Activity, Translocation, and Persistence of Isopyrazam for Controlling Cucumber Powdery Mildew

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Abstract

A cotyledon bioassay was conducted to assess the activity of isopyrazam against *Podosphaera xanthii* (Castagne) U. Braun & N. Shishkoff, causal agent of cucumber powdery mildew. Results showed that isopyrazam has protective and curative activity against *P. xanthii*, with EC_{50} values of 0.04 and 0.05 mg liter⁻¹, respectively. These activities are higher than those for hexaconazole, difenoconazole, pyraclostrobin, kresoxim-methyl, and azoxystrobin, fungicides currently used against cucumber powdery mildew. Isopyrazam at 0.5 mg liter⁻¹ damaged conidiophores. Results of inoculation tests in greenhouse pots indicate that isopyrazam demonstrates a level of systemic movement in cucumber plants, especially regarding translaminar and

transverse translocation. Efficacy following translaminar and transverse translocations on cotyledons and leaves treated with 60 mg liter⁻¹ was 94.40% and 88.96%, and 95.26% and 82.83%, respectively. In addition, isopyrazam at 60 mg liter⁻¹ exhibited a long duration of efficacy against cucumber powdery mildew, almost 2 to 3 weeks longer than that of triazoles and strobilurins. Similar trends in residual durations were observed during 2014 and 2015 greenhouse trials. Isopyrazam at 30 and 60 a.i. g ha⁻¹ provided efficacy ranging from 83.27 to 90.83% 20 days following treatment. In conclusion, isopyrazam has translaminar and transverse translocation in cucumber leaves, and long duration of activity against cucumber powdery mildew.

Powdery mildew is one of the most destructive foliar diseases of cucurbits, causing huge production losses worldwide (Cohen et al. 2004; Zhang et al. 2008). Cucumber powdery mildew is caused by the fungal pathogens *Podosphaera xanthii* (Castagne) U. Braun & N. Shishkoff (syn. *Sphaerotheca fusca* (Fr.) S. Blumer, formerly *Sphaerotheca fuliginea* (Schldt.: Fr.) Pollacci) and *Golovinomyces chichoracearum* (formerly *Erysiphe chichoracearum*), with the former being more common around the world than the latter (Elad et al. 1996). The pathogen appears as white colonies on leaves and may also develop on petioles, stems, and fruits, affecting both the yield and quality (Elad et al. 1998). Application of fungicides is presently the principal practice for managing this disease (McGrath and Staniszevska 1996; Pérez-García et al. 2009). Fungicides that have been used in China include benzimidazole, triazole, and strobilurin fungicides. However, as these fungicides have been frequently used to manage cucurbit powdery mildew, resistance of cucurbit powdery mildew pathogens to these fungicides has been observed in many countries (Fernández-Ortuño et al. 2006; Ishii et al. 2001; O'Brien 1994; Schepers 1983). Cucurbit powdery mildew pathogens have shown resistance to carbendazim and kresoxim-methyl in China (Lin et al. 2014). The development of new and highly active chemicals for controlling cucumber powdery mildew is imperative.

Isopyrazam is a novel broad-spectrum foliar fungicide (Harp et al. 2011). It is a succinate dehydrogenase inhibitor (SDHI) that targets complex-II (or succinate dehydrogenase) in the mitochondrial respiration chain, hindering energy metabolism (Ajigboye et al. 2014; Dubos et al. 2013). Isopyrazam, which was developed and patented by Syngenta Crop Protection in 2008, has been labeled to control *Septoria tritici*, rusts, and other diseases of cereals including wheat and barley (Harp et al. 2011). Isopyrazam has low solubility in water and high lipophilic solubility and can persist in the lipophilic wax layer of the plant during irrigation or rain. It has high activity against Ascomycota and Basidiomycota, and has demonstrated good efficacy against powdery mildew on cereals (*Erysiphe graminis*) (Sattler

et al. 2010); however, its systemic activity against powdery mildew on cucumber (*Podosphaera xanthii*) has not been reported.

In this work, the protective and curative activity, systemic translocation, and persistence of isopyrazam for controlling cucumber powdery mildew were investigated and compared with hexaconazole, difenoconazole, pyraclostrobin, kresoxim-methyl, and azoxystrobin.

Materials and Methods

Fungicides. Fungicides used in experiments with cotyledons and seedlings were isopyrazam (99%; Sigma-Aldrich [Shanghai] Co. Ltd., China); hexaconazole (95%; Jiangsu Sevencontinent Green Chemical Co. Ltd., Jiangsu, China); difenoconazole (92%) and azoxystrobin (93%) (Syngenta Crop Protection UK Ltd.); and pyraclostrobin (97%) and kresoxim-methyl (94%) (BASF SE., Ludwigshafen, Germany). Isopyrazam 125 g liter⁻¹ EC (Reflect), difenoconazole 10% WG (Score), azoxystrobin 250 g liter⁻¹ SC (Amistar), hexaconazole 5% ME (Cuili), pyraclostrobin 250 g liter⁻¹ EC (Cabrio), and kresoxim-methyl 50% WG (Stroby) were used in greenhouse experiments. Fungicide stock solutions were prepared at a concentration of 10 mg ml⁻¹ and stored at -20°C until use.

Preparation of the conidial suspension. An isolate (TA1) of *P. xanthii* used in this study was obtained from a powdery mildew infected cucumber plant (cv. Xintai Mici) grown in a cucumber greenhouse located at the horticulture station of Shandong Agricultural University. The isolate was single-spore isolated according to the method described by Fernández-Ortuño et al. (2006) and maintained in vitro (Álvarez and Torés 1997). Conidial concentration was enumerated with a bright-line hemocytometer, and consistent conidial suspensions (1×10^5 conidia ml⁻¹) were applied in all cotyledon bioassays and greenhouse experiments.

Protective and curative activities. To determine protective and curative activities, a cotyledon bioassay technique described previously (Jia et al. 2006) was used with minor modifications. Cucumber (cv. Xintai Mici) seeds were surface sterilized by soaking in 2.5% sodium hypochlorite for 3 min and washed three times with sterile water. The surface sterilized seeds were pregerminated in a moist chamber at 25°C and sown in seedling-raising trays filled with a 3:1 mixture of autoclaved sandy loam and compost. Subsequent to the unfolding of cotyledons, the two cotyledons were cut off of the plant, and petioles were inserted into a 0.1% water agar medium. Six cotyledons were placed on each of three replicated plates. Each stock solution of the fungicides was diluted to seven concentrations in sterile deionized water as follows: 0, 0.0125, 0.025, 0.05, 0.25, 0.5,

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and 1 mg liter⁻¹ for isopyrazam; 0, 0.04, 0.2, 1, 5, 25, and 50 mg liter⁻¹ for hexaconazole; 0, 0.2, 1, 5, 25, 50, and 100 mg liter⁻¹ for difenoconazole and pyraclostrobin; 0, 1, 5, 10, 50, 100, and 200 mg liter⁻¹ for kresoxim-methyl; and 0, 5, 10, 50, 100, 200, and 400 mg liter⁻¹ for azoxystrobin. Each diluent was sprayed evenly onto the cucumber cotyledons until runoff 24 h before inoculation (protective activity) and 96 h after inoculation (curative activity) using a settling tower for uniformity. Each concentration was repeated in triplicate. Inoculation was performed by spraying the conidial suspension (1×10^5 conidia ml⁻¹). After the liquid had dried, all treatments were placed in an incubator at 25 ± 2°C with a 16 h light, 8 h dark photoperiod for 7 to 10 days. The number of colonies were counted and the inhibition rate of colony numbers was calculated. The EC₅₀ values of fungicides tested against *P. xanthii* were calculated by regression of the percent relative growth against the log₁₀ fungicide concentrations. The inhibition rate was calculated with the following equation:

Inhibition rate of colony(%)

$$= (\text{colony numbers in control check} - \text{colony numbers in treatment groups}) / \text{colony numbers in control check} \times 100 \quad (1)$$

Effect of isopyrazam on the morphology of mycelium and conidiophores of *P. xanthii*. Five days after inoculation, 10-day-old cotyledons were randomly divided into two groups to be treated with distilled water and isopyrazam 0.5 mg liter⁻¹ diluents, respectively. Following incubation for another 48 h, cotyledon tissues were stained with Coomassie brilliant blue (Wolf and Fric 1981). The effect of isopyrazam on the morphology of mycelium and conidiophores of *P. xanthii* were observed under an optical microscope at 100× magnification.

Systemic translocation in cucumber cotyledons and leaves. Cucumber (cv. Xintai Mici) seeds were soaked in a water bath at 40°C for 6 h and transferred to an incubator at 25°C. When the hypocotyl length reached 3 to 4 mm, each seed was sown in a 20 cm pot, which contained autoclaved sandy loam and compost (3:1), and kept on glasshouse shelves at 25°C under a 16-h photoperiod, 3,800 lx intensity, and 80% relative humidity. A method of systemic translocation in cucumber cotyledons and leaves described previously (Ren et al. 2011) was used with minor modifications. Isopyrazam at 60 mg liter⁻¹ was sprayed on the right half of the adaxial surface of two cotyledons and the abaxial surface of two cotyledons to determine the transverse and translaminar movement in cotyledons, respectively. Cucumber seedlings at the three-leaf stage were sprayed with fungicide treatments applied either: (i) on the right half adaxial surface of two leaves to determine the transverse activity by evaluating disease severity of the adaxial surface of the left half of the leaves; (ii) on the abaxial surface of two leaves to assess the translaminar activity by evaluating disease severity of the adaxial surface of the leaves; (iii) on the lower leaf to monitor the acropetal translocation activity by evaluating disease severity of the top leaves on the plant; and (iv) on the top leaf to examine the basipetal translocation activity by evaluating disease severity of the lower leaves on the plant. Isopyrazam at the concentration of 60 mg liter⁻¹ was applied by spraying, along with the water control. Inoculations were performed by spraying the

conidial suspension (1×10^5 conidia ml⁻¹) on the adaxial leaf surface 24 h after treatment. Plants were arranged with row spacing at 30 cm. Fifteen plants were used per treatment and each experiment was repeated three times. Disease severity was determined after 10 days of incubation with the use of a 0 to 9 rating scale in which 0 = no visible symptoms, 1 = less than 5% foliage covered with powdery mildew, 3 = 6 to 10% foliage covered with powdery mildew, 5 = 11 to 20% foliage covered with powdery mildew, 7 = 21 to 40% foliage covered with powdery mildew, and 9 = more than 40% foliage covered with powdery mildew (IITP 2000). The disease index and the efficacy were calculated using the following equations:

Disease index (%)

$$= \left[\frac{\sum (\text{the number of diseased plant leaves in this rating scale} \times \text{the disease rating scale})}{\text{total plant leaves examined}} \times 9 \right] \times 100 \quad (2)$$

Efficacy (%)

$$= \left[\frac{(\text{the disease index of water control} - \text{the disease index of treatment})}{\text{the disease index of water control}} \right] \times 100 \quad (3)$$

Systemic translocation from roots to cotyledons. Seedlings that were 5 days old and at the cotyledon stage were drenched at the soil line with 25 ml solutions containing 0, 30, 60, 120, and 240 mg liter⁻¹ isopyrazam, respectively. Plants were arranged at a density of four plants per square meter. After treatment and inoculation, the treated plants were maintained at 25°C under a 16-h photoperiod, 3,800 lx intensity, and 80% relative humidity for 10 days, and evaluated using the same methods as Equations 2 and 3. Six seedlings were used per treatment. Each test was repeated three times and three replications were made for each treatment.

Duration of efficacy. Cucumber plants with three leaves were used in the pot experiment in 2014. The concentrations of test fungicides used were those recommended for use in the field in China. Ten milliliter solutions of isopyrazam at 60 mg liter⁻¹, hexaconazole at 30 mg liter⁻¹, difenoconazole at 100 mg liter⁻¹, pyraclostrobin at 150 mg liter⁻¹, kresoxim-methyl at 150 mg liter⁻¹, and azoxystrobin at 337.5 mg liter⁻¹ were sprayed onto each plant with sterile water as a control. Plants were separated into seven groups and arranged following a randomized complete block design. Each treatment was repeated three times (35 plants per treatment). Red string was used to distinguish treated from newly emerged leaves. Inoculation was conducted at 1, 7, 14, 21, 28, and 35 days after treating with fungicides. All leaves were inoculated by spraying a conidial suspension (1×10^5 conidia ml⁻¹), and were maintained at constant humidity of 80% for 24 h. The upper and lower leaves were examined for disease development after the plants were incubated for 7 to 10 days in a greenhouse of Shandong Agricultural University. Data collection, and calculation of disease index and efficacy were the same as for systemic translocation in cucumber cotyledons and leaves.

Greenhouse experiments. Greenhouse tests in May of 2014 and 2015 were carried out on cucumber cv. Xintai Mici and planted according to standard agronomic practices in a greenhouse situated in Tai'an City in Shandong Province. *P. xanthii* inoculum was abundant from widespread powdery mildew occurrence in surrounding

Table 1. The protective activity of six fungicides against *Podosphaera xanthii*

Treatment ^x	Slope ± SE ^y	EC ₅₀ for protective activity (mg liter ⁻¹)		EC ₉₀ for protective activity (mg liter ⁻¹)	
		Range	Mean ^z	Range	Mean
Isopyrazam	1.02 ± 0.05	0.03–0.05	0.04 f	0.54–0.89	0.70 f
Hexaconazole	1.11 ± 0.21	0.18–0.69	0.35 e	1.60–15.22	4.94 e
Difenoconazole	0.63 ± 0.07	0.38–1.28	0.69 d	32.37–154.84	70.80 d
Pyraclostrobin	0.78 ± 0.09	1.24–3.22	2.00 c	39.15–197.74	87.99 c
Kresoxim-methyl	0.99 ± 0.16	4.66–14.58	8.25 b	61.48–408.93	158.57 b
Azoxystrobin	0.97 ± 0.15	7.85–21.63	13.04 a	97.13–753.52	250.54 a

^x Each diluent was sprayed onto cucumber cotyledons until runoff 24 h before inoculations. A settling tower was used to insure uniformity of the application.

^y Slope represents the slope of the regression curve and SE means the standard error.

^z Mean values followed by the same letter within the same column were not significantly different by Fisher's LSD (least significant difference) tests at $P = 0.05$.

fields. The eight treatment applications were: isopyrazam (EC at 125 g liter⁻¹) at (i) 30 and (ii) 60 g a.i. ha⁻¹; (iii) hexaconazole (ME 5%) at 30 g a.i. ha⁻¹; (iv) difenoconazole (WG 10%) at 100 g a.i. ha⁻¹; (v) pyraclostrobin (EC at 250 g liter⁻¹) at 100 g a.i. ha⁻¹; (vi) kresoxim-methyl (WG 50%) at 150 g a.i. ha⁻¹; (vii) azoxystrobin (SC at 250 g liter⁻¹) at 337.5 g a.i. ha⁻¹; and (viii) a water treatment control. The eight treatment groups were laid out as a randomized complete block design with four replicates; each plot covered an area of 15 m² (5 m × 3 m), containing about 60 plants per block. The universal MATABI-16 knapsack hand atomizer was used at 0.3 MPa.

Fungicides were first applied on about 100-day-old cucumber plants, with two applications at 7 day intervals. The spray volume was 675 liters ha⁻¹. The disease index was recorded at initial treatment as well as 10 days and 20 days following the last treatment. The grading standard, the disease index and the calculation of the efficacy were recorded as follows.

Disease index (%)

$$= \left[\frac{\sum (\text{the number of diseased plant leaves in this rating scale} \times \text{the disease rating scale})}{\text{total plant leaves examined} \times 9} \right] \times 100 \quad (2)$$

Fig. 1. Curative activity of six fungicides against *Podosphaera xanthii* 10 days after treating with fungicides. **A**, control, **B**, azoxystrobin at 1 mg liter⁻¹, **C**, kresoxim-methyl at 1 mg liter⁻¹, **D**, pyraclostrobin at 1 mg liter⁻¹, **E**, difenoconazole at 1 mg liter⁻¹, **F**, hexaconazole at 1 mg liter⁻¹, and **G**, isopyrazam at 1 mg liter⁻¹.

Table 2. The curative activity of six fungicides against *Podosphaera xanthii*

Treatment ^x	Slope ± SE ^y	EC ₅₀ for the curative activity (mg liter ⁻¹)		EC ₉₀ for the curative activity (mg liter ⁻¹)	
		Range	Mean ^z	Range	Mean
Isopyrazam	1.24 ± 0.23	0.03–0.10	0.05 f	0.22–1.33	0.54 f
Hexaconazole	0.66 ± 0.080	0.29–0.89	0.50 e	15.28–125.04	43.71 e
Difenoconazole	0.78 ± 0.048	1.23–2.10	1.60 d	46.76–107.94	70.05 d
Pyraclostrobin	0.85 ± 0.11	1.32–3.95	2.28 c	29.75–183.74	73.94 c
Kresoxim-methyl	0.95 ± 0.14	6.30–16.33	10.14 b	92.28–561.55	227.64 b
Azoxystrobin	0.94 ± 0.12	10.73–24.97	16.37 a	150.96–969.79	382.63 a

^x Each diluent was sprayed onto cucumber cotyledons until runoff 96 h after inoculations. A settling tower was used to insure uniformity of the application.

^y Slope represents the slope of the regression curve and SE means the standard error.

^z Mean values followed by the same letter within the same column were not significantly different by Fisher's LSD (least significant difference) tests at *P* = 0.05.

Fig. 2. Effects of isopyrazam on conidiophore and mycelium morphology of *Podosphaera xanthii*. **A**, control and **B**, isopyrazam at 0.5 mg liter⁻¹.

$$\text{Efficacy}(\%) = [1 - (D_{C0} \times D_{T1}) / (D_{C1} \times D_{T0})] \times 100 \quad (4)$$

Where D_{T0} = the disease index of treatment before application, D_{T1} = the disease index of treatment after application, D_{C0} = the disease index of water control before application, and D_{C1} = the disease index of water control after application.

Statistical analysis. The EC_{50} values were calculated by regression of the percent relative growth against the $\log_{10}$ fungicide concentrations. In order to determine whether there were statistically significant differences among the test data, analyses of variance (ANOVA) were carried out followed by the LSD test (SPSS v. 13.0 for Windows) ($P = 0.05$).

Results

Protective activity of isopyrazam against *P. xanthii*. Isopyrazam exhibited the best protective activity among the tested fungicides, with an EC_{50} of 0.04 mg liter⁻¹. Compared with the EC_{50} for hexaconazole, difenoconazole, pyraclostrobin, kresoxim-methyl, and azoxystrobin, the EC_{50} of isopyrazam was the lowest concentration in the protective activity test for the control of powdery mildew. Moreover, the effectiveness of the strobilurin fungicides was relatively low. These included pyraclostrobin, kresoxim-methyl, and azoxystrobin, for which the EC_{50} values were 2.00, 8.25, and 13.04 mg liter⁻¹, respectively (Table 1).

Curative activity of isopyrazam against *P. xanthii*. Similar to the protective activity, the curative activity of isopyrazam was also apparent, with an EC_{50} value of 0.05 mg liter⁻¹. This was significantly lower than hexaconazole, difenoconazole, pyraclostrobin,

kresoxim-methyl, and azoxystrobin (Fig. 1). Additionally, the curative activity of pyraclostrobin, kresoxim-methyl, and azoxystrobin was inferior to their protective activity, with curative values of 2.28, 10.14, and 16.37 mg liter⁻¹, respectively (Table 2).

The effect of isopyrazam on the morphology of mycelium and conidiophores. Mildew colonies on cotyledons were observed following treatment. Dense and plump conidial chains were microscopically evident for the control check at 100× magnification (Fig. 2). With the isopyrazam treatment at 0.5 mg liter⁻¹, both the mycelium and conidiophores were clearly damaged. Conidiophores were shrunk or collapsed, and this may have hindered the formation of conidia.

Systemic translocation of isopyrazam in cucumber cotyledons and leaves. For cucumber seedlings at the cotyledon stage, the efficacy of isopyrazam at 60 mg liter⁻¹ was 94.40 and 88.96% for the translaminar and transverse translocations, respectively (Fig. 3). The isopyrazam treatment at 60 mg liter⁻¹ also had high efficacy for translaminar and transverse translocations at the three-leaf stage. However, basipetal movement was not evident. The efficacy of the acropetal movement and the basipetal movement were 16.54 and 1.50%, respectively. Thus, isopyrazam exhibited favorable properties of translaminar and transverse translocation, with the former being more evident, as shown in Figure 4. The transverse translocation distance of isopyrazam appears limited (Fig. 4).

Systemic translocation of isopyrazam from roots to leaves. The efficacies of root-drenching with isopyrazam at the levels of 30, 60, 120, and 240 mg liter⁻¹ were 32.94, 45.04, 60.77, and 76.22%, respectively. The efficacy of isopyrazam when irrigated to the roots was significantly enhanced as concentration increased (Fig. 5). This demonstrates that isopyrazam can be absorbed by the cucumber root and control powdery mildew of the leaves.

Duration of isopyrazam activity. The efficacy of isopyrazam at 60 mg liter⁻¹ 28 days after treatment was 97% control, and the efficacy after 35 days was 95%. For hexaconazole at 30 mg liter⁻¹, there was a significant decrease in efficacy 14 days after treatment. Efficacy decreased significantly 7 days after application for pyraclostrobin at 150 mg liter⁻¹, kresoxim-methyl at 150 mg liter⁻¹, and azoxystrobin at 337.5 mg liter⁻¹, which offered no control 21 days after application (Fig. 6). Thus, the duration of efficacy of isopyrazam is 2 weeks longer than that of hexaconazole at 30 mg liter⁻¹ and 3 weeks longer than difenoconazole at 100 mg liter⁻¹, pyraclostrobin at 150 mg liter⁻¹, kresoxim-methyl at 150 mg liter⁻¹, and azoxystrobin at 337.5 mg liter⁻¹. However, all treatments failed to control powdery mildew on leaves that emerged after treatment, which indicated that all of the tested fungicides had weak translocation ability from one leaf to another.

Greenhouse experiments. The disease index estimated at 10 days after the last fungicide application indicated that the efficacy of isopyrazam at 30 and 60 g a.i. ha⁻¹ was 87.96 and 93.83% in 2014 and 86.63 and 92.10% in 2015, respectively. The efficacy of isopyrazam was

Fig. 3. Systemic translocation of isopyrazam at 60 mg liter⁻¹ in cucumber plant. A, translaminar movement on cotyledon, B, transverse movement on cotyledon, C, translaminar movement on leaf, D, transverse movement on leaf, E, acropetal uptake transmission on leaf, and F, basipetal uptake transmission on leaf.

Fig. 4. Systemic translocation of isopyrazam at 60 mg liter⁻¹ in cucumber leaves. A, control, B, translaminar movement, and C, transverse movement.

significantly higher than that of hexaconazole at 30 g a.i. ha⁻¹, difenoconazole at 100 g a.i. ha⁻¹, pyraclostrobin at 150 g a.i. ha⁻¹, kresoxim-methyl at 150 g a.i. ha⁻¹, and azoxystrobin at 337.5 g a.i. ha⁻¹ in both trials. At 20 days after the last fungicide treatment, the efficacy

Fig. 5. Efficacy of isopyrazam root-drench for controlling cucumber powdery mildew.

of isopyrazam at 30 and 60 g a.i.ha⁻¹ was 83.44 and 90.83% in 2014 and 83.27 and 89.09% in 2015, respectively; significantly higher efficacy than all the other fungicides tested in both trials. The efficacy of isopyrazam at 30 and 60 g a.i. ha⁻¹ was not significantly different when compared 10 and 20 days after treatment. However, for the other fungicide treatments, the efficacy 20 days after treatment was lower than 10 days after treatment (Table 3). Thus, the efficacy of isopyrazam for cucumber powdery mildew was significantly higher than that of hexaconazole, difenoconazole, pyraclostrobin, kresoxim-methyl, and azoxystrobin. In addition, the effective activity of isopyrazam appears to be of longer duration.

Discussion

Currently, the most common and effective fungicides used for managing cucumber powdery mildew in China are triazoles (such as hexaconazole and difenoconazole) and strobilurins (pyraclostrobin, kresoxim-methyl, and azoxystrobin). In particular, hexaconazole appears most efficacious (Jia et al. 2006). Triazole and strobilurin fungicides both exhibit protective and curative activity (Holb and Schnabel 2007). However, our results indicated that protective and curative activities of isopyrazam were significantly higher than those of hexaconazole, difenoconazole, pyraclostrobin, kresoxim-methyl, and azoxystrobin. In addition, our results showed that isopyrazam at concentration of 0.5 mg liter⁻¹ damages conidiophores, which influences conidial formation. Our results indicate that isopyrazam is

Fig. 6. Efficacy of isopyrazam at 60 mg liter⁻¹ for controlling cucumber powdery mildew over 35 days.

Table 3. Efficacy of six fungicides for controlling cucumber powdery mildew under natural infection conditions in a greenhouse (cv. Xintai Mici)^w

		2014					2015				
		IDI	10 days ^y		20 days		IDI	10 days		20 days	
Fungicides	Dose ^x (a. i. g ha ⁻¹)		DI	CK (%) ^z	DI	CK (%)		DI	CK (%)	DI	CK (%)
125 g liter ⁻¹ isopyrazam EC	30	4.38	1.64	87.96 abc	2.85	83.44 c	4.59	1.93	86.63 b	3.11	83.27 b
	60	4.55	1.07	93.83 a	1.74	90.83 ab	4.30	1.04	92.10 a	1.89	89.09 a
5% hexaconazole ME	30	3.69	2.08	85.05 bc	3.75	75.91 de	3.89	2.15	83.77 bc	4.26	76.07 c
10% difenoconazole WG	100	3.67	2.08	84.64 bc	4.45	71.55 de	3.52	2.33	82.93 c	4.78	73.80 c
250 g liter ⁻¹ pyraclostrobin EC	150	4.19	2.22	85.36 bc	5.24	70.41 ef	4.15	2.44	82.54 c	4.33	76.24 c
50% kresoxim-methyl WG	150	4.22	3.78	76.51 de	6.65	62.82 g	4.41	2.93	76.08 d	5.44	66.25 d
250 g liter ⁻¹ azoxystrobin SC	337.5	4.15	3.48	77.19 d	6.14	64.88 fg	4.19	2.59	77.03 d	5.22	63.14 d
Control	—	4.22	15.92	—	17.92	—	4.52	14.48	—	19.04	—

^w The experiments were performed in Tai'an city in Shandong province in 2014 and 2015. IDI = initial disease index, DI = disease index, CK = control efficacy.

^x Dose means the effective concentration.

^y Efficacy 10 and 20 days after the last fungicide application. Fungicides were first applied on approximately 100-day-old cucumber plants with two applications at 7 day intervals.

^z Values followed by the same letter within the same column were not significantly different according to Fisher's LSD test at $P = 0.05$.

effective as a protectant and a curative, and limits sporulation of *P. xanthii*.

Although there are many reports about strong systemicity of SDHIs, such as carboxin, boscalid, thifluzamide, and flutolanil (Berggren and Pinckard 1973; Ferreira et al. 2006; Hirooka et al. 1989; Klittich et al. 2008; Snel and Edgington 1970), our study is the first to report the systemic translocation of isopyrazam in cucumber. The results demonstrate that isopyrazam has strong transverse and translaminar translocation, which is superior to the translocation from roots to leaves. Studies by McGrath and Staniszevska (1996) found that systemic fungicides can be transported to abaxial leaf surfaces, so they have an excellent protective, curative effect and high efficacy, which is consistent with the activity of isopyrazam. However, the translocation rate and distance of isopyrazam in cucumber plants still requires further studies using HPLC/UVD (Kim et al. 2013).

The long-lasting efficacy of isopyrazam was reported for controlling gray mold (Song et al. 2016) and *Zymoseptoria tritici* (Dubos et al. 2013), which was mainly due to the unique 'benzonorbornene ring' structure, which confers isopyrazam a strong attraction and accumulation in the wax layer of the plant leaf as well as a strong affinity to succinate dehydrogenase of the fungus (Harp et al. 2011). Based on the recommended concentrations of fungicides that growers use on cucurbits in China, our results indicate that isopyrazam has a longer duration of efficacy than hexaconazole, difenoconazole, pyraclostrobin, kresoxim-methyl, and azoxystrobin for controlling cucumber powdery mildew. Isopyrazam spray intervals can be extended to 2 to 3 weeks for controlling cucumber powdery mildew; this may delay the development of resistance in *P. xanthii*.

Although triazole and strobilurin fungicides were commonly used against cucumber powdery mildew, triazole fungicides often had negative effects on the growth of cucumber, so the application rate of triazoles must be less than the recommended dose (Burden et al. 1987). Strobilurin fungicides are no longer recommended to control cucurbit powdery mildew in the U.S.A. because resistant strains have been detected in many cases, and there is cross resistance among other strobilurin fungicides (Lebeda et al. 2010). In our study, isopyrazam was more efficient and longer-lasting than triazoles and strobilurins on cucumber plants in pot and greenhouse experiments. Isopyrazam is considered to have a medium to high risk of resistance development by the Fungicide Resistance Action Committee (McKay et al. 2011). There is no evidence of cross-resistance for isopyrazam with triazoles and strobilurins (Sattler et al. 2010).

Ajigboye et al. (2014) reported that the use of isopyrazam had a positive influence on the yield of wheat. More studies are required to determine if isopyrazam is capable of improving the yield of cucumber compared with other fungicides. In addition, the relationship between the degradation rate of isopyrazam and its efficacy duration against powdery mildew should also be determined.

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