Oil Adjuvants Enhance the Efficacy of Pyraclostrobin in Managing Cucumber Powdery Mildew (*Podosphaera xanthii*) by Modifying the Affinity of Fungicide Droplets on Diseased Leaves

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Abstract

Adding adjuvants improved the affinity of fungicide droplets to cucumber leaves infected with powdery mildew (*Podosphaera xanthii*) and subsequent efficacy of fungicide treatments in reducing the disease. The affinity of oil adjuvants was quantified by adhesional tension and “work of adhesion”. Oil adjuvant-fungicide mixtures were applied to plants in field experiments to evaluate their effectiveness in disease prevention. Both the adhesional tension and work of adhesion of the adjuvants at selected concentrations increased on powdery-mildew-infected cucumber leaves more than on healthy cucumber leaves. The adjuvant GY-Tmax (GYT) displayed the best surface activity or “surfactivity” in enhancing the affinity and adherence of droplets to powdery-mildew-infected cucumber leaves, while epoxidized soybean oil (ESO), methyl oleate, and biodiesel exhibited much

lower effects in terms of the surface tension, contact angle, adhesional tension, and work of adhesion. Field experiments determined that the combination of GYT at 1,000 mg liter⁻¹ and pyraclostrobin (150 g a.i. ha⁻¹) was most effective (91.52%) in controlling cucumber powdery mildew. Pyraclostrobin with ESO was also highly effective (ranging from 77.54 to 89.65%). The addition of oil adjuvants, especially GYT and ESO, to fungicide applications can be an effective strategy to enhance the efficacy of pesticides in controlling plant diseases by modifying the affinity of fungicide droplets to symptomatic leaves.

Keywords: affinity, efficiency, hydrophobic interface, oil adjuvants, powdery mildew.

Powdery mildew is one of the most widespread and destructive foliar diseases of cucurbits and has caused yield loss in many countries (Cohen et al. 2004; Zhang et al. 2008). Cucumber powdery mildew is caused by *Podosphaera xanthii* (Castagne) U. Braun & N. Shishkoff (syn. *P. fusca* (Fr.) U. Braun & Shishkoff) and *Golovinomyces orontii* (Castagne) V. P. Heluta (syn. *G. cichoracearum* (D.C.) V. P. Heluta or *Erysiphe cichoracearum* D.C.) (Braun et al. 2002). White, circular powdery spots develop on the upper and lower cucumber leaf surface and then gradually increase in size, coalescing as the disease progresses. Healthy cucumber leaves are naturally hydrophilic but, after infection, they become hydrophobic and are difficult to wet when sprayed with pesticides. When cucumber leaves are covered by mycelium and conidia of *P. xanthii*, pesticide droplets have greater contact angles and minimal contact areas with the hydrophobic interfaces. Thus, wetting of powdery-mildew-infected leaves with pesticide droplets is difficult because droplets collide, bounce, and coalesce when being applied, thus inhibiting uniform deposition to the target (Zhou et al. 2017). To achieve high treatment efficacy, adhesion of pesticide droplets to leaf surfaces is required regardless of whether cucumber leaves have hydrophilic or hydrophobic

surfaces (Xu et al. 2011). Adding spray adjuvants may be an effective approach for higher retention of pesticide droplets, improved coverage, and better efficacy (Hess and Foy 2000; Kudsk and Mathiassen 2007; Nairn et al. 2011; Ramsey et al. 2005, 2006).

Some adjuvants can serve as humectants to slow the drying of spray droplets and, thus, increase the retention of active ingredients (Xu et al. 2011). Adjuvants such as cottonseed, cocoa, and soybean oils are multifunctional and can reduce the surface tension and contact angle of spray droplets. Oil adjuvants can also enhance the affinity of pesticides for plant leaves and improve the penetration of pesticides into target organisms (Hess and Foy 2000; Kirkwood 1993; Kudsk and Mathiassen 2007; Xu et al. 2011). They can also increase the efficacy of pesticides two to three times (Cabanne et al. 1999).

Liu (2004) demonstrated that the absorptivity of plants to pesticides increased with increasing concentration of adjuvants, and the maximum absorptivity was obtained using a concentration of 0.5%. Others reported that, when the adjuvant concentration was 1%, the retention of pesticides on plants improved severalfold compared with not using adjuvants (De Ruiter et al. 1990). High concentrations of adjuvants in spray applications, however, can be phytotoxic (Downs 2008). Therefore, it is necessary to limit the concentration of adjuvants added to the pesticides, and concentrations may differ between adjuvants.

Measurements of contact angle and surface tension are quick and convenient methods for determining surface affinity (Gaskin et al. 2005; Hess and Foy 2000), which is affected by surface and adjuvant characteristics. The measurement of contact angle is taken at the liquid–solid interface, and a smaller contact angle indicates a better wetting effect (Zhu et al. 2014). During the spreading of a liquid, the force that causes the spread of the surface tension against the liquid is known as the adhesion tension; it reflects the ability of liquids to replace gases on solid surfaces. When the adhesion tension between liquid and solid is greater than the surface tension of the liquid

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Funding: This research was funded by the National Key R&D Program of China (2017YFD0200307).

*The e-Xtra logo stands for “electronic extra” and indicates that five supplementary tables are published online.

The author(s) declare no conflict of interest.

Accepted for publication 28 February 2019.

itself, liquid can be spread easily onto solid surfaces (Adamson and Gast 1997). The wettability and affinity of liquid to solid can be expressed as “work of adhesion”. The work of adhesion indicates that the liquid–solid interface per unit area is expanded, as is work required to generate gas–liquid surfaces and gas–solid surfaces per unit area during the wetting process (Adamson and Gast 1997). Hence, adhesion tension and work of adhesion are two important parameters for determining the wettability and affinity of liquid pesticides to the surface of plants (Wang et al. 2014).

Previous studies have focused mainly on the adhesion of adjuvants to leaves of a variety of healthy plants or on the wettability of adjuvants on the leaves of plants at different growth stages (Xu et al. 2011; Zhu et al. 2014). However, no study has systematically compared adhesional tension and work of adhesion of adjuvants between healthy and powdery-mildew-infected cucumber leaves.

The objectives of the current study were to use contact angles, surface tension, adhesive tension, and adhesive work (Zhao et al. 2005) to evaluate the properties of different adjuvants. The effects of adjuvants in enhancing the efficacy of pyraclostrobin against powdery mildew were also assessed under field conditions. Additionally, the relationship between the dried pesticide-adjuvant residues and the efficiency of surface coverage was evaluated. Thus, by studying the affinity to leaves combined with treatment efficacy in the field, a basis for determining the optimal formulation is provided.

Materials and Methods

Plant materials. Cucumber plants (cultivar Xintai Mici) were cultivated in a greenhouse at the experimental station of Shandong Agricultural University under conditions suitable for the occurrence of powdery mildew (i.e., 75 to 85% relative humidity and a temperature range of 15 to 35°C). Powdery mildew caused by *P. xanthii* developed naturally without inoculation.

Preparation of adjuvant solutions. Four different research-grade oil adjuvants were used in this study: methyl oleate (MO), biodiesel (BD), epoxidized soybean oil (ESO), and GY-Tmax (GYT). MO and GYT were provided by Beijing Grand AgroChem Co., Ltd. (Beijing, China), while BD and ESO were purchased from the Shandong Dezhou Longguang Grease-processing Plant (Shandong, China). The chemical compositions of the adjuvants are as follows: MO (methyl oleate and oleic acid 85%; surfactant content 15%), BD (methyl palmitate, methyl stearate, methyl oleate, methyl linoleate, and methyl linolenate 85%; surfactant content 15%), ESO (epoxidized soybean oil, linoleic acid, stearic acid, and oleic acid 85%; surfactant content 15%), and GYT (methyl soybean oil, linoleic acid, stearic acid, and oleic acid 85%; surfactant content 15%). Adjuvants were diluted with distilled water to final concentrations of 0.001, 0.01, 0.1, 1, 10, 100, or 1,000 mg liter⁻¹, for measurements of contact angle and surface tension. A commercial emulsifiable concentrate formulation of pyraclostrobin (Headline; 250 g of active ingredient [a.i.] liter⁻¹; BASF Crop Protection; Jiangsu Co., Ltd., Jiangsu, China) was used to evaluate the oil adjuvants.

Contact angle measurements. Healthy, young, fully expanded cucumber leaves and leaves of the same age infected with powdery mildew at a similar severity (disease index, 0.5 to 1.5%) were selected for measurements (four leaves per treatment). Leaf strips (approximately 0.7 by 5.0 cm) without veins were attached to a glass slide using double-sided tape and pressed lightly to prevent curling (Zhu et al. 2014). Care was taken to ensure that the leaf surface was not damaged. Droplets (5 µl) of each oil adjuvant test solution were placed on the leaf surface, and the contact angles were measured using a static optical contact angle goniometer (JC2000C2; Shanghai Zhongchen Digital Technology Apparatus Co., Ltd., Shanghai, China) at 25°C and 35 to 45% relative humidity. Each experiment was repeated twice with four replicates per treatment.

Surface tension measurements. Surface tensions of dilutions of oil adjuvants were measured by the Wilhelmy plate (made of Pt-Ir alloy) method (Zwertvaegher et al. 2014) in a Petri dish using an automatic surface tensiometer (BZY-1; Shanghai Hengping Instrument

and Meter Factory, Shanghai, China) at 25°C and 35 to 45% relative humidity. Each concentration was replicated four times, and the experiment was repeated twice.

Leaf surface affinity analysis. Contact angle measurements and frontal optical images of cucumber leaves were used to compare the affinity of droplets on healthy and powdery mildew-infected cucumber leaves (powdery mildew disease index, 0.5 to 1.5%). A 5-µl droplet of each oil adjuvant (1,000 mg liter⁻¹) was placed onto the cucumber leaves (avoiding leaf veins), and frontal optical images were obtained using a digital camera after the droplets stabilized (approximately 10 s). The droplet of each oil adjuvant was replicated four times, and the droplet was observed twice. The degree of leaf affinity is determined based on the wetting area and contact angle of the droplet on cucumber leaves.

Adhesional tension and work of adhesion. Adhesional tension and work of adhesion were used to evaluate the interaction of droplets with the leaf surface (Chibowski 2003). The treatments were replicated four times and the experiment was repeated twice. According to the basic theory of the wetting equation (Adamson and Gast 1997), adhesional tension and work of adhesion can be expressed using equation 1 and equation 2, respectively:

$$\beta = \gamma \cos \theta \quad (1)$$

$$W_a = \gamma (\cos \theta + 1) \quad (2)$$

where θ is the contact angle (°), γ is the surface tension (mN m⁻¹), β is the adhesional tension (mN m⁻¹), and W_a is the work of adhesion (mJ m⁻²).

Effect of pyraclostrobin-adjuvant mixtures on the morphology of *P. xanthii*. Cucumber leaves infected with powdery mildew at a similar severity (disease index, 0.5 to 1.5%) were sprayed with adjuvants (1,000 mg liter⁻¹), pyraclostrobin at 75 g a.i. ha⁻¹, pyraclostrobin at 75 g a.i. ha⁻¹ with adjuvants (1,000 mg liter⁻¹), or water. The spray volume was 900 liters ha⁻¹. The sprayer used in this test was 3WBD-20L knapsack electric sprayer (Taizhou Huang Yan Yifan sprayer factory, Taizhou, Zhejiang) and the pressure of the sprayer was set to 0.3 MPa. After 2 days, leaf tissues were sampled (approximately 2.0-by-4.0-cm leaf strips) and stained using a slightly modified protocol of Wolf and Fric (1981). Leaf samples were cleared for 30 min at 70°C using a 3:1 (vol/vol) mixture of ethanol and trichloromethane amended with 0.15% trichloroacetic acid. After rinsing with distilled water, tissues were stained in a 1:1 (vol/vol) mixture of 0.15% trichloroacetic acid and 0.6% Coomassie brilliant blue R-250 (Beijing Solarbio Science and Technology Co., Ltd., Beijing, China) (dissolved in 99% methanol) for 20 min. Tissues were then rinsed with distilled water and stored in glacial acetic acid-glycerol-water (1:4:15, vol/vol/vol). Fungal structures (the number of conidia in chains, detached conidia, and the length of conidial chains) of *P. xanthii* were observed by light microscopy (Olympus IX-71; Tokyo, Japan) at ×100 magnification. Four leaves were evaluated per treatment and the experiment was repeated twice.

Efficacy of pyraclostrobin mixed with adjuvants for the control of *P. xanthii*. Grafted cucumber plants (Xintai Mici) were grown in a greenhouse in Tai'an, Shandong, China (117.208°N, 36.035°E) in 2017. The plants were transplanted in two greenhouses (one greenhouse in Fangcun, the other in Dongdawu) on 8 March 2017 and cultivated according to recommended agronomic practices. Treatments included the adjuvants MO, BD, ESO, and GYT at 550, 775, or 1,000 mg liter⁻¹; pyraclostrobin at 75, 100, or 150 g a.i. ha⁻¹; pyraclostrobin at 75, 100, or 150 g a.i. ha⁻¹ with the addition of MO, BD, ESO, or GYT (1,000 mg liter⁻¹); and water as a control. Each treatment was applied three times on 9, 16, and 23 May to four replicate plots, each consisting of 60 plants in a 15 m² plot. The spray volume was 900 liters ha⁻¹ and treatments were applied using a 3WBD-20L knapsack electric sprayer and the pressure of the sprayer was set to 0.3 MPa.

Maximum retention (R_m) and evaporation time (T) of pyraclostrobin applied with adjuvants at low doses were measured immediately after application (within 1.5 h) (Supplementary Table S1). Disease

severity after natural infection with *P. xanthii* was recorded before the first fungicide application and 7 days after each application. Mature leaves of 20 randomly selected plants (6th to 10th fully expanded leaves of each plant, total 100 leaves per plot) were evaluated for disease severity. Disease severity of each leaf was scored using the following scale: 0 = no lesions and 1 = <5, 2 = 5 to 25, 3 = 25 to 50, 4 = 50 to 75, and 5 = >75% of the leaf area with powdery mildew lesions (Tanaka et al. 2017). The disease index and control efficacy were calculated using the following equations: Disease index (%) = $[\sum(\text{number of leaves with each disease severity} \times \text{disease severity})]/(\text{total number of leaves evaluated} \times 5) \times 100$, and Control efficacy (%) = $[1 - (D_{C0} \times D_{T1})/(D_{C1} \times D_{T0})] \times 100$, where D_{C0} is the disease index of control leaves before application, D_{C1} is the disease index of control leaves after 7 days of incubation, D_{T0} is the disease index of treated leaves before application, and D_{T1} is the disease index of treated leaves after 7 days of incubation.

Affinity pattern of pyraclostrobin with adjuvants. Glass (microscope slides; Zhengzhou Zhongtian Laboratory Instrument Co., Ltd., Zhengzhou, China) and membrane (polytetrafluorethylene; Shenzhen Xiong Yihua Plastic Material Co., Ltd., Shenzhen, China) surfaces were selected as representatives of hydrophilic and hydrophobic interfaces, respectively. The surfaces were cleaned with ethanol before the experiment. Droplets (5 μ l) of pyraclostrobin alone at 83.3 mg liter⁻¹ (75 g a.i. ha⁻¹) and pyraclostrobin at 83.3 mg liter⁻¹ with oil adjuvants at 1,000 mg liter⁻¹ were applied to each surface and allowed to dry for 2 h before observation with a light microscope (Olympus IX-71). Each experiment was repeated twice with four replicates. The patterns of residue distribution after droplet drying (the size of dry crystals, periphery, and oil droplets in dry crystals) were specifically observed. The size of the dry crystals were measured (in micrometers), the breadth of periphery was assessed, and the presence or absence of dry crystals in the oil droplets was recorded.

Statistical analyses. Data of repeated experiments were pooled for analysis, because variances between experiments were homogeneous through the homogeneity test of variance. Statistical analyses were performed using SPSS software (version 13.0 for Windows; SPSS Inc., Chicago, IL, U.S.A.). Differences among the treatments were evaluated with the Fisher's least significant difference test for the following variables: contact angle, surface tension, and control efficacy. The percent control efficacy was arcsine transformed prior to analyses. Analyses of covariance were performed to compare the slopes of regression line between surface tension and adhesional tension of different adjuvants on healthy and powdery-mildew-infected cucumber leaves.

Results

Surface tension. Surface tension values of the four oil adjuvants decreased with increasing concentration (Fig. 1). At concentrations

Fig. 1. Surface tension changes of four oil-based adjuvants at concentrations of 0.001 to 1,000 mg liter⁻¹ (in 10 $\times$ increments). Bars denote the standard error. Each data point represents the average of four replicates pooled over two surface tension experiments.

higher than 10 mg liter⁻¹, slopes of BD and GYT were greater than at lower concentrations. In contrast, surface tensions of ESO and MO decreased more gradually with increasing concentrations, indicating that their effects were almost constant. The surface tension of the four oil adjuvants was very similar at a concentration of 1,000 mg liter⁻¹ ($P = 0.936$).

Leaf affinity profiling. Contact angles and stereomicroscope images of adjuvant droplets at a concentration of 1,000 mg liter⁻¹ on healthy and powdery-mildew-infected cucumber leaves are shown in Figure 2 in comparison with the water control. The affinity of droplets on powdery-mildew-infected leaves was significantly lower than that on healthy leaves regardless of whether an adjuvant was used ($P = 0.021$). The affinity of different oil adjuvants on healthy and powdery-mildew-infected leaves was significantly different among adjuvants ($P < 0.01$). The GYT adjuvant showed the highest affinity, while the BD showed the lowest ($P < 0.01$). Droplets of all treatments tested in this study were wettable on leaves with contact angle (θ) < 90°. The four adjuvants showed wettability on powdery-mildew-infected leaves ($\theta < 90^\circ$); however, water alone did not ($\theta = 125.7^\circ$). The contact angle of GYT was the lowest ($P < 0.01$), and droplets covered a comparatively large area of healthy or powdery-mildew-infected leaves. The contact angle of ESO on powdery-mildew-infected leaves was intermediate; those of MO and BD were approximately 80°, and only small portions of the leaves were covered by circular droplets. In particular, the affinity effect of adjuvants on powdery-mildew-infected leaves was more influential (Fig. 2).

Adhesional tension. When comparing surface tension with adhesional tension for each adjuvant on healthy cucumber leaves (Fig. 3A), MO, BD, and ESO showed strong correlations, while GYT showed the weakest correlation ($R^2 = 0.681$). The small slope of the regression line for MO was -0.589 ; the slopes of the regression lines for the other adjuvants were very similar ($P = 0.217$), which showed that the increase of adhesional tension was similar with the decrease of surface tension (Supplementary Table S2).

Relationship between surface tension and adhesional tension for each adjuvant on powdery-mildew-infected cucumber leaves was evaluated (Fig. 3B). Similarly, the effect of MO on powdery-mildew-infected cucumber leaves was also minimal, and the slope of MO was -1.121 (data not shown). The slopes of the regression lines of BD and GYT were nearly identical ($P = 0.395$) but the slope of ESO was the highest on powdery-mildew-infected leaves ($P < 0.05$), and had the greatest effect on improving adhesional tension (Supplementary Table S3).

Work of adhesion. Work of adhesion for ESO and MO on healthy and powdery-mildew-infected cucumber leaves is shown in Figure 4A and B. On healthy leaves, the ranges of the work of adhesion were between 46.44 and 50.46 mJ m⁻² for adjuvant concentrations higher than 0.01 mg liter⁻¹, whereas the ranges for BD and GYT were inconsistent (Fig. 4C and D). BD and GYT at concentrations of 10 mg liter⁻¹ on healthy leaves showed the highest work of adhesion ($P = 0.017$), while the highest work of adhesion of GYT on powdery-mildew-infected leaves reached 61.07 mJ m⁻², and other adjuvants did not significantly affect the work of adhesion.

Effect of pyraclostrobin with adjuvants on the morphology of *P. xanthii*. The effect of pyraclostrobin at 75 g a.i. ha⁻¹ and pyraclostrobin at 75 g a.i. ha⁻¹ with adjuvants (1,000 mg liter⁻¹) on the morphology of *P. xanthii* was investigated. The morphology of untreated powdery mildew included conidial chains consisting of 6 to 10 conidia (typical hyphal growth; conidiophores grow naturally, conidium are fully developed) (Fig. 5A). The conidial chains of *P. xanthii* were unchanged after treatment with adjuvants alone; no detached conidia were observed; however, some distortion of mycelia was observed, such as mycelium thinning and bending (data not shown). The length of conidial chains of *P. xanthii* treated with pyraclostrobin were much shorter than that of the untreated powdery mildew, consisting of 6 to 10 conidia, and the number of conidia on the chain decreased (Fig. 5B). The conidiophores and conidial chains of *P. xanthii* treated with pyraclostrobin supplemented with adjuvants were inhibited to varying degrees (Fig. 5C to F); conidiophores were formed on the

mycelia but conidia were not produced (Fig. 5E) and the mycelia were distorted and deformed (Fig. 5F).

Control efficacy of pyraclostrobin with adjuvants for the control of *P. xanthii*. The four oil adjuvants alone reduced powdery mildew disease severity on cucumber (Table 1). Seven days after the last treatment, the efficacy of the four adjuvants alone was evaluated and the level of disease control ranged from 40.6 to 62.8%. The efficacy of the GYT group was the highest when applied at

1,000 mg liter⁻¹ ($P = 0.031$). This was consistent with its highest affinity for powdery-mildew-infected leaves. The control efficacy of ESO was slightly numerically lower than that of GYT at each concentration ($P = 0.085$), and there was no significant difference in control efficacy between MO and BD at the three tested concentrations ($P = 0.193$). There was also no statistical interaction between adjuvants and application concentrations ($P = 0.765$) (Supplementary Table S4).

Fig. 2. Contact angle (degree) and affinity as visually photographed using a stereomicroscope of **A** to **D**, GY-Tmax (GYT); **E** to **H**, epoxidized soybean oil (ESO); **I** to **L**, methyl oleate (MO) (**I**-**L**); and **M** to **P**, biodiesel (BD) with a concentration of 1,000 mg liter⁻¹ and **Q** to **T**, water (CK) on healthy cucumber leaves (**A**, **B**, **E**, **F**, **I**, **J**, **M**, **N**, **Q**, and **R**) and powdery-mildew-infected cucumber leaves (**C**, **D**, **G**, **H**, **K**, **L**, **O**, **P**, **S**, and **T**). Bar indicates 5 mm. The contact angle represents the average of four replicates pooled over two repeated contact angle experiments.

Fig. 3. Relationship between surface tension and adhesional tension for four oil adjuvants on **A**, healthy cucumber leaves and **B**, powdery-mildew-infected cucumber leaves. Each data point represents the average of four replicates adhesional tension under the corresponding surface tension of oil adjuvants pooled over surface tension and contact angle experiments.

Fig. 4. Work of adhesion for each adjuvant at concentrations of 0.001 to 1,000 (in 10 $\times$ increments) on healthy cucumber leaves and powdery-mildew-infected cucumber leaves. **A**, Epoxidized soybean oil (ESO); **B**, methyl oleate (MO); **C**, biodiesel (BD); and **D**, GY-Tmax (GYT). Bars denote the standard error. Each data point represents the average of four replicates work of adhesion under the corresponding concentration of oil adjuvants pooled over surface tension and contact angle experiments.

The control efficacy of pyraclostrobin (three tested concentrations) when combined with each adjuvant was higher than the adjuvant alone at 1,000 mg liter⁻¹ ($P = 0.018$) (Table 1). The efficacy increased as the rate of pyraclostrobin increased. These trials confirmed that the control efficacy of pyraclostrobin with adjuvants was higher than 70%, except when the fungicide was mixed with MO and BD at the lowest concentration tested. The efficacy of

pyraclostrobin with GYT and ESO was significantly higher than that of fungicide-alone treatments ($P = 0.028$), and the efficacy of pyraclostrobin with MO and BD was equally effective compared with the fungicide applied at rates of 75 and 100 g a.i. ha⁻¹. There was no statistical interaction between pyraclostrobin + adjuvants and application concentrations ($P = 0.600$) (Supplementary Table S5).

Fig. 5. Effect of pyraclostrobin (Pyra) at 75 g a.i. ha⁻¹ and Pyra at 75 g a.i. ha⁻¹ with oil adjuvant at 1,000 mg liter⁻¹ on the morphology of *Podosphaera xanthii* was observed by light microscopic. **A**, Water; **B**, Pyra; **C**, Pyra + biodiesel; **D**, Pyra + methyl oleate; **E**, Pyra + epoxidized soybean oil; and **F**, Pyra + GY-Tmax. Conidia, chains of conidia, and mycelium growth of *P. xanthii* are shown. Bars indicate 50 μ m. The morphology of each treatment represents the results of four replicates pooled over morphological experiments.

Table 1. Efficacy of oil adjuvants and pyraclostrobin (Pyra) with oil adjuvants (1,000 mg liter⁻¹) against powdery mildew on cucumber leaves^x

Treatments	Conc (mg liter ⁻¹)	IDI (%) ^y	7 days after last treatment		Treatments	Dose (g a.i. ha ⁻¹)	IDI (%) ^y	7 days after last treatment	
			DI (%)	CE (%) ^z				DI (%)	CE (%) ^z
GYT	550	1.89	5.04	47.46 ef	Pyra + GYT	75	1.85	1.89	79.87 d
	775	1.99	4.36	56.83 bc	...	100	2.08	1.21	88.54 ab
	1,000	1.95	3.68	62.82 a	...	150	2.09	0.90	91.52 a
ESO	550	1.99	5.42	46.34 f	Pyra + ESO	75	1.93	2.20	77.54 de
	775	1.82	4.30	53.45 cd	...	100	2.01	1.53	85.00 bc
	1,000	1.97	4.01	59.89 ab	...	150	1.96	1.03	89.65 ab
MO	550	1.90	5.46	43.38 fg	Pyra + MO	75	1.85	3.15	66.45 g
	775	1.95	5.12	48.27 def	...	100	2.08	2.97	71.87 f
	1,000	2.04	4.84	53.25 cd	...	150	2.09	2.06	80.58 cd
BD	550	2.02	6.09	40.60 g	Pyra + BD	75	2.04	3.56	65.62 g
	775	2.12	5.77	46.37 f	...	100	2.00	2.86	71.82 f
	1,000	1.99	4.86	51.88 cde	...	150	1.93	2.07	78.87 d
Water control	...	1.99	10.10	...	Pyra	75	2.10	3.74	64.91 g
	...	...	...	...	...	100	1.93	3.02	69.17 fg
	...	...	...	...	...	150	1.89	2.59	73.00 ef

^x Abbreviations: Conc = application concentration, IDI = initial disease index, DI = disease index, CE = control efficacy, Dose = application dose, GYT = GY-Tmax, ESO = epoxidized soybean oil, MO = methyl oleate, and BD = biodiesel.

^y DI (%) was derived from [Σ (number of leaves with each disease severity $\times$ disease severity)]/(total number of leaves investigated $\times$ 5) $\times$ 100.

^z Fisher's least significant difference tests were conducted at $P = 0.05$. The same letters indicate no significant difference.

Affinity pattern of pyraclostrobin with oil adjuvants. The dried droplet residue of pyraclostrobin alone and pyraclostrobin with oil adjuvants at 1,000 mg liter⁻¹ on membrane surfaces or a slide glass was characterized. Crystals in the dried residue of pyraclostrobin with oil adjuvants (Fig. 6A to D) on the two evaluated surfaces were smaller than those of pyraclostrobin alone (Fig. 6E and F) on both glass and membrane surfaces. With the exception of pyraclostrobin + GYT, the dried residue of other treatments formed obvious peripheries (the edges of dry droplets, where the crystal particles congregate). The distribution of all treatments on the hydrophilic interfaces (slide glasses) was more uniform than that on the hydrophobic interfaces (membranes). The characteristics of pyraclostrobin + ESO on the glass slide (Fig. 6B1) included the formation of large oil droplets in the center.

Discussion

A new idea has been put forward in this study, in that it was difficult to wet cucumber leaves infected with powdery mildew by pesticide droplets. We demonstrated that adding oil-based adjuvants to pyraclostrobin can improve the affinity of spray droplets on powdery-mildew-infected leaves. The affinity of pyraclostrobin with or without the oil-based adjuvants on powdery-mildew-infected leaves was significantly different but there was no significant difference on healthy leaves. The use of adjuvants can significantly improve the efficacy of pesticides and, thus, decrease the usage of pesticides (Abbott and Beckerman 2018).

Quinone outside inhibitor and sterol demethylation inhibitor fungicides are currently the main classes of fungicides used for the prevention and control of cucumber powdery mildew. However, high levels of resistance of *P. xanthii* to the two types of fungicides have been observed due to their frequent use (Fernández-Ortuño et al. 2006; López-Ruiz et al. 2010). The weak wettability of fungicides on powdery-mildew-infected leaves is an important factor because the leaves become hydrophobic interfaces when infected with powdery mildew, which hinders the wetting process of spray droplets. Previous studies have demonstrated that the type of liquid has a significant influence on the surface (Zhu et al. 2014). Our results also showed that adding oil adjuvants can improve the affinity of fungicides on healthy and powdery-mildew-infected leaves of cucumber. In previous studies, the splash of droplets on superhydrophobic interfaces can be greatly suppressed by adding a vesicular surfactant or adjuvant (Song et al. 2017). In terms of contact angle, lower values indicate better surface wetting (Good 1992). Both the type of spray solution and the polarity of the leaf surface affect the spreading area of spray droplets (Nairn et al. 2016). Xu et al. (2011) reported that the

wetting area of spray per volume increased from oil adjuvants and that the amount of spray mixtures can be reduced. In our current study, stereomicroscope images showed that the affinity of different oil adjuvants was different, and the wetting area of GYT was the largest on healthy and powdery-mildew-infected leaves, which suggested that GYT can effectively alter the affinity of the fungicides.

Adhesion tension and work of adhesion are two important evaluation indexes of interface affinity. Interestingly, our results showed that there was a relationship between surface tension and adhesional tension for four oil adjuvants on healthy cucumber leaves and powdery-mildew-infected cucumber leaves. Wang et al. (2014) found that the adhesion of water was correlated with the retention of water on different leaf surfaces. Due to the influence of the rough interface formed by powdery mildew, the correlation between surface tension and adhesional tension is altered. The work of adhesion is an important measurable indicator for agricultural chemists. The work of adhesion not only changed with the degree of roughness of the interface (Nairn and Forster 2017) but also varied with the addition of different adjuvants. Our results showed that, as the concentration of ESO and BD increased, the ratio of the work of adhesion on powdery-mildew-infected leaves to that on healthy leaves was closer to 1 (Fig. 4A and C). In contrast, the work of adhesion for MO did not change across different concentrations, whereas GYT has a modal relationship with concentrations evaluated. Our results demonstrated that, within the concentration ranges evaluated, the higher the concentration of adjuvants, the less the effect of interface roughness was on the work of adhesion. Considering adhesion properties on healthy and powdery-mildew-infected leaves, GYT was the best among all tested adjuvants.

Because the pathogen that causes powdery mildew is an obligate and biotrophic ectoparasite, the pathogen cannot be cultured in in vitro culture medium. Thus, to investigate the effects of fungicides on the morphology of *P. xanthii*, morphological changes of the pathogen on cucumber leaves were observed using an optical microscopy in this study and others (He et al. 2017; Tanaka et al. 2017). This research found that the sporulation ability of *P. xanthii* treated with adjuvants decreased; only fungicides can produce fungicidal effects, while adjuvants provide small effects. Also, the results indicated that all the treatments that contained adjuvants had caused the deformation of the conidial chain. Therefore, adding adjuvants can contribute to the fungicide efficacy. The conclusion of our analysis and observations was that the higher the inhibition of conidial chain formation by pyraclostrobin–oil adjuvant mixtures, the greater the suppression of the occurrence of powdery mildew.

Fig. 6. Characteristic distribution patterns ($n = 4$) of pyraclostrobin (Pyra) at 83.3 mg liter⁻¹ (75 g a.i. ha⁻¹) and Pyra at 83.3 mg liter⁻¹ with oil adjuvants at 1,000 mg liter⁻¹ on 1, slide glass surfaces and 2, membrane surfaces. Images in the lower right corner of each picture are the edge of the droplet after drying. A, Pyra + GY-Tmax; B, Pyra + epoxidized soybean oil; C, Pyra + methyl oleate; D, Pyra + biodiesel; and E, Pyra. Bars indicate 10 μ m. The characteristic distribution patterns of each treatment represents the results of four replicates pooled over droplets drying experiments.

To evaluate the practical application effect of oil adjuvants, we added adjuvants to pyraclostrobin to verify their field efficacy. Under the same size and speed of the liquid droplets, the efficacy of pyraclostrobin supplemented with adjuvants was much higher than that of pyraclostrobin alone ($P = 0.028$). This indicated that the addition of adjuvants had a significant role in the management of powdery mildew. The mechanism of the improvement in the field control effect lies in the addition of adjuvants to regulate the affinity and spreadability of the liquid on the plant leaves, thus ensuring deeper penetration and absorption of the liquid on the target (DiTomaso 1999; Xu et al. 2011). Our results demonstrated that the low dose of pyraclostrobin with adjuvants can be used to prevent and reduce cucumber powdery mildew development. Oil adjuvants can reduce both the surface tension and contact angle of spray droplets, increase the deposition amount of liquid on a leaf surface, and delay the evaporation time of spray droplets (Harrison and Bode 1986; Xu et al. 2011). This information is very helpful for improving the application of pesticides.

Previous studies have shown that artificial surfaces used in the study of deposition and deposition sediment of pesticide with or without adjuvants were highly similar to the sedimentary models on plant leaves (Hunsche and Noga 2012; Kraemer et al. 2009). Evaluations of the crystal distribution of pyraclostrobin with or without oil adjuvants on hydrophilic or hydrophobic surfaces indicated the deposition of the fungicide on both surfaces. The clustering of crystal particles of pyraclostrobin decreased in fungicide-oil adjuvants mixtures. The mixture of pyraclostrobin + ESO formed oil droplets on glass slides. The advantage of this phenomenon was that it can extend the evaporation time of the pesticide but the disadvantage was the uneven distribution of the active ingredient of the pesticide. In this study, the hydrophobic interface represented the powdery mildew surface, and pyraclostrobin with oil adjuvants reduced the marginalization and aggregation of its active ingredient and increased its uniform distribution on the interface.

Literature Cited

- Abbott, C. P., and Beckerman, J. L. 2018. Incorporating adjuvants with captan to manage common apple diseases. *Plant Dis.* 102:231-236.
- Adamson, A. W., and Gast, A. P. 1997. Pages 452-469 in: *Physical Chemistry of Surfaces*, 6th ed. XII. Friction, Lubrication, and Adhesion and XIII. Wetting, Flotation, and Detergency. Wiley, New York.
- Braun, U., Cook, R. T. A., Inman, A. J., and Shin, H. D. 2002. The taxonomy of the powdery mildew fungi. Pages 13-55 in: *The Powdery Mildews. A Comprehensive Treatise*. R. R. Bélanger, W. R. Bushnell, A. J. Dik, and T. L. W. Carver, eds. American Phytopathological Society, St Paul, MN, U.S.A.
- Cabanne, F., Gauudry, J. C., and Streibig, J. C. 1999. Influence of alkyl oleates on efficacy of phenmedipham applied as an acetone: Water solution on *Galium aparine*. *Weed Res.* 39:57-67.
- Chibowski, E. 2003. Surface free energy of a solid from contact angle hysteresis. *Adv. Colloid Interface Sci.* 103:149-172.
- Cohen, R., Burger, Y., and Katzir, N. 2004. Monitoring physiological races of *Podosphaera xanthii* (syn. *Sphaerotheca fuliginea*), the causal agent of powdery mildew in cucurbits: Factors affecting race identification and the importance for research and commerce. *Phytoparasitica* 32:174-183.
- De Ruiter, H., Uffing, A. J. M., Meinen, E., and Prins, A. 1990. Influence of surfactants and plant species on leaf retention of spray solution. *Weed Sci.* 38:567-572.
- DiTomaso, J. M. 1999. Barriers to foliar penetration and uptake of herbicides. *Proc. Calif. Weed Sci. Soc.* 51:150-155.
- Downs, T. E. 2008. The phytotoxic effects of herbicide and adjuvant application by physiological timing on the woody shrub salal (*Gaultheria shallon*). Master's thesis, Oregon State University.
- Fernández-Ortuño, D., Pérez-García, A., López-Ruiz, F. J., Romero, D., de Vicente, A., and Torés, J. A. 2006. Occurrence and distribution of resistance to QoI fungicides in populations of *Podosphaera fusca* in south central Spain. *Eur. J. Plant Pathol.* 115:215-222.
- Gaskin, R. E., Steele, K. D., and Forster, W. A. 2005. Characterising plant surfaces for spray adhesion and retention. *N. Z. Plant Prot.* 58:179-183.
- Good, R. J. 1992. Contact angle, wetting, and adhesion: A critical review. *J. Adhes. Sci. Technol.* 6:1269-1302.
- Harrison, S. K., and Bode, L. E. 1986. Influence of adjuvants and application variables on postemergence weed control with bentazon and sethoxydim. *Weed Sci.* 34:462-466.
- He, L., Cui, K., Ma, D., Shen, R., Huang, X., Jiang, J., Mu, W., and Liu, F. 2017. Activity, translocation and persistence of isopyrazam for controlling cucumber powdery mildew. *Plant Dis.* 101:1139-1144.
- Hess, F. D., and Foy, C. L. 2000. Interaction of surfactants with plant cuticles. *Weed Technol.* 14:807-813.
- Hunsche, M., and Noga, G. 2012. Effects of relative humidity and substrate on the spatial association between glyphosate and ethoxylated seed oil adjuvants in the dried deposits of sessile droplets. *Pest Manage. Sci.* 68: 231-239.
- Kirkwood, R. C. 1993. Use and mode of action of adjuvants for herbicides: A review of some current work. *Pestic. Sci.* 38:93-102.
- Kraemer, T., Hunsche, M., and Noga, G. 2009. Surfactant-induced deposit structures in relation to the biological efficacy of glyphosate on easy- and difficult-to-wet weed species. *Pest Manage. Sci.* 65:844-850.
- Kudsk, P., and Mathiassen, S. K. 2007. Analysis of adjuvant effects and their interactions with variable application parameters. *Crop Prot.* 26: 328-334.
- Liu, Z. Q. 2004. Effects of surfactants on foliar uptake of herbicides—A complex scenario. *Colloids Surf. B* 35:149-153.
- López-Ruiz, F. J., Pérez-García, A., Fernández-Ortuño, D., Romero, D., García, E., de Vicente, A., Brown, J. K. M., and Tores, J. A. 2010. Sensitivities to DMI fungicides in populations of *Podosphaera fusca* in south central Spain. *Pest Manage. Sci.* 66:801-808.
- Naim, J. J., and Forster, W. A. 2017. Methods for evaluating leaf surface free energy and polarity having accounted for surface roughness. *Pest Manage. Sci.* 73:1854-1865.
- Naim, J. J., Forster, W. A., and van Leeuwen, R. M. 2011. Quantification of physical (roughness) and chemical (dielectric constant) leaf surface properties relevant to wettability and adhesion. *Pest Manage. Sci.* 67:1562-1570.
- Naim, J. J., Forster, W. A., and van Leeuwen, R. M. 2016. Effect of solution and leaf surface polarity on droplet spread area and contact angle. *Pest Manage. Sci.* 72:551-557.
- Ramsey, R. J. L., Stephenson, G. R., and Hall, J. C. 2005. A review of the effects of humidity, humectants, and surfactant composition on the absorption and efficacy of highly water-soluble herbicides. *Pestic. Biochem. Physiol.* 82: 162-175.
- Ramsey, R. J. L., Stephenson, G. R., and Hall, J. C. 2006. Effect of humectants on the uptake and efficacy of glufosinate in wild oat (*Avena fatua*) plants and isolated cuticles under dry conditions. *Weed Sci.* 54:205-211.
- Song, M., Ju, J., Luo, S., Han, Y., Dong, Z., Wang, Y., Gu, Z., Zhang, L., Hao, R., and Jiang, L. 2017. Controlling liquid splash on superhydrophobic surfaces by a vesicle surfactant. *Sci. Adv.* 3:e1602188.
- Tanaka, K., Fukuda, M., Amaki, Y., Sakaguchi, T., Inai, K., Ishihara, A., and Nakajima, H. 2017. Importance of prumycin produced by *Bacillus amyloliquefaciens* SD-32 in biocontrol against cucumber powdery mildew disease. *Pest Manage. Sci.* 73:2419-2428.
- Wang, H., Shi, H., Li, Y., and Wang, Y. 2014. The effects of leaf roughness, surface free energy and work of adhesion on leaf water drop adhesion. *PLoS One* 9:e107062.
- Wolf, G., and Fric, F. 1981. A rapid staining method for *Erysiphe graminis* f. sp. *hordei* in and on whole barley leaves with a protein-specific dye. *Phytopathology* 71:596-598.
- Xu, L., Zhu, H., Ozkan, H. E., Bagley, W. E., and Krause, C. R. 2011. Droplet evaporation and spread on waxy and hairy leaves associated with type and concentration of adjuvants. *Pest Manage. Sci.* 67:842-851.
- Zhang, Z. Y., Dai, G. H., Zhuge, Y. Y., and Li, Y. B. 2008. Protective effect of *Robinia pseudoacacia* Linn1 extracts against cucumber powdery mildew fungus, *Sphaerotheca fuliginea*. *Crop Prot.* 27:920-925.
- Zhao, Q., Liu, Y., Wang, C., Wang, S., and Müller-Steinhagen, H. 2005. Effect of surface free energy on the adhesion of biofouling and crystalline fouling. *Chem. Eng. Sci.* 60:4858-4865.
- Zhou, Z., Cao, C., Cao, L., Zheng, L., Chao, M. A., Li, F., and Huang, Q. 2017. Research advances of droplet evaporation on different interfaces and the efficiency of pesticide utilization. *Chin. J. Pest. Sci.* 19:9-17.
- Zhu, Y. Q., Yu, C. X., Li, Y., Zhu, Q. Q., Zhou, L., Cao, C., Yu, T. T., and Du, F. P. 2014. Research on the changes in wettability of rice (*Oryza sativa*) leaf surfaces at different development stages using the OWRK method. *Pest Manage. Sci.* 70:462-469.
- Zwertvaegher, I. K., Verhaeghe, M., Brusselman, E., Verboven, P., Lebeau, F., Massinon, M., Nicolai, B. M., and Nuytens, D. 2014. The impact and retention of spray droplets on a horizontal hydrophobic surface. *Biosyst. Eng.* 126:82-91.