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Fujita, Kentaro
Haga, Yuki
Yoshihara, Ryouhei
Matsumura, Chisato
Inui, Hideyuki

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Kentaro Fujita[1], Yuki Haga[2], Ryouhei Yoshihara[3, a], Chisato Matsumura[2], Hideyuki Inui[1,3,*]

[1]Graduate School of Agricultural Science, Kobe University, 1-1 Rokkodaicho, Nada-ku, Kobe, Hyogo 657-8501, Japan

[2]Hyogo Prefectural Institute of Environmental Sciences, 3-1-18 Yukihiracho, Suma-ku, Kobe, Hyogo 654-0037, Japan

[3]Biosignal Research Center, Kobe University, 1-1 Rokkodaicho, Nada-ku, Kobe, Hyogo 657-8501, Japan

[a]Present address: Faculty of Science, Saitama University, 255 Shimo-okubo, Sakura-ku, Saitama 338-8570, Japan.

*Corresponding Author Hideyuki Inui, Biosignal Research Center, Kobe University, 1-1 Rokkodaicho, Nada-ku, Kobe, Hyogo, 657-8501, Japan

E-mail: hinui@kobe-u.ac.jp, Telephone number: +81-78-803-5863

Abstract

Hydrophobic pollutants have become widely distributed across the world. From an agricultural perspective, their accumulation in crops from contaminated soil threatens food security and quality, leading to many diseases in humans. The Cucurbitaceae family can accumulate high concentrations of hydrophobic pollutants in their aerial parts. The Cucurbitaceae family contains major latex-like proteins (MLPs) as transporting factors for hydrophobic pollutants. *MLP* genes are expressed in the roots in which the MLPs bind hydrophobic pollutants. MLPs transport these hydrophobic pollutants to the aerial parts of the plant through the xylem vessels. As a result, hydrophobic pollutant contamination occurs in the Cucurbitaceae family. In this study, we suppressed the expression of *MLP* genes in the roots and reduced the amounts of MLPs with pesticide treatments. First, the fungicides Benlate and Daconil that deceased the hydrophobic pollutant, perylene, concentration in the xylem sap of zucchini plants were selected. Daconil suppressed the transcription activity of *MLP* in the roots. In the Daconil treatment, the amount of MLPs in the roots and xylem sap of zucchini plants was decreased, and the concentrations of the hydrophobic pollutants, pyrene and dieldrin, were significantly decreased. Our research contributes to the production of safer crops.

Key words

Cucurbita pepo; hydrophobic pollutant; major latex-like protein; pesticide; gene suppression

1. Introduction

Some industrial materials, pesticides, and unintentional products have been recognized as hydrophobic pollutants, such as persistent organic pollutants (POPs) and polycyclic aromatic hydrocarbons. Since their toxicity was revealed, their use and production have been prohibited. However, hydrophobic pollutant contamination is still detected worldwide (Bartrons et al., 2016). Because of their high bioaccumulation ability, living organisms at the top of the food chain, such as human beings, are likely to accumulate high concentrations of hydrophobic pollutants. The intake of contaminated crops is a major route in the accumulation of hydrophobic pollutants. Agricultural soil can be contaminated by hydrophobic pollutants through biochar treatment (Fabbri et al., 2013), the incomplete combustion of straw (Jenkins et al., 1996), and POP-insecticide treatment, such as dieldrin, dichloro-diphenyl-trichloroethane, and β-hexachlorocyclohexanes (Namiki et al., 2013). The intake of hydrophobic pollutants by human beings occurs through the uptake of these pollutants by crops. The accumulation of hydrophobic pollutants in the human body leads to many diseases, such as respiratory syndromes (Bortey-Sam et al., 2017), digestive tract cancer (Abdur Rehman et al., 2017), and Alzheimer's (Yan et al., 2016). Therefore, it is critically important to inhibit the uptake of hydrophobic pollutants into crops at the first step of the food chain.

The Cucurbitaceae family, which includes cucumbers (*Cucumis sativus*), squashes (*Cucurbita maxima*), and zucchinis (*Cucurbita pepo*), can accumulate high concentrations of hydrophobic pollutants in their fruits (Parrish et al., 2006; Otani et al., 2007). It has been reported that the Cucurbitaceae family accumulates high concentrations of drins (dieldrin and endrin) (Otani et al., 2007), polychlorinated dibenzo-*p*-dioxins, polychlorinated dibenzofurans (Hülster et al., 1994; Inui et al., 2008), polychlorinated biphenyls (Inui et al., 2008), dichlorodiphenyldichloroethylene (Lunney et al., 2004), chlordane (Mattina et al., 2004), and pyrene (Parrish et al., 2006). Various approaches for the reduction of contamination through soil remediation were attempted to inhibit the accumulation of

hydrophobic pollutants in crops. For example, POPs are adsorbed by active carbon (Murano et al., 2009), and POP-degrading microorganisms were screened (Yamazaki et al., 2014). However, their POP removal efficiency was low. Therefore, we developed a new approach, using plant functions to produce low-contamination crops in contaminated soil.

It has been reported that major latex-like proteins (MLPs) play a crucial role in the accumulation of POPs in the Cucurbitaceae family (Inui et al., 2013). MLPs belong to Bet v 1 family (Fernandes et al., 2013) and are thought to function as resistance against pathogens (Yang et al., 2015) and as transporters of hydrophobic compounds using an internal hydrophobic cavity. *MLP* genes were highly expressed in the roots of the Cucurbitaceae family, and MLPs were detected in the roots and xylem sap (Goto et al., 2019). Furthermore, MLPs bind POPs in the root cells using an internal hydrophobic cavity (Goto et al., 2019). The resultant MLP-POP complexes are translocated into the xylem vessels, and the POPs are transported to the aerial parts of the plant (Goto et al., 2019). As a result, contamination by hydrophobic pollutants occurs. Therefore, the expression of *MLP* genes in the roots is a crucial step of contamination in the Cucurbitaceae family.

In this study, we focused on the expression of *MLP* genes in the roots. We attempt to develop a strategy for the cultivation of low-contamination crops, even in soil contaminated by hydrophobic pollutants. Previously, the amount of MLPs in zucchini plants was decreased by environmental factors, such as cultivation temperature and day length, leading to a decrease in pyrene concentration (Inui et al., 2020). Therefore, it is possible to reduce hydrophobic pollutant contamination in the Cucurbitaceae family by reducing the amount of MLPs. We attempted to suppress the expression of *MLP* genes in the roots using pesticide treatments in zucchini plants with the highest ability to accumulate the hydrophobic pollutants in the Cucurbitaceae family (Otani et al., 2007). Pesticides are essential products in agriculture and subject to safety tests. To accomplish our purpose, we selected the fungicide Daconil with the ability to suppress *MLP* gene expression and inhibit the accumulation of the hydrophobic

pollutants, pyrene and dieldrin, in the Cucurbitaceae family. This study enables farmers to produce safer crops in the contaminated farmland with the hydrophobic pollutants, although farmers have to apply the pesticide to reduce crop contamination even if they do not need it. The pesticide application is confirmed in safety and low cost compared with conventional manners such as the application of the active carbon and microorganisms.

2. Materials & Methods

2.1 Plant materials & pesticides

The seeds of the *C. pepo* subspecies *pepo* cultivars 'Magda' (MG) and 'Raven' (RA) were purchased from Johnny's Selected Seeds (Albion, ME, USA). The insecticides Guardbait (Sankei Chemical Co., Ltd., Kagoshima, Japan), Starkle (Hokko Chemical Industry Co., Ltd., Tokyo, Japan), and Diazinon (Sankei Chemical Co., Ltd.) and the fungicides Benlate (Sumika Agrotech Co., Ltd., Osaka, Japan) and Daconil (Kumiai Chemical Industry Co., Ltd., Tokyo, Japan) were purchased from a market (Table S1).

2.2 Collection of xylem sap

The soil (Hyponex Japan Corp., Ltd., Osaka, Japan) was autoclaved for 15 min at 120 °C and completely dried. Then, 1 kg of dry soil was spiked with 500 mL of 2.5 mM perylene, 2.5 mM pyrene, or 25 μM dieldrin in acetone, and the acetone completely evaporated in a draft chamber. The seed coats of the MG and RA cultivar seeds were removed, and the seeds soaked in tap water overnight at 4 °C. Two or three seeds were sown in glass jars supplemented with 200 g of the contaminated soil. They were cultivated at 26 °C under a 16/8 h light/dark cycle. One healthy seedling was selected, and the others removed around 1 week after sowing. During cultivation, pesticides were applied to the soil with the recommended number of times according to the manufacturer's instructions, either at the

recommended dose or triple the recommended dose (Table S3 and S4). The powder of Guardbait, Starkle, Diazinon, and Benlate was applied, and Daconil was applied after 1,000 times dilution with tap water. After 27 days, xylem sap was collected, as previously reported (Inui et al., 2013). The stem was cut 1 cm below the cotyledon. A glass tube, washed with acetone or hexane, was set against the stem, and around 500 µL of xylem sap was collected. The collected xylem sap was stored at 4 °C until use. After the collection of xylem sap, the roots were carefully washed with tap water to remove soil particles. The washed roots were stored at −80 °C until use.

2.3 Cloning of the promoter region of the MLP *gene*

The promoter region of the *MLP-GR3* gene was cloned by thermal asymmetric interlaced (TAIL) PCR (Liu et al., 1995). First, a 751-bp upstream region of the *MLP-GR3* gene was amplified with a random primer and iPCR-MLP-as1. The other two specific antisense primers, TAIL-MLP-P-as2 and TAIL-MLP-P-as3, were designed by referring 751-bp regions to amplify further upstream regions. The sequences of these primers were listed in Supplementary Table 2. Genomic DNA extracted from *C. pepo* ssp. *ovifera* cv. 'Patty Green' was used as the template, and the PCR reaction was performed with 2xQuick Taq HS DyeMix (Toyobo. Co., Ltd., Osaka, Japan). Although the reaction conditions of the TAIL-PCR followed the PCR conditions described by Liu and colleagues (Liu et al., 1995), the annealing temperature of the random hexamer and specific primers were changed to 33 °C and 53 to 56 °C, respectively.

A 1-month-old leaf of the MG cultivar was sampled, and genomic DNA was extracted using an Isoplant kit (Nippon Gene Co., Tokyo, Japan). The DNA fragment, including the promoter region of the *MLP* gene, was amplified by KOD FX Neo polymerase (Toyobo Co., Ltd.) with 100 ng of genomic DNA and the primers *MG-Pro-s* and *MG-Pro-as* under the following conditions: 5 min at 94 °C; 32 cycles of 30 s at 94 °C, 30 s at 56 °C, and 2 min at 72 °C; and 7 min at 72 °C (Table S2). The amplified

145 fragment was purified using the Gel/PCR DNA Isolation System (VIOGENE BIOTEK Co., New
146 Taipei City, Taiwan). The 2,005-bp fragment was finally amplified and then inserted into the vector
147 pBI221, digested with *Hin*d III and *Xba* I using an In-Fusion HD Cloning Kit (Takara Bio Inc., Shiga,
148 Japan). After sequencing, the fragment containing the *MLP* gene promoter and *β-glucuronidase* (*GUS*)
149 gene cut by *Hin*d III and *Sac* I was ligated with the plant expression vector pGWB402Ω (provided by
150 Dr. Tsuyoshi Nakagawa at Shimane University), digested by both restriction enzymes (Nakagawa et
151 al., 2007).
152
153 *2.4 Transformation of tobacco plants (*Nicotiana tabacum*)*
154 The plant expression plasmid containing the *MLP* gene promoter and *GUS* gene was introduced into
155 the *Rhizobium radiobacter* strain LBA4404, and the leaf disk method was employed to produce
156 transgenic tobacco lines because tobacco plants are commonly employed for GUS activity assay
157 (Horsch et al., 1985). The T_4 generation of the transgenic tobacco plants was used for the GUS assay
158 under the pesticide treatments.
159
160 *2.5 GUS assay*
161 The seeds of transgenic tobacco plants containing the *pMLP-GR3::GUS* fusion were sown on
162 Murashige and Skoog medium and incubated for 1 day at 4 °C in the dark. Then, they were incubated
163 at 24 °C under a 16-h light/8-h dark cycle. After two weeks, the seedlings were transferred to water
164 and incubated at 24 °C for another day. They were transferred to water containing 0.22 μM, 0.43 μM,
165 or 0.58 μM benomyl (Wako Pure Chemical Industries, Ltd., Osaka, Japan), an active compound in
166 Benlate, or 0.019 μM, 0.038 μM, or 0.11 μM tetrachloroisophthalonitrile (TPN) (Wako Pure Chemical
167 Industries, Ltd.), an active compound in Daconil, with 1% dimethyl sulfoxide (DMSO), and cultivated
168 at 24 °C under a 16-h light/8-h dark cycle. The concentration gradients for pesticides were selected

because the concentrations of benomyl and TPN influencing physiological changes in transgenic tobacco plants were unknown. After two days, the roots of each plant were collected, frozen in liquid nitrogen, and stored at −80 °C until use. Root samples in a protein extraction buffer [50 mM sodium phosphate buffer (pH7.0), 10 mM EDTA (pH 8.0), 0.1% (v/v) Triton X-100, 0.1% (w/v) *N*-lauroylsarcosine sodium salt, and 0.072% (v/v) 2-mercaptoethanol] were homogenized. After centrifugation at 4 °C at 20,700 × *g* for 5 min, the supernatants were collected and centrifuged again under the same conditions. The supernatants were subjected to GUS assay, as reported previously (Kodama et al., 2007).

2.6 Western blot analysis

Roots from the MG cultivar were ground in liquid nitrogen, and an extraction buffer [50 mM sodium phosphate buffer (pH 7.0), 10 mM EDTA, 0.1% (v/v) Triton X-100, 0.1% (v/v) *N*-lauroylsarcosine sodium salt] supplemented with 10 mM 2-mercaptoethanol was added to the root powder. The suspension was vigorously mixed, and the supernatant was collected after centrifugation at 20,700 × *g* for 15 min at 4 °C, as reported previously (Goto et al., 2019). The concentrations of proteins in the xylem sap and of the extracted root proteins were quantified using the Bradford method (Bradford, 1976). Sample buffer solution with reducing reagent for SDS-PAGE (Nacalai Tesque, Inc., Kyoto, Japan) was added to the samples, and they were subjected to SDS-PAGE on 15% acrylamide gel. Western blot analysis was performed with anti-MLP-PG1 and anti-MLP-GR3 antibodies, as reported previously (Goto et al., 2019). Briefly, polyvinylidene difluoride membranes with proteins transferred from gels were reacted with the anti-MLP-PG1 and anti-MLP-GR3 antibodies (Medical & Biological Laboratories Co., Ltd., Aichi, Japan) in TTBS buffer [20 mM Tris−HCl (pH 7.5), 150 mM NaCl, 0.05% (v/v) Tween 20]. After the reaction with alkaline phosphatase-conjugated goat anti-rabbit IgG

192 (Sigma-Aldrich, St. Louis, MO) in TTBS buffer, MLPs were detected. The band intensities were
193 quantified using ImageJ software (Schneider et al., 2012).
194
195 *2.7 Quantification of perylene, pyrene, and dieldrin*
196 The obtained xylem sap (50 µL) and 50 µL of DMSO were mixed in a 96-well black microtiter plate.
197 The mixture was scanned using a microplate reader (Microplate Reader SH-9000, Corona Electric Co.,
198 Ltd., Hitachinaka, Ibaraki, Japan). The fluorescences of perylene and pyrene were measured at a 410
199 nm excitation wavelength and 445 nm emission wavelength, and a 330 nm excitation wavelength and
200 at 390 nm emission wavelength, respectively.
201 The xylem sap (250 µL) was mixed with 20 µL of 200 ng/mL ^{13}C-labeled dieldrin (Cambridge Isotope
202 Laboratories, Inc., Tewksbury, MA, USA), offered by the Tohoku Ryokka Kankyohozen Co., Ltd.
203 (Sendai, Japan) as an internal standard. Dieldrin was extracted twice using 1 mL of hexane. After
204 dehydration with sodium sulfate dehydrate, 50 µL of nonane and 10 µL of 50 ng/mL MBP-15, 70, 101,
205 153 (Wellington Laboratories Inc., Guelph, Canada) as a syringe spike was added, and the samples
206 were concentrated using nitrogen gas at 35 °C until the total amount reached approximately 100 µL.
207 The concentration of dieldrin was quantified by high-resolution gas chromatography and high-
208 resolution mass spectrometry system [HRGC/HRMS: GC, 6890 N (Agilent Technologies, Tokyo,
209 Japan); MS, JMS-800D (JEOL Ltd., Tokyo, Japan)] equipped with a DB-5MS column (Kanto
210 Chemical Co. Inc., Tokyo, Japan).
211
212 *2.8 Statistical analysis*
213 One-way analysis of variance was performed, and Dunnett's multiple comparison test was applied to
214 judge significant differences.
215

3. Results

3.1 Selection of pesticides to decrease perylene concentration in the xylem sap

The insecticides Guardbait, Starkle, and Diazinon and the fungicides Benlate and Daconil were selected as applicable pesticides for Cucurbitaceae family plants. The fresh weights of the aerial parts of the plants did not significantly differ between treatments after 27 days of incubation (Figure S1A). The perylene concentration in the xylem sap was not significantly lower in any of the tested pesticide treatments, but seemed likely to decrease in the Benlate and Daconil treatments (Figure 1A). Furthermore, the perylene concentrations of the plants in the triple-dose pesticide treatments were also investigated. The fresh weight of the aerial parts did not differ between treatments (Figure S1B). Perylene concentrations were significantly decreased in the triple-dose Diazinon, Benlate, and Daconil treatments (Figure 1B). Benlate and Daconil were selected as pesticides with the ability to suppress the expression of *MLP* genes.

3.2 Decrease in the transcription activity of the MLP *gene*

GUS activity in the roots treated with benomyl did not significantly decrease, but tended towards decreasing after treatment with 0.43 μM benomyl (Figure 2). In contrast, GUS activity was significantly decreased, by 45% and 36%, after the treatment with 0.019 μM and 0.11 μM TPN, respectively (Figure 2). Thus, Daconil was selected as an *MLP* gene-suppressing pesticide.

3.3 Decrease of pyrene concentration in the xylem sap

The MG cultivar was cultivated in soil contaminated with pyrene and treated with Daconil. In the Daconil treatment with the recommended dose, the pyrene concentration in the xylem sap was significantly decreased by 36% (Figure 3). There were no significant differences in the fresh weights of the aerial parts between treatments (Figure S2).

240

241 *3.4 Decrease in the amount of MLPs in the roots and xylem sap*

242 In the roots, a band of over 17 kDa was detected after the reaction with the anti-MLP-PG1 antibody.
243 In the Daconil treatment, the relative band intensity of MLP-PG1 was decreased by 84% (Figure 4A).
244 Two bands of over 17 kDa were detected in the roots after the reaction with the anti-MLP-GR3
245 antibody. In the Daconil treatment, both band intensities were decreased, and the relative band
246 intensity of the lower band of MLP-GR3 was decreased by 93% (Figure 4A). Thus, the amount of
247 MLP-PG1 and MLP-GR3 in the roots was decreased in the Daconil treatment. Two bands of over 17
248 kDa were detected in the xylem sap after the reaction with the anti-MLP-PG1 antibody. In the Daconil
249 treatment, both bands disappeared (Figure 4B). A band below 17 kDa was detected in the xylem sap
250 after the reaction with the anti-MLP-GR3 antibody. In the Daconil treatment, the relative band
251 intensity of MLP-GR3 was decreased by 18% (Figure 4B). Thus, the amount of MLP-PG1 and MLP-
252 GR3 in the xylem sap was also decreased in the Daconil treatment (Figure 4B).

253

254 *3.5 Decrease of dieldrin concentration in xylem sap*

255 The RA cultivar was cultivated in soil contaminated with dieldrin and treated with Daconil. In the
256 Daconil treatment with the triple dose, the dieldrin concentration was significantly decreased by 52%
257 (Figure 5). In the Daconil treatment with the recommended dose, there was no significant decrease,
258 but the value was 13% lower (Figure 5). There were no significant differences in the fresh weights of
259 the aerial parts between treatments (Figure S3).

260

261 **4. Discussion**

262 The purpose of the present study was to reduce crop contamination by hydrophobic pollutants through
263 the suppression of the expression of *MLP* genes. The binding of POPs by MLPs in the roots is a crucial

step for the POP contamination in the Cucurbitaceae family (Inui et al., 2013; Goto et al., 2019). Therefore, suppression of the expression of *MLP* genes in the roots leads to a reduction of contamination. First, we focused on identifying pesticides that suppress *MLP* gene expression. Pesticides are widely used for crop cultivation and cause physiological changes in plants. This study proposes the novel use of pesticides to reduce contamination. Thus, the pesticides are not utilized for their insecticidal and fungicidal actions.

In this study, pesticides with the ability to suppress *MLP* gene expression were selected from five pesticides that can be used on the Cucurbitaceae family. Diazinon, Benlate, and Daconil caused a significant decrease in perylene concentration in the xylem sap in the triple-dose treatments. Benlate and Daconil caused a decrease of perylene in the xylem sap even with the recommended dose. Thus, these pesticides were used for further analysis (Figure 1A). Pesticides contain active compounds for insecticidal and fungicidal action, spreading agents, and detergent. Transgenic tobacco plants, containing the *GUS* gene downstream of the promoter of the *MLP-GR3* gene, were treated with active compounds to confirm that an active compound suppresses *MLP* gene expression. TPN, an active compound in Daconil, significantly decreased GUS activity in the roots. The results clearly show that TPN suppressed *MLP* gene transcription in the roots. We did not clarify the mechanisms underlying the suppression of *MLP* gene expression. The detailed mechanisms underlying this gene suppression should be clarified, as it could lead to the production of safer crops.

There are several reports that pesticides changed the expression level of genes in plants (Lu et al., 2016). TPN up-regulated more than 500 genes, including *cytochrome P450* and *glutathione S-transferase,* and down-regulated more than 400 genes in the leaves of tomato plants (Zhou et al., 2015). Some pesticides induce the expression of genes involved in resistance against pathogens. For example, the pesticides probenazole and benzothiadiazole induced the genes for UDP: glucose salicylic acid glucosyltransferase and WRKY45, respectively, in rice (Shimono et al., 2007; Umemura et al., 2009).

288 Therefore, it is possible that *MLP* gene expression was suppressed by the pesticide treatments. MLPs
289 have been identified in many plants, such as *Arabidopsis thaliana* (Lytle et al., 2009), soybeans
290 (Strömvik et al., 1999), cotton (Chen and Dai, 2010), grapes (Zhang et al., 2018), and mulberries (Gai
291 et al., 2018). Thus, the expression of *MLP* in various plant species could probably be controlled using
292 pesticide treatments.
293 Our results clearly show that a decrease in the amount of MLP-PG1 in the roots led to a decrease in
294 the amount of MLP-PG1 in the xylem sap translocated from the roots. However, the amount of MLP-
295 GR3 in the xylem sap was only slightly decreased, although that of the MLP-GR3 in the roots was
296 remarkably decreased. One possible reason for this is that the stability of MLP-GR3 is relatively high
297 in xylem sap (the accumulation of MLP-GR3 was found even in the early growth stage of *C. pepo*),
298 whereas the expression level of the *MLP-GR3* gene is low (Goto et al., 2019). It is thought that the
299 decrease of two MLPs in the xylem sap additively produced a significant decrease in the
300 concentrations of pyrene and dieldrin. Since xylem vessels are the main pathway connecting the roots
301 and aerial parts of the plant, such as the leaves and fruits, the concentration of these pollutants in the
302 fruits would also be decreased.
303
304 **5. Conclusion**
305 Based on these results, in the fungicide Daconil treatment, the transcription of *MLP* in the roots was
306 suppressed, and the amount of MLPs in the roots decreased. Subsequently, the amount of MLPs
307 translocated into the xylem vessels from the roots also decreased. As a result, the concentration of
308 hydrophobic pollutants in the xylem sap of zucchini plants was significantly decreased, leading to a
309 decrease in pollutant concentrations in the aerial parts of the plant. We are now trying to clarify the
310 mechanisms underlying the suppression of *MLP* in the fungicide Daconil treatment. This is the first
311 report to reduce crop contamination through the suppression of *MLP* expression using pesticide

312 treatment. This study proposes a new usage of pesticides. Since it is easy and low-cost, this approach
313 is applicable and could potentially replace the remediation of soil by biological, chemical, and physical
314 methods. It is notable that farmers can potentially cultivate crops in contaminated soil and produce
315 safe crops using pesticide treatments.
316
317 **Conflicts of interest**
318 The authors declare that they have no known competing financial interests or personal relationships
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320
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324 dieldrin, respectively.
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Figure legends

Figure 1. Perylene concentration in xylem sap from *Cucurbita pepo* 'Magda' (MG) after treatment with pesticides at the recommended (A) and triple the recommended (B) dose.

The MG cultivar was cultivated in soil contaminated with perylene (1.25 mmol/kg) under a 16/8 h light/dark cycle at 26 °C. During cultivation, pesticides were applied under various dosage conditions (Table S3). After 27 days, xylem sap was collected from each plant, and the perylene concentration quantified by measuring the fluorescence at 410 nm excitation and 445 nm emission. –, cultivation in soil not contaminated with perylene or no pesticide treatment; +, cultivation in soil contaminated with perylene; GB, SK, DZ, BL, and DN, Guardbait, Starkle, Diazinon, Benlate, and Daconil treatment, respectively. Error bars indicate standard deviations (n = 3–4). Asterisks indicate significant differences compared to perylene concentration in samples cultivated in soil contaminated with perylene without pesticide treatment (*, p<0.05; **, p<0.01).

Figure 2. Suppression of reporter gene expression in the roots of transgenic tobacco plants after treatment with the active compounds of pesticides.

Two-week-old transgenic tobacco plants expressing the *β-glucuronidase* (*GUS*) gene downstream of the *MLP-GR3* promoter were incubated in water containing 0.22 μM (+), 0.43 μM (++), or 0.58 μM (+++) benomyl or 0.019 μM (+), 0.038 μM (++), or 0.11 μM (+++) TPN under a 16/8 h light/dark cycle at 24 °C for 2 days. The proteins from the roots were extracted, and GUS assay was performed.

Error bars indicate standard deviation (n = 4–5). Asterisks indicate significant differences compared to GUS activity in samples incubated in water without pesticides (*, p<0.05).

Figure 3. Pyrene concentration in the xylem sap of *Cucurbita pepo* 'Magda' (MG) after Daconil treatment.

The MG cultivar was cultivated in soil contaminated with pyrene (1.25 mmol/kg) under a 16/8 h light/dark cycle at 26 °C. The fungicide Daconil was applied at the recommended dose. During cultivation, pesticides were applied under various dosage conditions (Table S4). After 27 days, xylem sap was collected from each plant, and the pyrene concentration was quantified by measuring the fluorescence at 330 nm excitation and 390 nm emission. –, cultivation in soil not contaminated with pyrene or no Daconil treatment; +, cultivation in soil contaminated with pyrene or treatment of Daconil at the recommended dose. Error bars indicate standard deviation (n = 7–8). Asterisks indicate significant differences compared to the pyrene concentration of samples cultivated in soil contaminated with pyrene without Daconil treatment. (**, p<0.01).

Figure 4. Major latex-like proteins in the roots (A) and xylem sap (B) of *Cucurbita pepo* 'Magda' (MG) after Daconil treatment.

The MG cultivar was cultivated in soil contaminated with pyrene (1.25 mmol/kg) under a 16/8 h light/dark cycle at 26 °C. The fungicide Daconil was applied at the recommended dose. During cultivation, pesticides were applied under various dosage conditions (Table S4). After 27 days, the roots and xylem sap were collected, and the proteins in the roots were extracted. Root extracts and xylem sap were subjected to SDS-PAGE. MLP-PG1 and MLP-GR3 were detected by western blot analysis using the anti-MLP-PG1 antibody and anti-MLP-GR3 antibody, respectively. –, no Daconil treatment; +, Daconil treatment. Band intensities were quantified by ImageJ.

Figure 5. Dieldrin concentration in the xylem sap of *Cucurbita pepo* 'Raven' (RA) after Daconil treatment.

The RA cultivar was cultivated in soil contaminated with dieldrin (12.5 μmol/kg) under a 16/8 h light/dark cycle at 26 °C. The fungicide Daconil was applied at the recommended dose and triple the recommended dose. During cultivation, pesticides were applied under various dosage conditions (Table S4). After 27 days, xylem sap was collected, and dieldrin concentration was quantified by high-resolution gas chromatography and high-resolution mass spectrometry. –, cultivation in soil not contaminated with dieldrin or no Daconil treatment; +, cultivation in soil contaminated with dieldrin or Daconil treatment at the recommended dose; +++, Daconil treatment at triple the recommended dose. Error bars indicate standard deviation (n = 5–8). Asterisks indicate significant differences compared to the dieldrin concentration of samples cultivated in soil contaminated with dieldrin with no Daconil treatment (***, p<0.001).

Supplementary Figure 1. Fresh weight of the aerial parts of *Cucurbita pepo* 'Magda' (MG) after pesticide treatment at recommended (A) and triple (B) doses.

The MG cultivar was cultivated in soil contaminated with perylene (1.25 mmol/kg) under a 16/8 h light/dark cycle at 26 °C. During cultivation, pesticides were applied under various dosage conditions (Table S3). After 27 days, the fresh weights of the aerial parts were measured. –, cultivation in soil not contaminated with perylene or no pesticide treatment; +, cultivation in soil contaminated with perylene; GB, SK, DZ, BL, and DN, Guardbait, Starkle, Diazinon, Benlate, and Daconil treatment, respectively. Error bars indicate standard deviation (n = 3–4).

524 Supplementary Figure 2. Fresh weight of the aerial parts of *Cucurbita pepo* 'Magda' (MG) after
525 Daconil treatment.
526 The MG cultivar was cultivated in soil contaminated with pyrene (1.25 mmol/kg) under a 16/8 h
527 light/dark cycle at 26 °C. The fungicide Daconil was applied at the recommended dose. During
528 cultivation, pesticides were applied under various dosage conditions (Table S4). After 27 days, the
529 fresh weights of the aerial parts were measured. –, cultivation in soil not contaminated with pyrene or
530 no Daconil treatment; +, cultivation in soil contaminated with pyrene or Daconil treatment at the
531 recommended dose. Error bars indicate standard deviation (n = 7–8).
532
533 Supplementary Figure 3. Fresh weight of the aerial parts of *Cucurbita pepo* 'Raven' (RA) after Daconil
534 treatment.
535 The RA cultivar was cultivated in soil contaminated with dieldrin (12.5 μmol/kg) under a 16/8 h
536 light/dark cycle at 26 °C. The fungicide Daconil was applied at the recommended dose and triple the
537 recommended dose. During cultivation, pesticides were applied under various dosage conditions
538 (Table S4). After 27 days, the fresh weights of the aerial parts were measured. –, cultivation in soil not
539 contaminated with dieldrin or no Daconil treatment; +, cultivation in soil contaminated with dieldrin
540 or Daconil treatment at the recommended dose; +++, Daconil treatment at triple the recommended
541 dose. Error bars indicate standard deviation (n = 5–8).

Pesticides

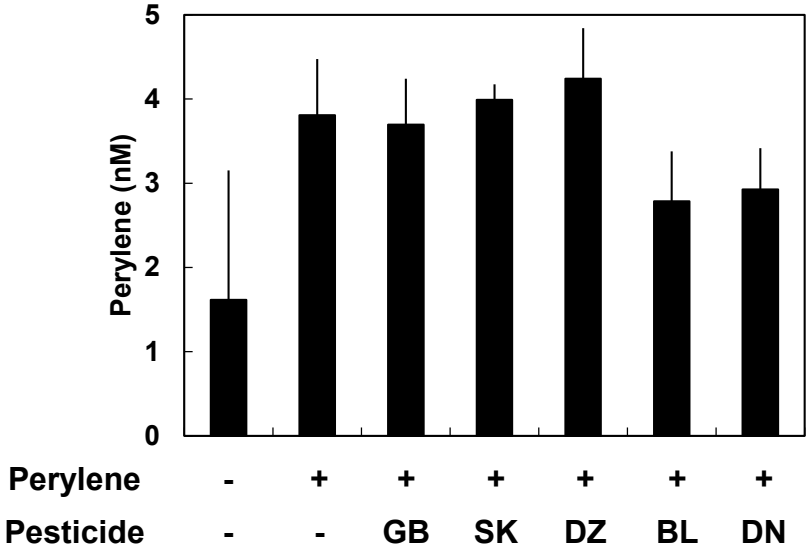
Toxic

Safe

MLP genes

Zucchini
(*Cucurbita pepo*)

(A)


Perylene (nM)
0
1
2
3
4
5
Perylene - + + + + + +
Pesticide - - GB SK DZ BL DN

(B)

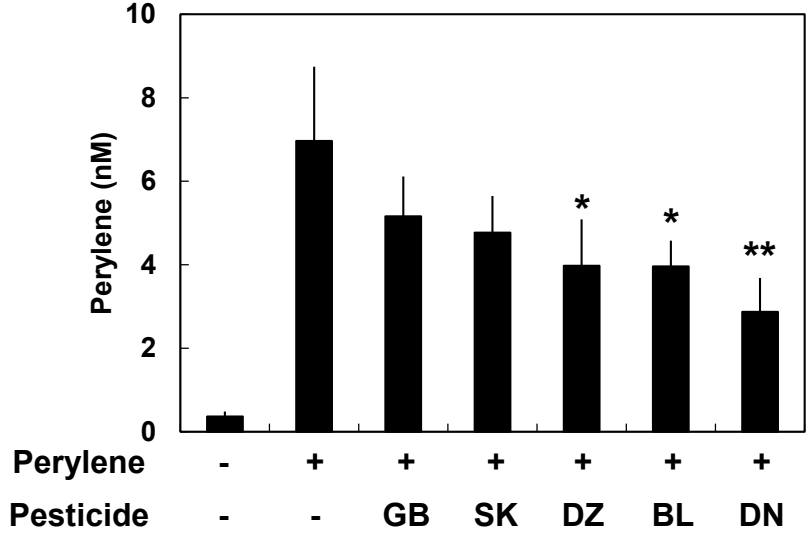

Perylene (nM)
0
2
4
6
8
10
*
*
**
Perylene - + + + + + +
Pesticide - - GB SK DZ BL DN

Figure 1

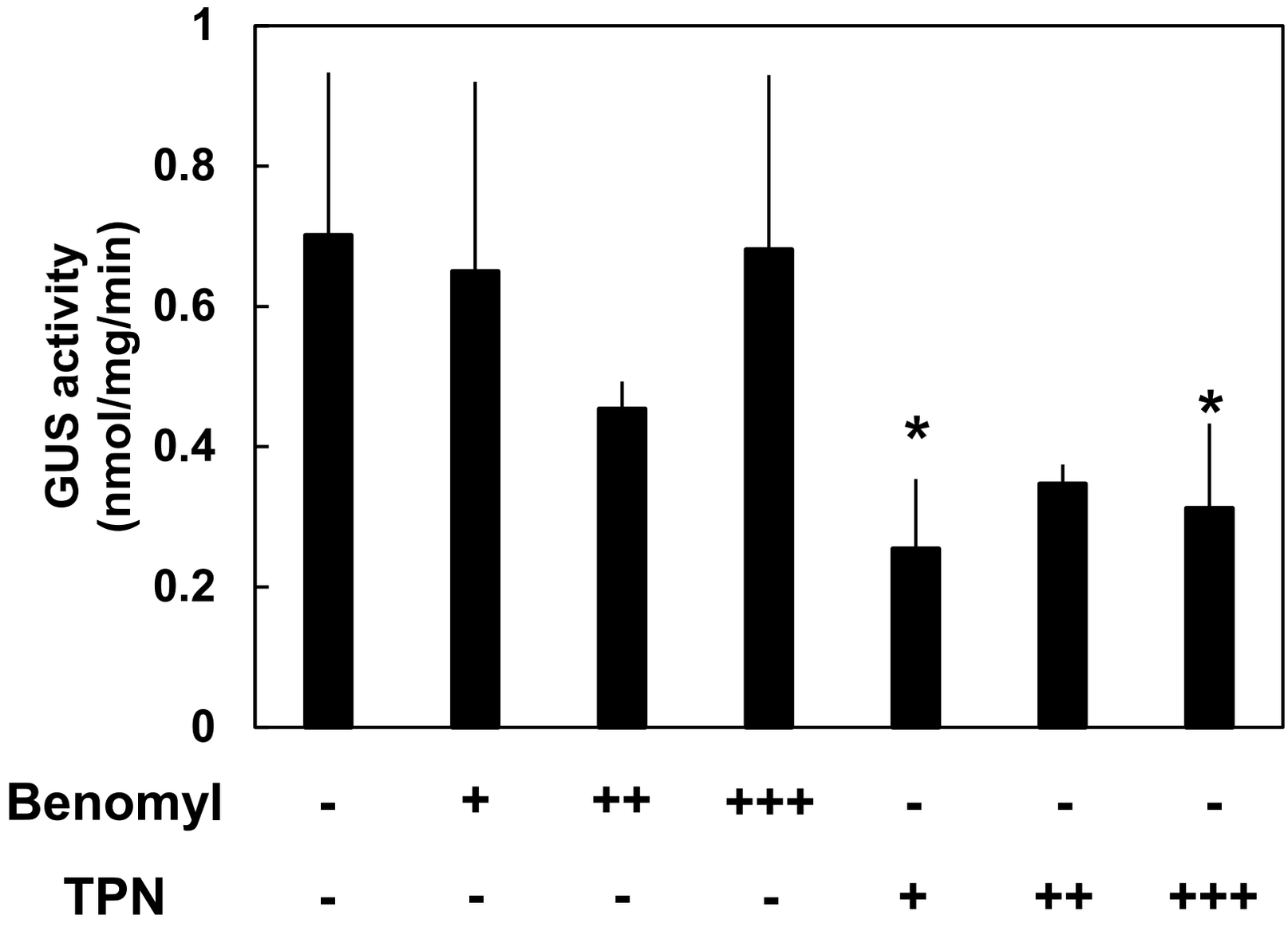


Figure 2

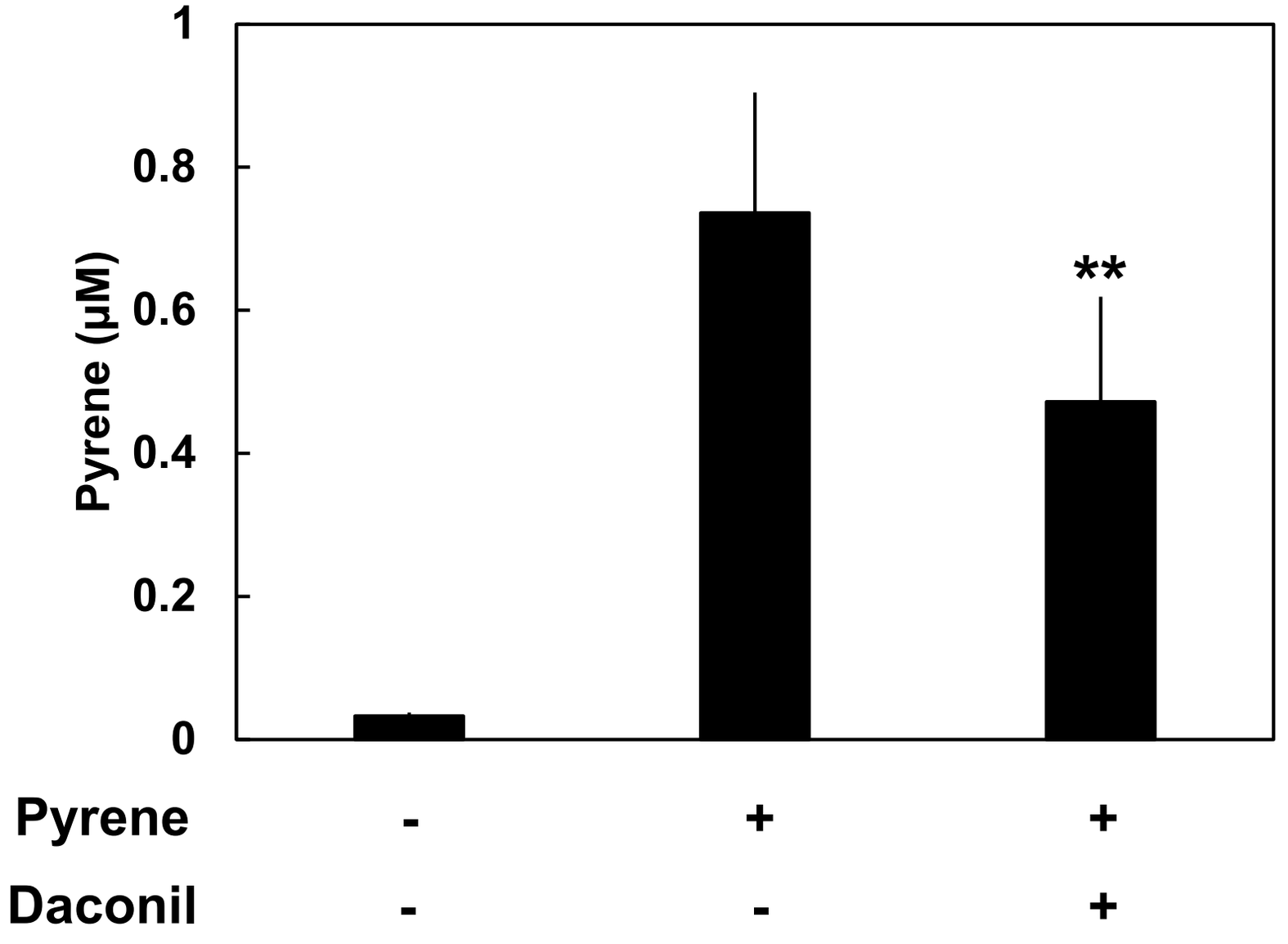


Figure 3

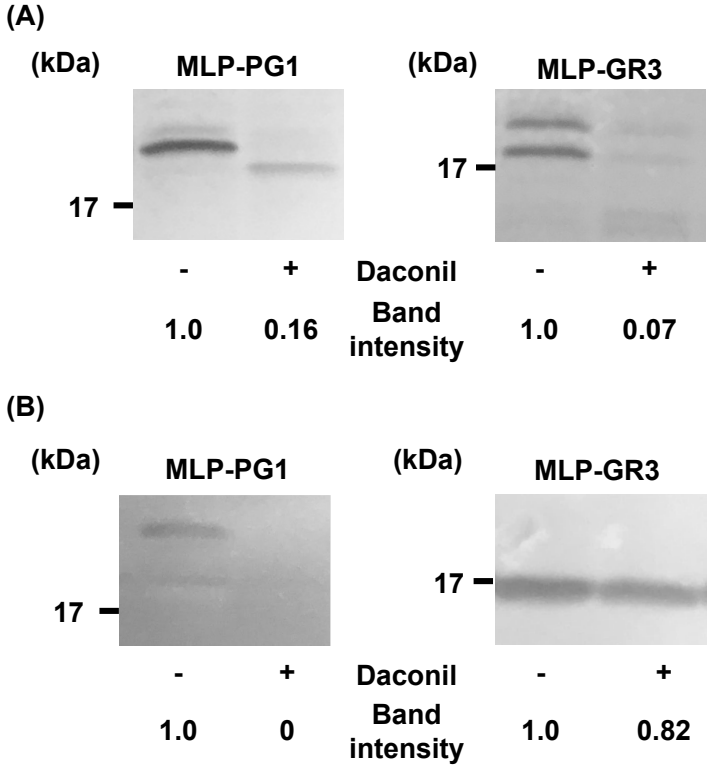

(A)
(kDa)
MLP-PG1
17
-
+
1.0
0.16
(kDa)
MLP-GR3
17
Daconil
-
+
Band intensity
1.0
0.07
(B)
(kDa)
MLP-PG1
17
-
+
1.0
0
(kDa)
MLP-GR3
17
Daconil
-
+
Band intensity
1.0
0.82

Figure 4

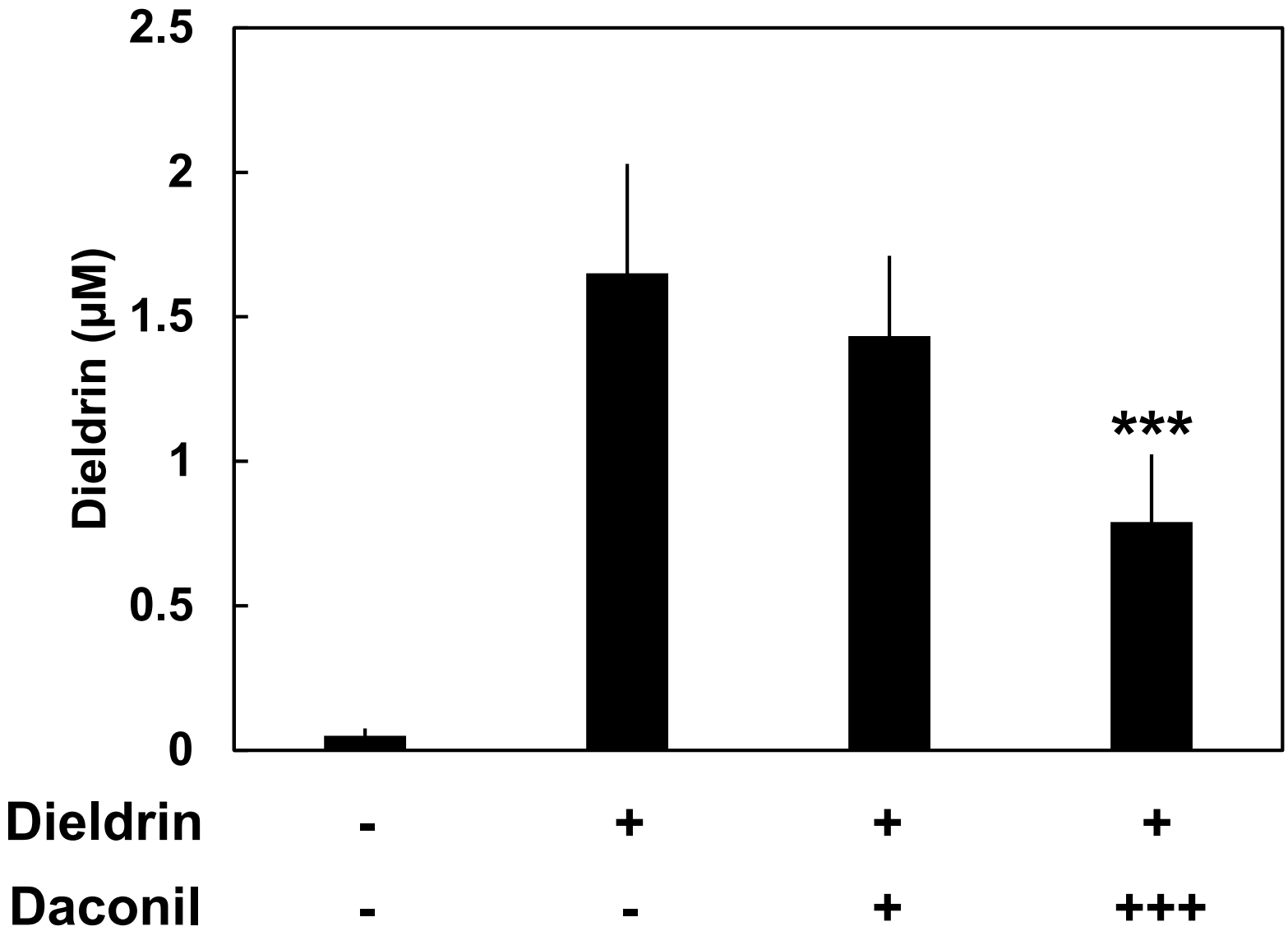
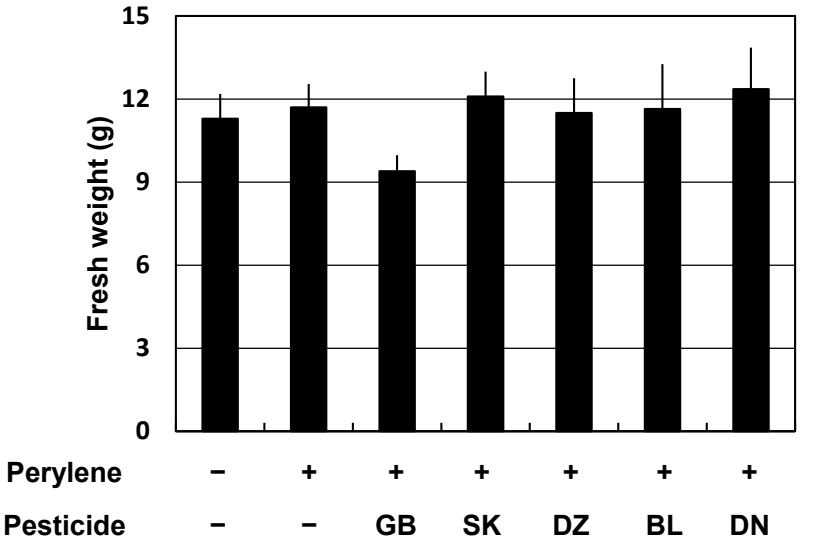
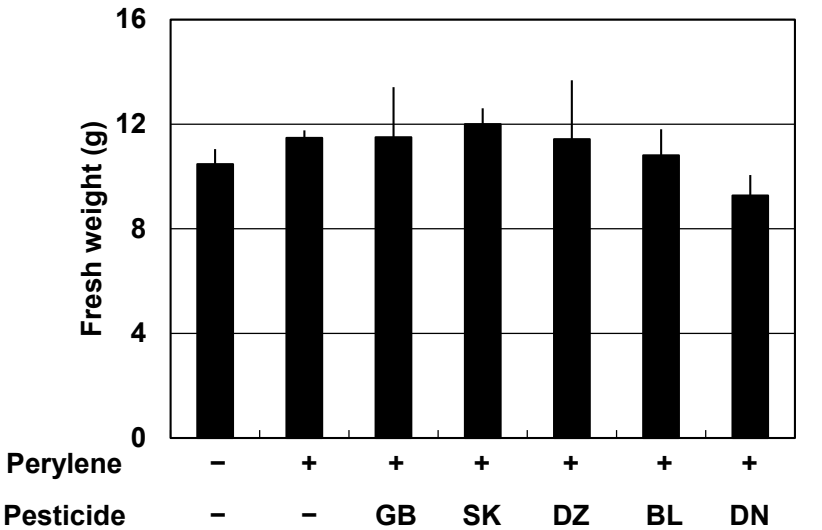


Figure 5

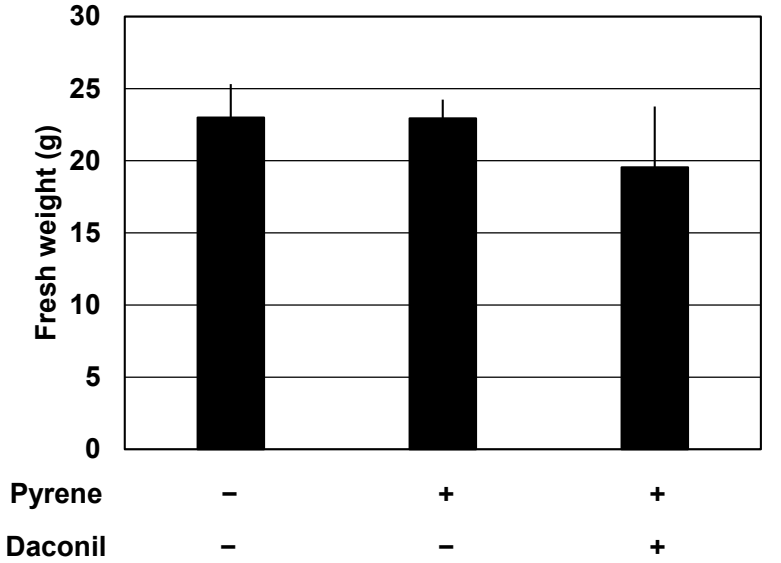
(A)



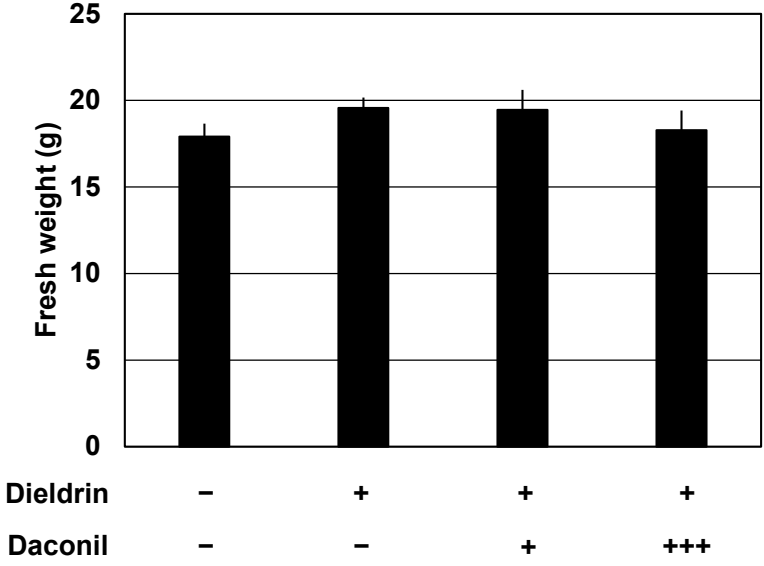
(B)



Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3

Supplementary Table 1. Physicochemical and toxic properties of pesticides.

Property	Pesticide				
	GB	SK	DZ	BL	DN
logK_{ow}	6.5	-0.549	3.81	2.12	3.05
LD_{50} (mg/kg)	430 - 4000	2000 - 2800	1340	>9590	>10000

The value for logK_{ow} and LD_{50} of GB, DZ, BL, and DN were from web service (https://pubchem.ncbi.nlm.nih.gov/). Those of SK were from papers (Corbel et al., 2004; Li et al., 2018).

Supplementary Table 2. Sequences of primers used in this study.

Primer name	Sequence
MG-Pro-s	5'-TGATTACGCCAAGCTTAAGCATTCAATAAGTTGTT-3'
MG-Pro-as	5'-CCGGGGATCCTCTAGTTTCTTTCGATGTGATACAA-3'
iPCR-MLP-as1	5’-CCTAAAATCTTCTCCAGAGA-3’
TAIL-MLP-P-as2	5’-ATCTTGTCTCCCTCTCCAAC-3’
TAIL-MLP-P-as3	5’-CTTCGTGAACTAAATGGGGC-3’

Supplementary Table 3. Applied amount of pesticides at the recommended dose during the cultivation of *Cucurbita pepo* 'Magda' (MG).

Cultivation period	Pesticide				
(day)	GB	SK	DZ	BL	DN
10	9 mg	0.45 mg	24 mg	300 mg	9 μL
13	9 mg	–	–	300 mg	9 μL
17	9 mg	–	–	300 mg	–
20	9 mg	–	–	–	–
24	9 mg	–	–	–	–

The MG cultivar was cultivated in soil contaminated with perylene (1.25 mmol/kg) under a 16/8 h light/dark cycle at 26°C. After 27 days, xylem sap was collected from each plant. The triple dose of the pesticides was applied at three times the amount described in the table. –, no pesticide treatment; GB, SK, DZ, BL, and DN, Guardbait, Starkle, Diazinon, Benlate, and Daconil treatment, respectively. The powder of GB, SK, DZ, and BL was applied, and DN was applied after 1,000 times dilution in tap water.

Supplementary Table 4. Applied amount of Daconil during the cultivation of *Cucurbita pepo* 'Magda' (MG) and 'Raven' (RA) at the recommended dose.

Cultivation period	Cultivar	
(day)	MG	RA
11	9 μL	–
12	–	9 μL
14	9 μL	–
15	–	9 μL
20	–	9 μL

The MG and RA cultivars were cultivated in soil contaminated with pyrene (1.25 mmol/kg) and dieldrin (12.5 μmol/kg) under a 16/8 h light/dark cycle at 26°C. After 27 days, xylem sap was collected from each plant. The triple dose of pesticides was applied at three times the amount described in the table. –, no pesticide treatment. DN was applied after 1,000 times dilution in tap water.

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