	0.82 ± 0.06	8.01 ± 1.44

Table S3.9 Experiment 5, no. of droplets per cm^2 on the adaxial side of detached chrysanthemum leaves, the leaf area covered by droplets (%), the droplet area (mm^2), and area of droplets that caught thrips (mm^2). All values are means ± SE. Adult thrips were added to the leaves to investigate if the droplets could trap them. The rightmost column gives an indication of the size of droplets that were successful in catching thrips.

Treatment	n	Droplets (no. cm^{-2})	Area covered by droplets (%)	Droplet area (mm^2)	Thrips catching droplet area (mm^2)
MIX (sprayed)	18	68.1 ± 3.4	11.0 ± 1.1	0.16 ± 0.01	3.57 ± 0.68