Supporting Information

Phloem Delivery of Fludioxonil by Plant Amino Acid Transporter-Mediated Polysuccinimide Nanocarriers for Controlling Fusarium Wilt in Banana

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1. Thermogravimetric analysis (TGA) of FLU@PGA nanoparticles

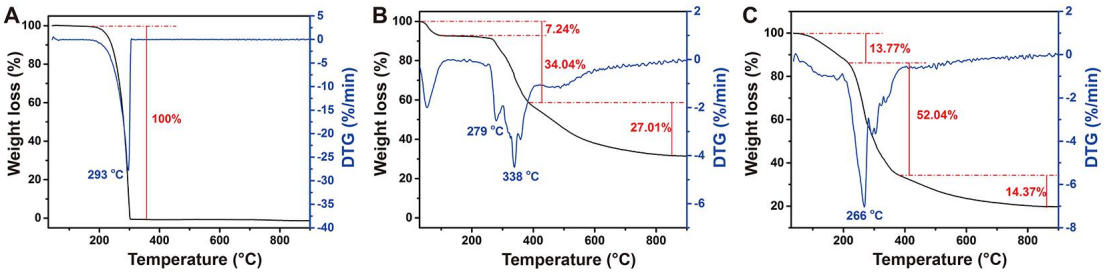


Figure S1. TGA profiles and derivative thermogravimetry (DTG) analysis of (A) FLU, (B) PGA nanoparticles, and (C) FLU@PGA nanoparticles.

For the thermal degradation curve of FLU, complete weight loss occurred at approximately 300 °C, and the temperature at which the initial weight loss occurred was 173 °C (Fig. S1A). As shown in Fig. S1B,C, FLU@PGA showed three distinct stages of weight loss, which was in line with the PGA. The first stage weight loss temperature of FLU@PGA occurred from 59 to 215 °C, whereas that of PGA was observed from 38 to 100 °C. Compared with the observed profiles for PGA, the weight loss of FLU@PGA was higher for the first and second stages, which could have been caused by the decomposition of FLU. The maximum weight loss temperature of FLU and PGA were 293 °C and 338 °C observed by DTG, respectively. However, the maximum weight loss temperature of FLU@PGA was reduced to 266 °C due to the main chain decomposition of PGA with a peak at 279 °C. In addition, the residue of FLU@PGA was decreased to 19.82%, while the residue of PGA was 31.71%.

2. Investigation of release mechanism of FLU@PGA nanoparticles

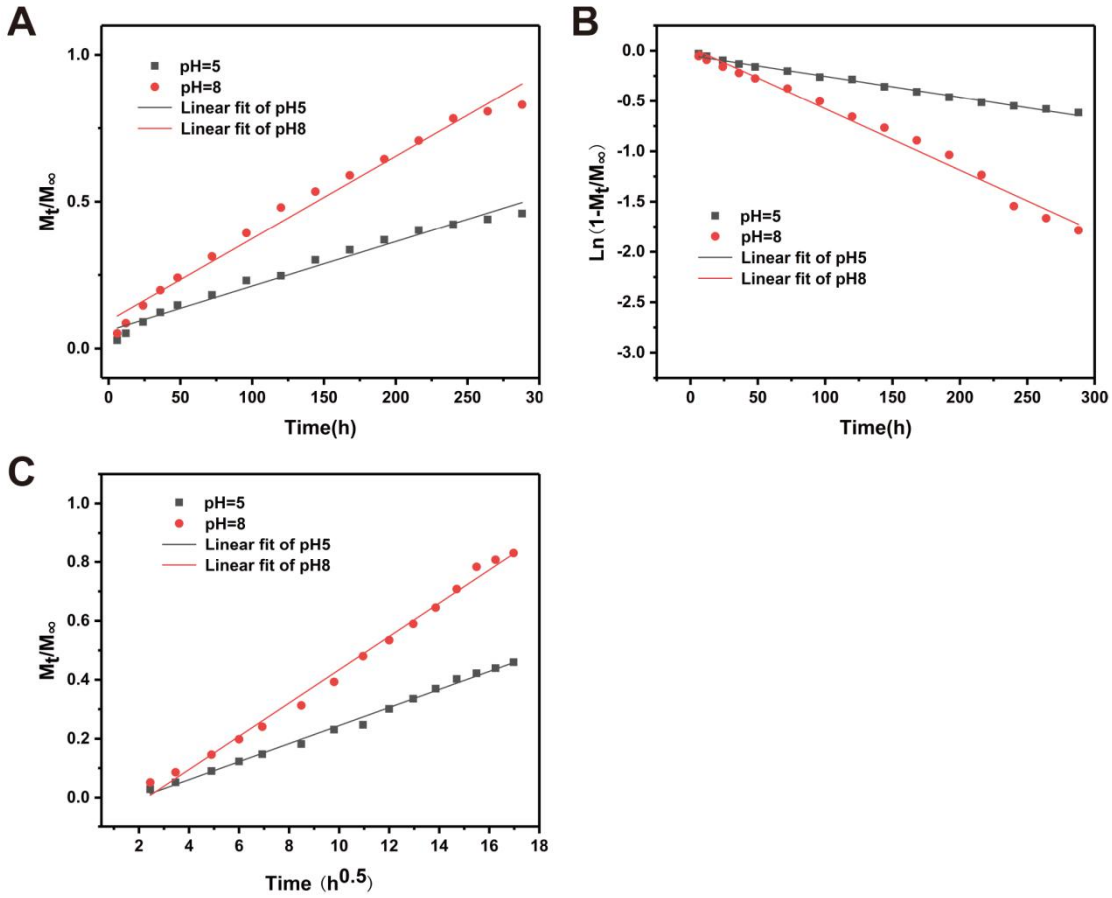


Figure S2. *In vitro* release study of FLU from FLU@PGA nanoparticles. The data obtained were curve-fitted using three different kinetic models: (A) Zero-order model, (B) First-order model, and (C) Higuchi model. Mt/M_{∞} defined as the accumulative release (%) of FLU from FLU@PGA nanoparticles.

Table S1. The fit parameters of mathematical models for the cumulative release rate of FLU@PGA at two different pH values.

Model	pH 5.0		pH 8.0	
	Fitting Equation	R^2	Fitting Equation	R^2
Zero-order	$M_t/M_\infty=0.0015t+0.0607$	0.9756	$M_t/M_\infty=0.0028t+0.0937$	0.9817
First-order	$Ln(1-M_t/M_\infty)=-0.0021t-0.0466$	0.9921	$Ln(1-M_t/M_\infty)=-0.0061t-0.0343$	0.9850
Higuchi	$M_t/M_\infty=0.0307t^{0.5}-0.0617$	0.9940	$M_t/M_\infty=0.0565t^{0.5}-0.1307$	0.9922

Mt/M_∞ defined as the accumulative release (%) of FLU from the FLU@PGA nanoparticles

3. Investigation of photostability of FLU@PGA nanoparticles

To evaluate the light stability of FLU@PGA, samples containing 4 mg of FLU@PGA or an equivalent dose of FLU (TC) were dispersed in 20 mL of ethanol solution, which was stirred at 120 r/min. The solutions were placed in a black box coupled with an 8 W ultraviolet lamp (excitation wavelength 254 nm), and the distance between the sample and the lamp was 20 cm. At given time intervals, a 0.5 mL aliquot was sampled, and the concentration of FLU was analyzed by the mentioned HPLC method in experimental section. The photostability tests were repeated three times.

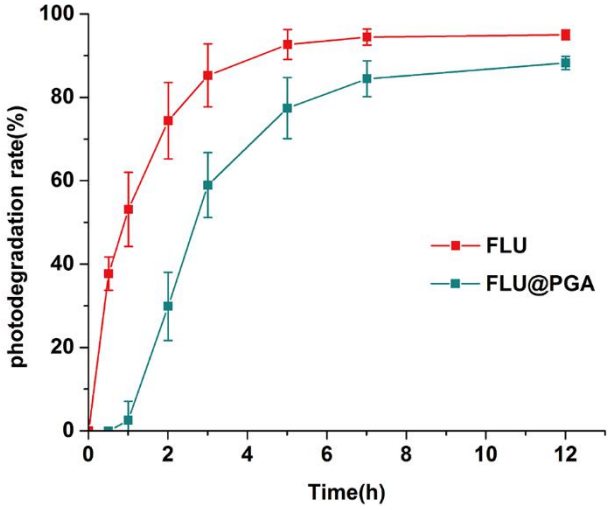


Figure S3. Photostability analysis of the FLU@PGA nanoparticles. The response curve of the photolysis rate of FLU (TC) and FLU@PGA under an ultraviolet lamp.

Photostability is a key property for the practical application of phenylpyrrole fungicides especially for their foliar application, because the leading compound pyrrolnitrin is very unstable in light.[1] Under our experimental conditions, it was not surprising that FLU (TC) was highly prone to degradation after exposure to UV light. As shown in Fig. S3, the photodegradation rate of the pure active ingredient was 53.2% after 1 h of continuous UV irradiation, while only 2.6% of FLU loaded in the PGA nanocarriers was degraded after the same time. The photodegradation of FLU@PGA was slower than that of FLU (TC) with reaching 50% at approximately 2.5 h (Fig. S3), which demonstrated that loading FLU into the PGA nanocarriers could effectively prevent its photodegradation. An improved photostability can prolong the retention and uptake time of FLU on the leaf surface, further improving its utilization efficiency.

4. *In vitro* antifungal activities of FLU@PGA against Foc TR4

Fungal strains were grown on potato dextrose agar (PDA) medium at 28 °C in the dark. A filter-paper method was used to investigate the effect of PGA nanocarriers on Foc TR4 growth. PGA (200 mg/L), FLU@PGA (200 mg/L, loading rate 27.9%) and FLU (55.8 mg/L, TC) were dissolved in DMSO (0.5% final concentration) and then suspended in sterile water containing 0.1% Tween 80. Four sterile filter paper discs were placed on a PDA plate at equidistance to the center, after which 10 μL of a suspension of nanoparticles or sterile water with 0.5% DMSO and 0.1% Tween 80 (control) was added to the filter paper discs. Each plate was inoculated centrally with a 5-mm diameter mycelial agar plug from the margin of an actively growing colony. The plates were incubated for 5 days at 28 °C in the dark. The experiment was performed in triplicate and repeated two times.

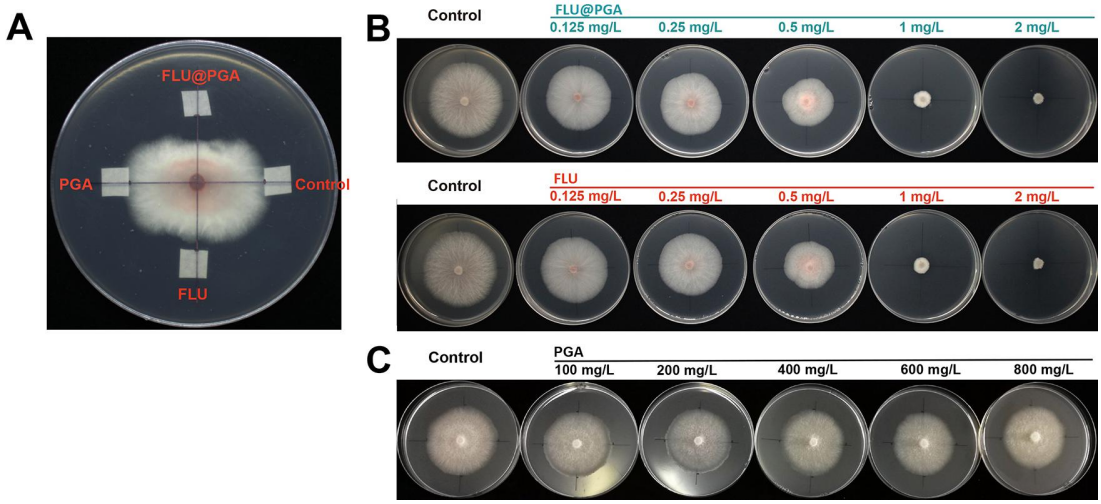


Figure S4. *In vitro* antifungal activities of the PGA and FLU@PGA (loading rate of 27.9%) nanoparticles against *Fusarium oxysporum* f. sp. *cubense* tropical race 4 on potato dextrose agar (PDA) media (5 days). (A) Four sterile filter paper discs were placed equidistantly from the center point, and then 10 μL of a solution containing

200 mg/L of PGA or FLU@PGA was added to the filter paper. Paper discs with an equivalent dose of FLU (55.8 mg/L, TC) and sterile water were used as chemical and untreated controls. (B) The same concentration of the FLU@PGA nanoparticles and FLU (50% WP) were added to PDA (27.9% vs 50 % of the FLU content, respectively). (C) the PGA nanoparticles were added to PDA at 5 concentration levels (100, 200, 400, 600, and 800 mg/L).

5. LC-MS/MS method validation

Banana plant samples (5 g) were homogenized in liquid nitrogen, after which 2 mL of water and 15 mL of ACN were added to each sample. Subsequently, the mixtures were placed inside an ultrasonic bath for 10 min and vigorously shaken on a vortex mixer for 1 min. Then, 4 g of MgSO4 and 1 g of NaCl were added, and the mixtures were immediately shaken for an additional 1 min. After centrifugation at 4000 rpm for 5 min, 1 mL of the supernatant was transferred to a centrifuge tube containing 25 mg of primary secondary amine (PSA), 25 mg of MgSO4 and 5 mg of graphitized carbon black (GCB). The tube was immediately shaken on a vortex mixer for 1 min, after which the supernatant was filtered through a 0.22 μm pore-sized syringe filter and then injected into the LC-MS/MS system.

Chromatographic separation was achieved using an ACQUITY UPLC BEH C18 column (1.7 μm particle size, 2.1 × 100 mm, Waters). The mobile phase consisted of MilliQ water (A) and acetonitrile (B) at a flow rate of 0.2 mL/min, using the following gradient in a total run time of 13 min: 0.0 to 3.0 min, holding at 80% A and 20% B; 3 to 8 min, conversion to 10% A and 90% B; 8 to 10 min, holding at 10% A and 90% B before returning to 80% A and 20% B at 10.5 min; and equilibration for an additional 2.5 min. The injection volume used was 5 μL. The optimized MS parameters were as follows: the capillary voltage was set at 2.0 kV, the source temperature at 600 °C and the desolvation gas flow was 500 L/h.

The method was validated following a validation procedure that included the

following parameters: linearity, limits of quantification (LOQs), accuracy and precision.

Table S2. Mass spectrometer (MS/MS) parameters for the MRM of fludioxonil.

Compound	Retention time (min)	Electrospray ionization	Cone (V)	MRM transition: parent ion > fragment ion (*m*/*z*)	Collision energy (V)
Fludioxonil	5.7	negetive	25	247.2 > 126[Q]	30
				247.2 > 180	25

[Q] means MRM transition used for quantification of the compounds.

Table S3. Fludioxonil linear calibration curves.

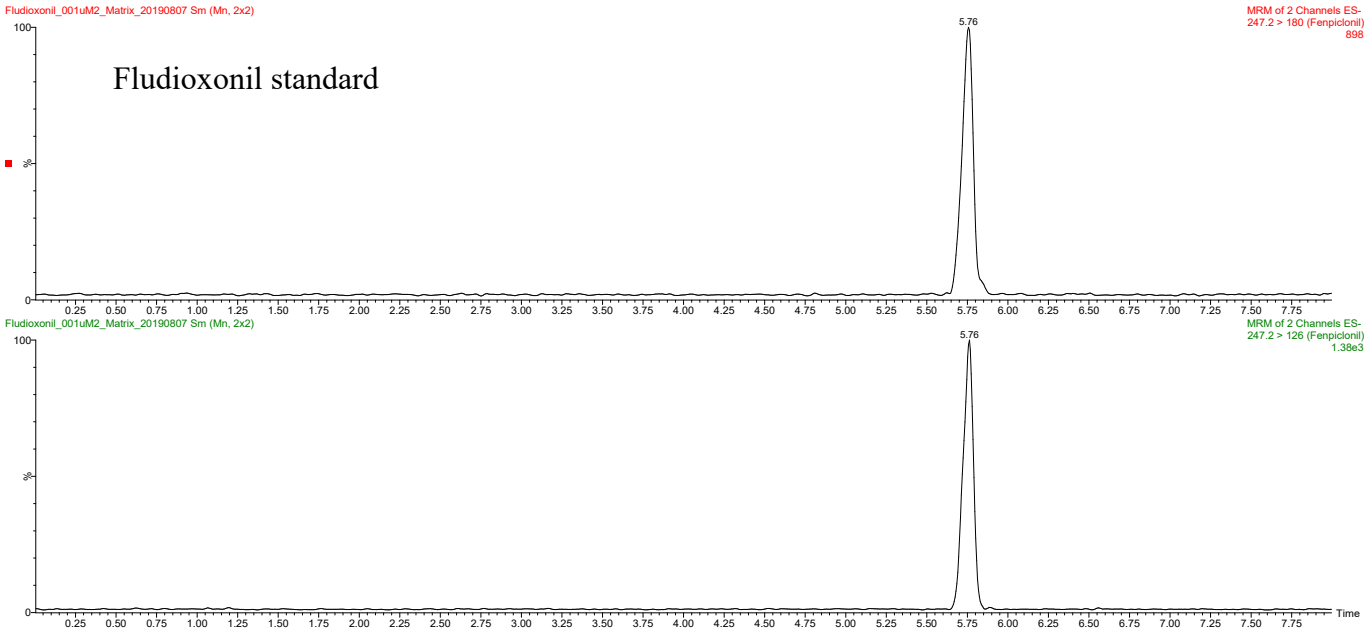
Compound	Sample	Regression Equation	R^2	LOQ (mg/kg)[a]
Fludioxonil	oocytes	y = 46133x + 1770.1	0.9942	0.001
	leaves	y = 27812x + 131.06	0.9914	0.001
	roots	y = 24165x + 110.13	0.9948	0.001

[a] LOQs were determined as the concentration that produced a signal to noise ratio (S/N) of 10 for the quantifier ion.

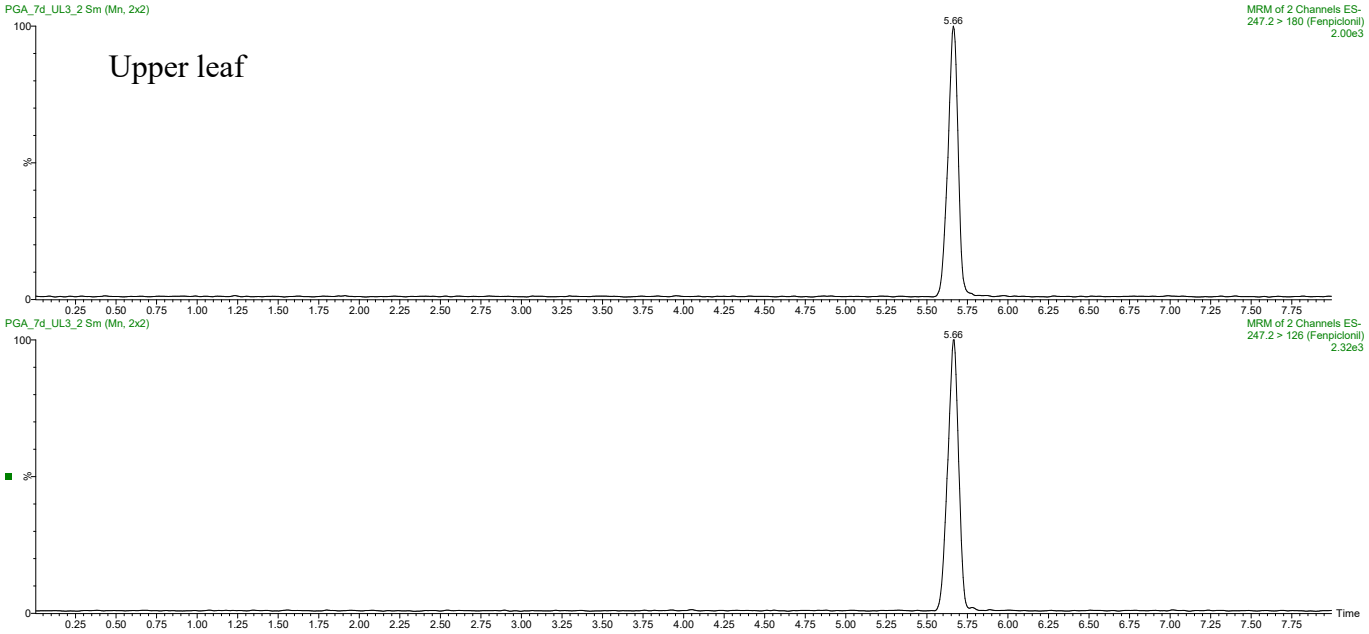
Table S4. Fludioxonil recoveries from spiked blank samples (n = 3).

Sample	spike level (mg/kg)	Average Recoveries (%)	RSD (%)
leaves	0.001	95	17
	0.01	89	9
	0.1	93	5
roots	0.001	96	13
	0.01	111	13
	0.1	98	9

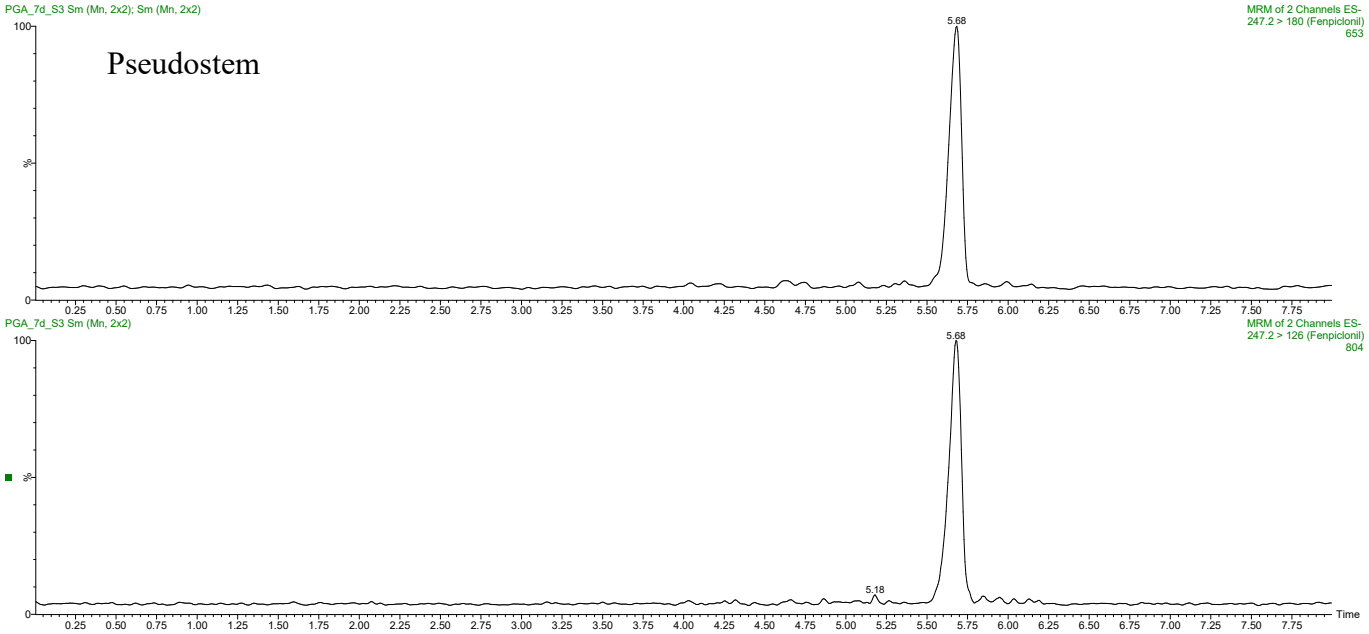
A


Fludioxonil_001uM2_Matrix_20190807 Sm (Mn, 2x2)
MRM of 2 Channels ES-
247.2 > 180 (Fenpiclonil)
898
Fludioxonil standard
5.76
Fludioxonil_001uM2_Matrix_20190807 Sm (Mn, 2x2)
MRM of 2 Channels ES-
247.2 > 126 (Fenpiclonil)
1.38e3
5.76
Time

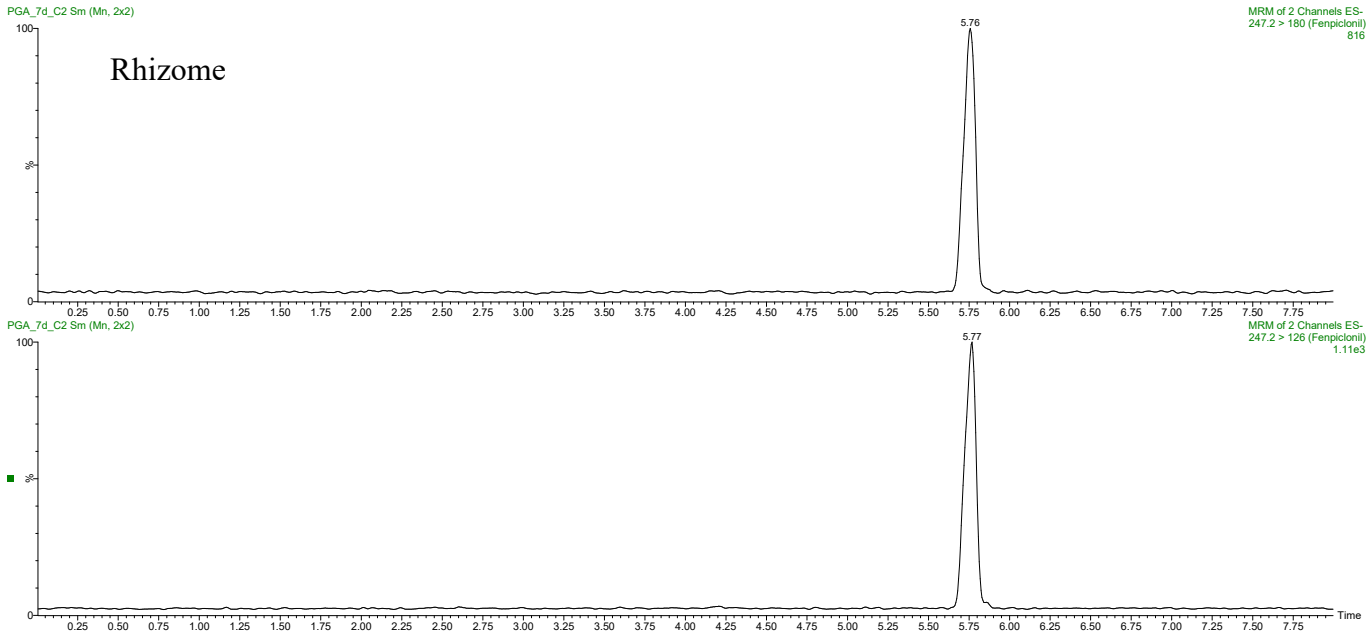
B


PGA_7d_UL3_2 Sm (Mn, 2x2)
MRM of 2 Channels ES-
247.2 > 180 (Fenpiclonil)
2.00e3
Upper leaf
5.66
PGA_7d_UL3_2 Sm (Mn, 2x2)
MRM of 2 Channels ES-
247.2 > 126 (Fenpiclonil)
2.32e3
5.66
Time

C


PGA_7d_S3 Sm (Mn, 2x2); Sm (Mn, 2x2)
MRM of 2 Channels ES-
247.2 > 180 (Fenpiclonil)
653
Pseudostem
5.68
PGA_7d_S3 Sm (Mn, 2x2)
MRM of 2 Channels ES-
247.2 > 126 (Fenpiclonil)
804
5.68
5.18
Time

D



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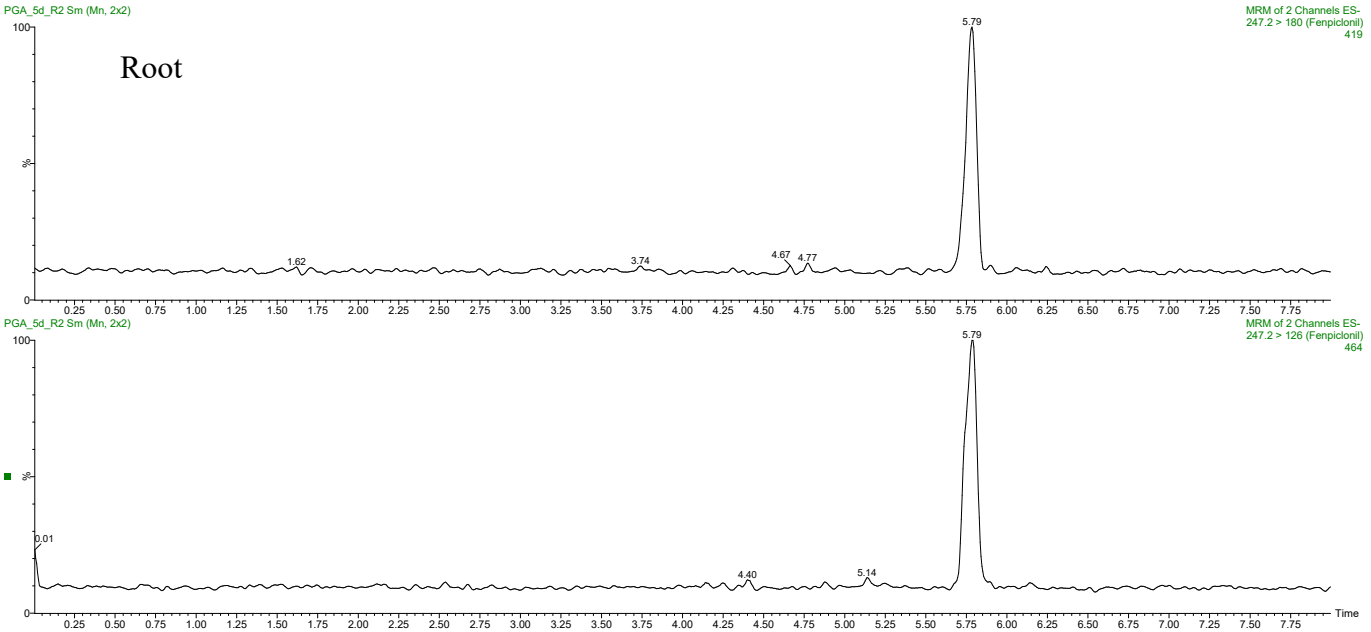

Figure S5. LC-MS/MS chromatograms (MRM) of fludioxonil in different parts of banana plants after the treatment with 200 mg/L FLU@PGA.

6. Characterization of FITC-cad labeled PGA nanoparticles (PGA-FITC)

PSI (50 mg) was dissolved in 2 mL of DMSO. Then, 77 mg of HGlyOMe, 5 mg of FITC-cad and 100 μL of DBA was dissolved in 2 mL of DMSO under vigorous stirring. The mixture was then added to the PSI solution, which was stirred vigorously for 12 h. To generate the PGA-FITC nanoparticles, the resulting solution was added to 60 mL of citric acid buffer (pH 2.5). Finally, the suspension was centrifuged at 10 000 r/min, and the precipitate was washed three times with deionized water and then freeze-dried overnight.

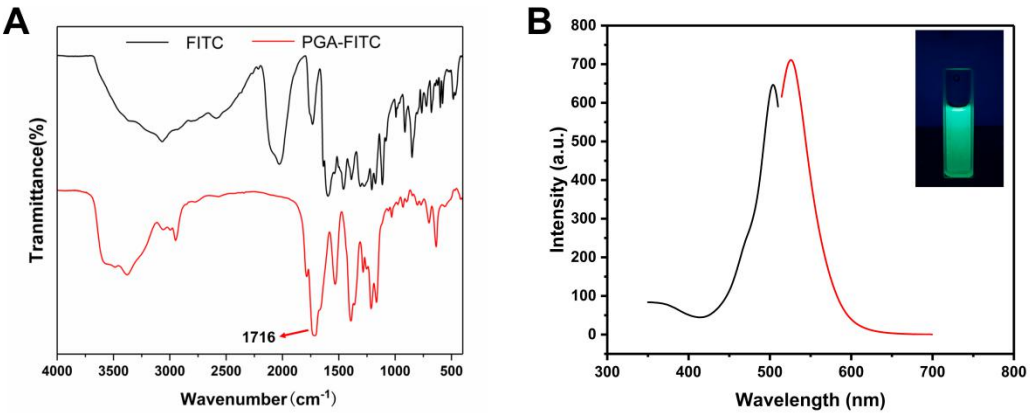

Figure S6. (A) FTIR spectra of PGA-FITC nanoparticles. Compared with the spectrum of FITC-cad, the peak at 1727 cm^{-1} of PGA-FITC was redshifted to 1716 cm^{-1}, and the amino peak at 1673 cm^{-1} was weakened, indicating that the PGA-FITC were successfully prepared. (B) Excitation (black) and emission (red) fluorescence spectra of PGA-FITC.

References:

(1) Nyfeler, R.; Ackermann, P. Phenylpyrroles, a New Class of Agricultural Fungicides Related to the Natural Antibiotic Pyrrolnitrin. In *Synthesis and Chemistry of Agrochemicals III*; Baker, D. R., Fenyes, J. G., Steffens, J. J., Eds.; ACS Symposium Series; American Chemical Society, 1992; Vol. 504, pp 36–395.