Pesticide treatment reduces hydrophobic pollutant contamination in Cucurbita pepo through competitive binding to major latex-like proteins

Fujita, Kentaro ; Kondoh, Yasumitsu ; Honda, Kaori ; Haga, Yuki ; Osada, Hiroyuki ; Matsumura, Chisato ; Inui, Hideyuki

(Citation)
Environmental Pollution,266(2):115179

(Issue Date)
2020-11

(Resource Type)
journal article

(Version)
Accepted Manuscript

Pesticide treatment reduces hydrophobic pollutant contamination in *Cucurbita pepo* through competitive binding to major latex-like proteins

Kentaro Fujita[1], Yasumitsu Kondoh[2], Kaori Honda[2], Yuki Haga[3], Hiroyuki Osada[2], Chisato Matsumura[3], Hideyuki Inui[1, 4, *]

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

[2]RIKEN Center for Sustainable Resource Science, RIKEN, 2-1 Hirosawa, Wako, Saitama, 351-0198, Japan

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

[4]Biosignal Research Center, Kobe University, 1-1 Rokkodaicho, Nada-ku, Kobe, Hyogo, 657-8501, 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 are still present in agricultural soil. The Cucurbitaceae family accumulates hydrophobic pollutants through roots, resulting in the contamination of aerial parts. Major latex-like proteins (MLPs), found in the Cucurbitaceae family, play an important role in the contamination by binding to these hydrophobic pollutants. Thus far, efficient cultivation methods for the production of safe crops with lower concentrations of hydrophobic pollutants have not been developed. Herein, we competitively inhibited the binding of MLPs to hydrophobic pollutants, pyrene and dieldrin, in roots by using MLP binding pesticides. By conducting a chemical array screening, we found that MLPs bound compounds with indole- and quinazoline-like structures. Commercially available pesticides

amisulbrom and pyrifluquinazon, which possess such structures, successfully inhibited the binding of MLPs to pyrene and dieldrin *in vitro*. When zucchini plants were cultivated in the contaminated soil with 1.25 mmol/kg pyrene and 12.5 μmol/kg dieldrin, the concentration of pyrene and dieldrin in xylem sap was significantly decreased by 30% and 15%, respectively. Our results demonstrate that the pesticides binding to MLPs competitively inhibited the binding of MLPs to pyrene and dieldrin in roots, resulting in the reduction of overall contamination. This study proposes a novel approach to cultivate safer crops and advances the utilization of unknown functions of pesticides.

(184 words)

Key words

competitive binding inhibition; *Cucurbita pepo*; hydrophobic pollutant; major latex-like protein; pesticides

Capsule

Pesticides binding major latex-like proteins competitively inhibited the binding of MLPs to hydrophobic pollutants and decreased the concentrations of them in xylem sap of zucchini plants.

1. Introduction

The contamination of crops by hydrophobic pollutants such as persistent organic pollutants (POPs) and polycyclic aromatic hydrocarbons (PAHs) is currently a severe problem in terms of food safety and quality. For example, the insecticides dieldrin and heptachlor, categorized as POPs, were detected in cucumber and pumpkin, respectively, over the residue limits, although they have been prohibited from use more than 40 years ago (Hashimoto, 2005). Hydrophobic pollutants registered as POPs were discontinued in 181 countries in 2020 because of their persistence, long-distance mobility, bioaccumulation, and toxicity. However, they were detected in crops, such as the Cucurbitaceae family, because of the contamination of agricultural soil by the use of insecticides determined as POPs and the incomplete combustion of straw after field burning (Shen et al., 2013). Hydrophobic pollutants are reported to show carcinogenicity (Bortey-Sam et al., 2017) and neurotoxicity (Shi et al., 2019). Therefore, the intake of such contaminated crops can lead to these diseases in humans.

It is well known that the Cucurbitaceae family such as cucumber, melon, squash, watermelon, pumpkin, and zucchini, can accumulate POPs in their stems and leaves (Otani et al., 2007). In addition, when dieldrin is detected in cucumber over the Japanese residue limit (0.02 ppm), farmers have to discard all crops cultivated in the same farmland, leading to enormous economic loss (Hashimoto, 2005). Thus, many approaches, such as degradation of POPs by microorganisms (Xiao et al., 2011) and adsorption of POPs by active carbon (Murano et al., 2009), have been attempted for the suppression of crop contamination. However, these methods show low efficiency and take a long time and high cost. Therefore, it is crucially important to develop novel technologies to produce safe crops efficiently.

The accumulation of hydrophobic pollutants in the Cucurbitaceae family is mainly due to the transport of them to aerial plant tissue. In certain Cucurbitaceae species, POPs such as chlordane were transported from roots to aerial tissues via the xylem sap (Mattina et al., 2004); in another study on

73 the uptake of the POP dieldrin by cucurbits, it was found that dieldrin-binding proteins were
74 responsible for the solubilization of dieldrin in the xylem sap and possibly the subsequent transport of
75 dieldrin to aerial tissues (Murano et al., 2010). Major-latex like proteins (MLPs) were identified as
76 one of the POP transporting factors in zucchini plants (Inui et al., 2013).
77 MLPs are a member of the birch pollen allergen (Bet v 1) family (Radauer and Breiteneder, 2007),
78 which includes pathogenesis-related proteins of class 10 (PR-10s) (Fernandes et al., 2013), cytokinin
79 specific-binding proteins (Pasternak et al., 2006), and fruit allergens such as kiwi allergen Act d 11
80 (D'Avino et al., 2011), and strawberry allergen (Ishibashi et al., 2017). Proteins in this family have a
81 similar 3D structure; they all contain three α helices and seven β sheets, but have a variable amino acid
82 sequence (Choi et al., 2015). Remarkably, these structural elements are assembled in such a way that
83 a large internal hydrophobic cavity, which enables the proteins to bind hydrophobic compounds such
84 as plant hormones and secondary metabolites, is created (Fernandes et al., 2013). For example, Bet v
85 1l (a naturally occurring isoform of Bev v 1) can bind brassinosteroids (Markovic'-Housley et al.,
86 2003), and Fra a proteins in strawberries can bind flavonoids such as quercetin, myricetin, and catechin
87 (Casañal et al., 2013). MLP-PG1 and MLP-GR3 proteins, identified in zucchini plants, exhibit binding
88 activity towards PCBs, dieldrin, and other hydrophobic pollutants (Goto et al., 2019). MLPs from the
89 Cucurbitaceae family are key factors in the hydrophobic pollutant contamination of their aerial tissue:
90 they bind hydrophobic pollutants in roots, which are then transported to fruits via xylem vessels (Goto
91 et al., 2019).
92 In this study, reduction of hydrophobic pollutant contamination was attempted in a Cucurbita species
93 via the use of targeted pesticides: by treatment with MLP binding pesticides, Raimei and Colt,
94 competitive inhibition could decrease the binding of MLPs to hydrophobic pollutants, pyrene and
95 dieldrin, resulting in their decreases in the xylem sap, and consequently a reduction in the
96 transportation of them to the aerial parts of plants. This approach proposes a practical reduction of

97 contamination reduction, as treatments consist of commercially available pesticides. To the best of our
98 knowledge, this is the first study to focus on the reduction of such contamination through competitive
99 inhibition of MLP and contaminant binding.
100
101 **2. Materials & Methods**
102 *2.1 Chemical array screening*
103 Chemical array screening was performed primarily to identify compounds with a positive response to
104 MLPs. The arrays were prepared using previously described method (Kondoh et al., 2015). We used
105 22,097 compounds from the RIKEN NPDepo for the screening. Recombinant MLP-PG1 and MLP-
106 GR3 proteins fused with His-tags at the C-terminus were produced using *Escherichia coli*, as described
107 previously (Inui et al., 2013). To prevent non-specific binding, the chemical arrays were incubated in
108 a blocking solution of TBS-T (10 mM Tris-HCl [pH 8.0], 150 mM NaCl, 0.05% [v/v] Tween 20) and
109 1% skimmed milk for 1 h at room temperature. After washing with TBS-T and potassium phosphate
110 buffer (20 mM potassium phosphate buffer [pH 5.6], 100 mM KCl, 1 mM $MgCl_2$, 0.2 mM $CaCl_2$, and
111 0.2 mM EDTA), 4 μM of recombinant MLP-PG1 or MLP-GR3 in potassium phosphate buffer was
112 applied onto the chemical arrays and incubated at 30°C for 1 h. After another washing with potassium
113 phosphate buffer and TBS-T, the chemical arrays were incubated with anti-His antibody (GE
114 Healthcare, Chicago, IL), followed by a second antibody (goat anti-mouse IgG) with Cy5 fluorescence
115 dye (Thermo Fisher Scientific, Waltham, MA). Finally, the chemical arrays were scanned with a
116 microarray scanner GenePix 4300A (Molecular Devices, Sunnyvale, CA), using the Cy5 channel, with
117 the fluorescence excitation wavelength set at 635 nm and emission wavelength set at 655–695 nm. A
118 structure search of MLP-binding compounds was performed in SciFinder
119 (https://www.cas.org/products/scifinder).
120

121 *2.2 Plant materials, growing conditions, and collection of xylem sap*

122 The seeds of *Cucurbita pepo* subspecies *pepo*, cultivars' Magda (MG)' and 'Raven (RA)', were
123 purchased from Johnny's Selected Seeds (Albion, ME). Soil was obtained from Hyponex Japan Corp.,
124 Ltd. (Osaka, Japan), autoclaved at 120°C for 15 min and completely dried. Two types of soil were
125 prepared: non-contaminated soil (1 kg of the dry soil mixed with 500 mL of acetone), and
126 contaminated soil (1 kg of the dry soil mixed with 500 mL of acetone with 2.5 mM pyrene or 25 μM
127 dieldrin). Acetone was then evaporated from the soil. The spiked concentration was six times higher
128 than that in the agricultural field (Hashimoto, 2005). After peeling the seed coat off to promote
129 germination, seeds of the two cultivars of *C. pepo* subsp. *pepo* were incubated in tap water at 4°C for
130 1 d and then sown in a glass jar supplemented with 200 g of non-contaminated or contaminated soil.
131 Zucchini plants cultivated in non-contaminated soil and contaminated soil without the treatment of
132 pesticides were regarded as the negative control and positive control, respectively.
133 After 27-day of cultivation at 26°C under a 16/8 h light/dark cycle, xylem sap was collected following
134 procedures described previously (Inui et al., 2013). Briefly, the stem was cut at 1 cm below the
135 cotyledon, and the weight of stem and leaf tissues was measured. A glass tube washed with acetone or
136 hexane (for the quantification of pyrene or dieldrin, respectively) was put on the stem until the amount
137 of xylem sap reached approximately 500 μL. Collected xylem sap was stored at 4°C until time of use.
138 The fungicide Raimei (Nissan Chemical Co., Ltd., Tokyo, Japan) and the insecticide Colt (Kumiai
139 Chemical Industry Co., Ltd., Tokyo, Japan), chosen based on the results of the chemical assay, were
140 applied to the plants by spray according to the manufacturer's instruction at the recommended timing
141 at two doses (the standard dose, or thrice the standard dose). The number of collected xylem sap ranged
142 from 5 to 12 and from 8 to 19 under the cultivation of contaminated soil with pyrene and dieldrin,
143 respectively.
144

145 *2.3 Quantification of pyrene and dieldrin*

146 The obtained xylem sap (50 μL) was mixed with 50 μL of dimethyl sulfoxide (DMSO) in a 96-well
147 black microtiter plate. Fluorescence of pyrene in the samples was measured using Microplate Reader
148 SH-9000 (Corona Electric Co., Ltd., Hitachinaka, Ibaraki, Japan) with the excitation wavelength set
149 at 330 nm and the emission wavelength set at 390 nm. Zucchini plants cultivated in non-contaminated
150 soil and contaminated soil without the treatment of pesticides were regarded as the negative control
151 and positive control.

152 The xylem sap (250 μL) was mixed with 20 μL of 200 ng/mL ^{13}C-labeled dieldrin as an internal
153 standard ([Cambridge Isotope Laboratories, Inc., Tewksbury, MA], offered by Tohoku Ryokka
154 Kankyohozen Co., Ltd. [Sendai, Japan]). Dieldrin was extracted using two washes of 1 mL hexane.
155 After dehydration by anhydrous sodium sulfate, 50 μL of nonane were added, and samples were
156 concentrated by nitrogen gases at 35°C until the total amount reached approximately 100 μL. We
157 added 10 μL of 50 ng/mL MBP-15, 70, 101, 153 (Wellington Laboratories Inc., Guelph, Canada) as a
158 syringe spike, and dieldrin concentration was quantified by high-resolution gas chromatography and
159 high-resolution mass spectrometry (HRGC/HRMS: GC, 6890N [Agilent Technologies, Tokyo, Japan];
160 MS, JMS-800D [JEOL Ltd., Tokyo, Japan]) equipped with a DB-5MS column (Agilent Technologies)
161 using DioK Ver. 4.02 (JEOL, Ltd., Tokyo, Japan). The values of the method detection limits and the
162 method quantification limits were 0.19 nM and 0.63 nM, respectively.

163

164 *2.4 Competitive binding assay*

165 Recombinant MLPs were diluted with a sodium phosphate buffer (50 mM sodium phosphate buffer
166 [pH 7.0], 200 mM NaCl, and 150 mM imidazole) at a final concentration of 0.2 mg/mL. Two different
167 quantities (5 mM or 10 mM) of amisulbrom (Wako Pure Chemical Industries, Ltd., Osaka, Japan) and
168 pyrifluquinazon (Wako Pure Chemical Industries), dissolved in DMSO, were added into MLP

169 solutions at a final concentration of 25 μM or 50 μM, respectively. Finally, pyrene (1 mM), dissolved
170 in DMSO, was added at a final concentration of 10 μM to the solutions. These solutions were incubated
171 with rotation at 20°C for 1 h. Fluorescence of pyrene was measured in 100 μL of each solution using
172 Microplate Reader SH-9000, as described above.
173 Dieldrin (200 mM), dissolved in dimethylformamide (DMF), was bound to 1 mg of epoxy beads
174 (Tamagawa Seiki, Co., Ltd, Nagano, Japan) according to the manufacturer's instruction. After
175 dispersion and spindown of dieldrin-binding beads, magnetic separation was performed to remove the
176 supernatant, and the binding buffer (50 mM potassium phosphate buffer [pH 5.6], 100 mM KCl, 1
177 mM $MgCl_2$, 0.2 mM $CaCl_2$, 0.2 mM EDTA, 10% [v/v] glycerol, 0.1% [v/v] Nonidet P-40, 1 mM
178 dithiothreitol, 0.2 mM phenylmethylsulfonyl fluoride, 0.1% Triton X-100, and 0.1% *N*-
179 lauroylsarcosine sodium salt) was added. Washing was performed three times. Recombinant MLP-
180 PG1 or MLP-GR3 was diluted with the binding buffer, until the final concentration of each MLP was
181 0.1 mg/mL. The solutions were centrifuged at 4°C for 30 min at 20,700 × *g*, and the supernatants were
182 collected. Then, 20 mM amisulbrom and pyrifluquinazon, dissolved in DMSO, were added into the
183 solutions at a final concentration of 0.2 mM. The solutions were incubated with rotation at 4°C for 2
184 h. After incubation, 200 μL of the solutions were added to 0.25 mg of beads and incubated at 4°C for
185 4 h. Magnetic separation was performed, the supernatant removed, and the binding buffer added.
186 Washing with binding buffer was repeated eight times. The binding buffer (35 μL) and 7 μL of Sample
187 Buffer Solution with Reducing Reagent for SDS-PAGE (Nacalai Tesque, Inc., Kyoto, Japan) were
188 added to the beads, and the beads with the solution were heated at 98°C for 5 min. After magnetic
189 separation, the samples were subjected to SDS-PAGE on a 15% acrylamide gel, and gels were stained
190 using a Silver Staining kit (Wako Pure Chemical Industries). Band intensities were measured using
191 the ImageJ software (Schneider et al., 2012).
192

193 *2.5 Western blot analysis*

194 Roots from RA were ground, and root proteins were extracted as reported previously (Goto et al.,
195 2019). Extracted root proteins were quantified by the Bradford method (Bradford, 1976)(Bradford,
196 1976). Sample Buffer Solution with Reducing Reagent for SDS-PAGE (Nacalai Tesque) was added to
197 the samples, and they were subjected to SDS-PAGE on 15% acrylamide gel. Western blot analysis was
198 performed with anti-MLP-PG1 and anti-MLP-GR3 antibodies, as reported previously (Goto et al.,
199 2019). Band intensities were quantified using the ImageJ software (Schneider et al., 2012).

200

201 *2.6 Statistical analysis*

202 Each experiment for quantification of pyrene and dieldrin was performed independently with at least
203 three biological replicates. One-way analysis of variance was performed, and Dunnett's multiple
204 comparison test was applied to judge significant differences among treatments using R software ver.
205 3.6.2 (https://www.R-project.org/).

206

207 **3. Results**

208 *3.1 Selection of compounds binding MLPs and selection of pesticides*

209 Chemical array screening was performed for the identification of positive signals after incubation with
210 MLPs. Of the 22,097 compounds from the RIKEN NPDepo, the compounds 166 and 176 had positive
211 signals with different fluorescence intensities for recombinant MLP-PG1 and MLP-GR3, respectively
212 (Table 1). The number of total compounds with positive signals was 242, and 100 compounds had
213 positive signals for both MLPs.

214 Thirty plant-related compounds, such as plant hormones and secondary metabolites, were chosen from
215 the 242 compounds by SciFinder, and two compounds with indole-like structures were proposed

(Table 2). Amisulbrom was finally selected as a pesticide containing an indole-like structure (Figure 1A).

Compounds whose positive signal showed ++ or +++ to both MLPs were selected. Amongst them, ten compounds that had quinazoline-, quinoxaline-, and quinoline-like structures were identified (Table 3). Pyrifluquinazon was finally selected as a pesticide with a quinazoline-like structure (Figure 1B).

3.2 Competitive binding activity of pesticides

When pyrene was incubated with MLPs, the fluorescence of pyrene dramatically increased (Figure 2). With MLP-PG1, the addition of 50 μM of amisulbrom led to a decrease of 24% in the fluorescence of pyrene, as compared to without pesticides (Figure 2A). With the addition of 25 μM and 50 μM of pyrifluquinazon, the fluorescence of pyrene was decreased by 26% and 61%, respectively (Figure 2A). With MLP-GR3, the addition of 25 μM and 50 μM of amisulbrom led to a decrease of 50% and 40% in the fluorescence of pyrene, as compared to without pesticides (Figure 2B). Similarly, with the addition of 25 μM and 50 μM of pyrifluquinazon, the fluorescence of pyrene was decreased by 32% and 28%, respectively (Figure 2B). Without pesticides, the fluorescence when incubated with MLP-GR3 roughly doubled as compared to that with MLP-PG1 (Figure 2). When pyrene was incubated with pesticides without MLPs, the fluorescence of pyrene was not increased (Figure S1A). When MLPs were incubated with pesticides without pyrene, the fluorescence was also not increased (Figure S1B).

In MLP-PG1 and MLP-GR3, a band was detected when dieldrin-binding beads were added (Figure 3). With the addition of amisulbrom, the band intensity was not clearly decreased in MLP-PG1, but was decreased by 49% in MLP-GR3 (Figure 3A). In contrast, with the addition of pyrifluquinazon, the band intensity was decreased by 19% in MLP-PG1 and by 78% in MLP-GR3, respectively (Figure

239 3B). No bands were detected in the beads without dieldrin (Figure 3). There were no bands in the
240 supernatant from the last washing step (Figure S2).
241
242 *3.3 Reduced contamination in zucchini plants cultivated in the contaminated soil*
243 The fungicide Raimei containing amisulbrom and the insecticide Colt containing pyrifluquinazon
244 were selected as pesticides to accomplish the reduction of pyrene and dieldrin contamination in
245 zucchini plants. In the Raimei treatment, pyrene concentration was significantly decreased by 40%
246 with the triple dose, but not with the usual dose (Figure 4A). In the Colt treatment, the pyrene
247 concentration was not significantly decreased with the triple doses but was significantly decreased by
248 30% with the usual dose (Figure 4B). The fresh weights of aerial parts in each treatment were not
249 significantly changed (Figure S3).
250 In the Raimei treatment during the cultivation of zucchini plants in dieldrin-contaminated soil, dieldrin
251 concentration was not significantly decreased, even upon the use of the triple dose (Figure 5). In the
252 Colt treatment, dieldrin concentration was significantly decreased by 15% upon administration of the
253 triple dose; the usual dose tended to also decrease the concentration, but not significantly so (Figure
254 5). The fresh weights of aerial parts in each treatment were not significantly changed (Figure S4).
255 Furthermore, with the treatment of the triple dose of Colt, the amount of MLP-PG1 and MLP-GR3 in
256 roots was not significantly decreased (Figure S5).
257
258 **4. Discussion**
259 Chemical array screening is a suitable method to identify compounds with a high ability to bind MLPs
260 as shown by Maeda *et al.* (2017) that the inhibitor for the synthase responsible for production of a
261 fungal toxin was successfully obtained (Maeda et al., 2017). The MLPs used in this study bound onto
262 242 compounds in the chemical arrays (Table 1). Almost all of the compounds had aromatic rings,

263 which suggested that MLPs were likely to bind to relatively hydrophobic compounds. It had been
264 reported that Act d 11, which has a similar 3D structure to MLPs, had a significant ability to bind
265 compounds with indole-like structures (Chruszcz et al., 2013). Thus, it was thought that MLPs could
266 bind indole compounds. Amongst 30 compounds, there were two compounds with indole-like
267 structures (Table 2), and amisulbrom (contained in the fungicide Raimei) was selected for the
268 competitive inhibition of MLP binding to hydrophobic pollutants (Figure 1). Amongst compounds
269 with a high affinity to bind MLPs, there were 10 compounds with quinazoline-like, quinoxaline-like,
270 and quinoline-like structures (Table 3), and the compound pyrifluquinazon (with a quinazoline-like
271 structure), contained in the insecticide Colt, was selected (Figure 1). Notably, this is the first report to
272 find MLPs that could bind compounds with quinazoline-like, quinoxaline-like, and quinoline-like
273 structures. Raimei and Colt are registered for the application on the Cucurbitaceae family, so treatment
274 with these pesticides is a practical method for reduction of hydrophobic pollutant contamination.
275 Amisulbrom competitively inhibited the binding of MLPs to pyrene and dieldrin. Inhibition activity
276 was higher in MLP-GR3 than in MLP-PG1 (Figure 2 and 3A). This result was supported by the result
277 of the chemical array screening. MLP-GR3 had a higher ability to bind compounds with indole-like
278 structures than MLP-PG1 (Table 2). Pyrifluquinazon also competitively inhibited the binding of MLPs
279 to pyrene and dieldrin (Figure 3B). Pyrifluquinazon was better at inhibiting the binding of MLP-PG1
280 to pyrene than that of MLP-GR3 to pyrene (Figure 2). It is thought that MLPs have a binding
281 preference toward certain compounds, due to their structural difference. Based on these results,
282 amisulbrom and pyrifluquinazon can both be applied to reduce the contamination by hydrophobic
283 pollutants in the Cucurbitaceae family.
284 The hydrophobic pollutant perylene was found to mainly localize in the plasma membrane of the
285 endodermis and pericycle of root tissue (Yamazaki et al., 2015). Therefore, MLPs produced in root
286 cells will bind hydrophobic pollutants on the plasma membrane of these tissues. Then, MLP-

hydrophobic pollutant complexes translocate to xylem vessel (Goto et al., 2019). The estimated log K_{ow} of amisulbrom and pyrifluquinazon is 4.4 and 3.12, respectively, which indicates their relative hydrophobic properties (https://pubchem.ncbi.nlm.nih.gov/). Based on these results, these pesticides must also localize in the plasma membrane of the endodermis and pericycle and competitively inhibit the binding of MLPs to hydrophobic pollutants.

Pyrene concentration in the xylem sap was significantly decreased by the treatment of Raimei and Colt, which suggests that amisulbrom and pyrifluquinazon competitively inhibited the binding of MLPs to pyrene in roots (Figure 4). Since POP concentration in xylem sap had a positive correlation with that in aerial parts, the amount of pyrene in aerial parts would also be decreased (Goto et al., 2019) (Figure 3). Dieldrin concentration was significantly decreased by the treatment of Colt, but not Raimei, although the mole of pyrifluquinazon applied to plants was 425 times lower than that of amisulbrom (Figure 5). One of the reasons for this is the difference in the competitive inhibition activity between amisulbrom and pyrifluquinazon. The inhibition activity of MLP binding to dieldrin by pyrifluquinazon was higher than that of MLPs to dieldrin by amisulbrom (Figure 3). Another reason for the high potency of Colt is the difference in the solubility of the two pesticides in water. The solubility of pyrifluquinazon is more than ten times higher than that of amisulbrom. Hence, pyrifluquinazon can easily spread to the rhizosphere and is taken up into roots. Furthermore, another possible cause is the different binding ability of MLPs toward these hydrophobic pollutants. Hydrophobicity of the compounds is a critical factor in explaining differences in binding affinity. However, the estimated log K_{ow} of pyrene and dieldrin is 5.18 (Miller et al., 1985) and 5.20 (Namiki et al., 2018), respectively, indicating that water solubility is almost the same. This suggests that these compounds in zucchini plants accumulate nearly at the same concentrations. In contrast, the difference in the bulkiness between hydrophobic pollutants such as PCBs made a big difference in their accumulation in zucchini plants. The amount of bulky PCBs containing chlorines at *ortho*-positions

311 such as 2,3′,4,4′,5-pentachlorobiphenyl (CB118) in zucchini plants was several times higher than that
312 of planner PCBs not containing such chlorines such as 3,3′,4,4′,5-pentachlorobiphenyl (CB126)
313 (Matsuo et al., 2011), although their estimated log K_{ow} is almost the same (Hawker and Connell, 1988).
314 Pyrene has a planner structure, but dieldrin has a bulky structure. Thus, in the study, MLPs had a higher
315 ability to bind dieldrin than pyrene. Therefore, with the Raimei treatment, pyrene concentration was
316 significantly decreased, while dieldrin concentration was not significantly decreased. Hence, only a
317 triple dose of Colt was effective in significantly decreasing dieldrin concentration in xylem sap.
318
319 **5. Conclusion**
320 In this study, the contamination of zucchini plants by the hydrophobic pollutants, pyrene and dieldrin,
321 in the agricultural field was reduced by the treatment of pesticides with the ability to bind MLPs. Our
322 findings show that selected pesticides competitively inhibited the binding of MLPs to pyrene and
323 dieldrin, and the treatment of Colt decreased the concentration of pyrene and dieldrin by 30% and
324 15%, respectively, in xylem sap, potentially leading to a decrease of contamination in the fruits,
325 although there are no reports on the positive correlation between the concentrations of pollutants in
326 the xylem sap and fruits. However, this study did not show the contamination levels in the fruits, and
327 analysis of the fruits should be undertaken. Selected pesticides showed highly selective toxicity, and
328 so they are safe for human beings. This is the first report to reduce crop contamination based on
329 molecular mechanisms in the uptake of pyrene and dieldrin. Previous studies attempted the
330 remediation of agricultural soil by physical and chemical methods for the production of safe crops.
331 This study proposes the novel method to reduce contamination based on the molecular mechanisms
332 of the uptake of hydrophobic pollutants by the Cucurbitaceae family. Since zucchini, pumpkin, and
333 cucumber have MLPs binding hydrophobic pollutants (Iwabuchi et al., 2020), this approach can apply
334 to many Cucurbitaceae family plants. With the treatment of pesticides that bind MLPs, safe crops can

be produced, even in contaminated soil. Furthermore, this study advances the utilization of unknown functions of pesticides.

CRediT authorship contribution statement

Kentaro Fujita: Data curation, Formal analysis, Writing - original draft, Writing - review & editing. **Kondoh Yasumitsu**: Data curation, Formal analysis. **Kaori Honda**: Data curation, Formal analysis. **Yuki Haga**: Data curation, Formal analysis. **Hiroyuki Osada**: Data curation, Formal analysis. **Chisato Matsumura**: Data curation, Formal analysis. **Hideyuki Inui**: Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Writing - original draft, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We thank Prof. Hirosato Takikawa (Graduate School of Agricultural Science, Kobe University), Prof. Masaki Kuse (Graduate School of Agricultural Science, Kobe University), and Prof. Daisuke Matsuoka (Graduate School of Agricultural Science, Kobe University) for useful discussion. This work was supported by Biosignal Research Center, Kobe University.

References

Bortey-Sam, N., Ikenaka, Y., Akoto, O., Nakayama, S.M.M., Asante, K.A., Baidoo, E., Obirikorang, C., Saengtienchai, A., Isoda, N., Nimako, C., Mizukawa, H., Ishizuka, M., 2017. Oxidative stress and

359 respiratory symptoms due to human exposure to polycyclic aromatic hydrocarbons (PAHs) in
360 Kumasi, Ghana. Environ. Pollut. 228, 311–320. https://doi.org/10.1016/j.envpol.2017.05.036
361 Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of
362 protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.
363 https://doi.org/10.1016/0003-2697(76)90527-3
364 Casañal, A., Zander, U., Muñoz, C., Dupeux, F., Luque, I., Botella, M.A., Schwab, W., Valpuesta, V.,
365 Marquez, J.A., 2013. The Strawberry Pathogenesis-related 10 (PR-10) Fra a Proteins Control
366 Flavonoid Biosynthesis by Binding to Metabolic Intermediates. J. Biol. Chem. 288, 35322–35332.
367 https://doi.org/10.1074/jbc.M113.501528
368 Choi, S.H., Hong, M.K., Kim, H.J., Ryoo, N., Rhim, H., Nah, S.Y., Kang, L.W., 2015. Structure of
369 ginseng major latex-like protein 151 and its proposed lysophosphatidic acid-binding mechanism.
370 Acta Crystallogr. Sect. D Biol. Crystallogr. 71, 1039–1050.
371 https://doi.org/10.1107/S139900471500259X
372 Chruszcz, M., Ciardiello, M.A., Osinski, T., Majorek, K.A., Giangrieco, I., Font, J., Breiteneder, H.,
373 Thalassinos, K., Minor, W., 2013. Structural and bioinformatic analysis of the kiwifruit allergen
374 Act d 11, a member of the family of ripening-related proteins. Mol. Immunol. 56, 794–803.
375 https://doi.org/10.1016/j.molimm.2013.07.004
376 D'Avino, R., Bernardi, M.L., Wallner, M., Palazzo, P., Camardella, L., Tuppo, L., Alessandri, C.,
377 Breiteneder, H., Ferreira, F., Ciardiello, M.A., Mari, A., 2011. Kiwifruit Act d 11 is the first
378 member of the ripening-related protein family identified as an allergen. Allergy Eur. J. Allergy
379 Clin. Immunol. 66, 870–877. https://doi.org/10.1111/j.1398-9995.2011.02555.x
380 Fernandes, H., Michalska, K., Sikorski, M., Jaskolski, M., 2013. Structural and functional aspects of PR-
381 10 proteins. FEBS J. 280, 1169–1199. https://doi.org/10.1111/febs.12114

382 Goto, J., Iwabuchi, A., Yoshihara, R., Kodama, N., Matsui, T., Hirota, M., Eun, H., Inui, H., 2019.
383 Uptake mechanisms of polychlorinated biphenyls in *Cucurbita pepo* via xylem sap containing
384 major latex-like proteins. Environ. Exp. Bot. 162, 399–405.
385 https://doi.org/10.1016/j.envexpbot.2019.03.019
386 Hashimoto, Y., 2005. Dieldrin Residue in the Soil and Cucumber from Agricultural Field in Tokyo. J.
387 Pestic. Sci. 30, 397–402. https://doi.org/10.1584/jpestics.30.397
388 Hawker, D.W., Connell, D.W., 1988. Octanol-Water Partition Coefficients of Polychlorinated Biphenyl
389 Congeners. Environ. Sci. Technol. 22, 382–387. https://doi.org/10.1021/es00169a004
390 Inui, H., Sawada, M., Goto, J., Yamazaki, K., Kodama, N., Tsuruta, H., Eun, H., 2013. A Major Latex-
391 Like Protein Is a Key Factor in Crop Contamination by Persistent Organic Pollutants. Plant Physiol.
392 161, 2128–2135. https://doi.org/10.1104/pp.112.213645
393 Ishibashi, M., Nabe, T., Nitta, Y., Tsuruta, H., Iduhara, M., Uno, Y., 2017. Analysis of major paralogs
394 encoding the Fra a 1 allergen based on their organ-specificity in *Fragaria* × *ananassa*. Plant Cell
395 Rep. 37, 411–424. https://doi.org/10.1007/s00299-017-2237-6
396 Iwabuchi, A., Katte, N., Suwa, M., Goto, J., Inui, H., 2020. Factors regulating the differential uptake of
397 persistent organic pollutants in cucurbits and non-cucurbits. J. Plant Physiol. 245, 1–7.
398 https://doi.org/10.1016/j.jplph.2019.153094
399 Kondoh, Y., Honda, K., Osada, H., 2015. Construction and Application of a Photo-Cross-Linked
400 Chemical Array. Methods Mol. Biol. 1263, 29–41. https://doi.org/10.1007/978-1-4939-2269-7
401 Maeda, K., Nakajima, Y., Motoyama, T., Kondoh, Y., Kawamura, T., Kanamaru, K., Ohsato, S.,
402 Nishiuchi, T., Yoshida, M., Osada, H., Kobayashi, T., Kimura, M., 2017. Identification of a
403 trichothecene production inhibitor by chemical array and library screening using trichodiene
404 synthase as a target protein. Pestic. Biochem. Physiol. 138, 1–7.
405 https://doi.org/10.1016/j.pestbp.2017.03.006

406 Markovic´-Housley, Z., Massimo, D., Doriano, L., Edda, von R.-L., Stephan, C., Markus, S., Ferreira,
407 F., Scheiner, O., Breiteneder, Heimo, 2003. Crystal Structure of a Hypoallergenic Isoform of the
408 Major Birch Pollen Allergen Bet v 1 and its Likely Biological Function as a Plant Steroid Carrier. J.
409 Mol. Biol. 2836, 123–133. https://doi.org/10.1016/S0022-2836(02)01197-X
410 Matsuo, S., Yamazaki, K., Gion, K., Eun, H., Inui, H., 2011. Structure-selective accumulation of
411 polychlorinated biphenyls in *Cucurbita pepo*. J. Pestic. Sci. 36, 363–369.
412 https://doi.org/10.1584/jpestics.G11-03
413 Mattina, M.I., Eitzer, B.D., Iannucci-Berger, W., Lee, W.Y., White, J.C., 2004. Plant uptake and
414 translocation of highly weathered, soil-bound technical chlordane residues: Data from field and
415 rhizotron studies. Environ. Toxicol. Chem. 23, 2756–2762. https://doi.org/10.1897/03-570
416 Miller, M.M., Wasik, S.P., Huang, G.L., Shlu, W.Y., Mackay, D., 1985. Relationships between Octanol-
417 Water Partition Coefficient and Aqueous Solubility. Environ. Sci. Technol. 19, 522–529.
418 https://doi.org/10.1021/es00136a007
419 Murano, H., Otani, T., Makino, T., Seike, N., 2009. Effects of the application of carbonaceous adsorbents
420 on pumpkin (*Cucurbita maxima*) uptake of heptachlor epoxide in soil. Soil Sci. Plant Nutr. 55, 325–
421 332. https://doi.org/10.1111/j.1747-0765.2009.00361.x
422 Murano, H., Otani, T., Seike, N., 2010. Dieldrin-dissolving abilities of the xylem saps of several plant
423 families, particularly *Cucurbita pepo* L. Environ. Toxicol. Chem. 29, 2269–2277.
424 https://doi.org/10.1002/etc.288
425 Namiki, S., Otani, T., Motoki, Y., Seike, N., Iwafune, T., 2018. Differential uptake and translocation of
426 organic chemicals by several plant species from soil. J. Pestic. Sci. 43, 96–107.
427 https://doi.org/10.1584/jpestics.D17-088

Otani, T., Seike, N., Sakata, Y., 2007. Differential uptake of dieldrin and endrin from soil by several plant families and *Cucurbita* genera. Soil Sci. Plant Nutr. 53, 86–94. https://doi.org/10.1111/j.1747-0765.2007.00102.x

Pasternak, O., Bujacz, G.D., Fujimoto, Y., Hashimoto, Y., Jelen, F., Otlewski, J., Sikorski, M.M., Jaskolski, M., 2006. Crystal Structure of Vigna radiata Cytokinin-Specific Binding Protein in Complex with Zeatin. Plant Cell 18, 2622–2634. https://doi.org/10.1105/tpc.105.037119

Radauer, C., Breiteneder, H., 2007. Evolutionary biology of plant food allergens. J. Allergy Clin. Immunol. 120, 518–525. https://doi.org/10.1016/j.jaci.2007.07.024

Schneider, C.A., Rasband, W.S., Eliceiri, K.W., 2012. NIH Image to ImageJ: 25 years of image analysis. Nat. Methods 9, 671–675. https://doi.org/10.1038/nmeth.2089

Shen, H., Huang, Y., Wang, R., Zhu, D., Li, W., Shen, G., Wang, B., Zhang, Y., Chen, Y., Lu, Y., Chen, H., Li, T., Sun, K., Li, B., Liu, W., Liu, J., Tao, S., 2013. Global atmospheric emissions of polycyclic aromatic hydrocarbons from 1960 to 2008 and future predictions. Environ. Sci. Technol. 47, 6415–6424. https://doi.org/10.1021/es400857z

Shi, X., Zha, J., Wen, B., Zhang, S., 2019. Diastereoisomer-specific neurotoxicity of hexabromocyclododecane in human SH-SY5Y neuroblastoma cells. Sci. Total Environ. 686, 893–902. https://doi.org/10.1016/j.scitotenv.2019.06.008

Xiao, P., Mori, T., Kamei, I., Kiyota, H., Takagi, K., Kondo, R., 2011. Novel metabolic pathways of organochlorine pesticides dieldrin and aldrin by the white rot fungi of the genus *Phlebia*. Chemosphere 85, 218–224. https://doi.org/10.1016/j.chemosphere.2011.06.028

Yamazaki, K., Tsuruta, H., Inui, H., 2015. Different uptake pathways between hydrophilic and hydrophobic compounds in lateral roots of *Cucurbita pepo*. J. Pestic. Sci. 40, 99–105. https://doi.org/10.1584/jpestics.D14-081

452 **Figure legends**

453 Figure 1. Structures of amisulbrom (the fungicide Raimei) (A) and pyrifluquinazon (the insecticide
454 Colt) (B).

455

456 Figure 2. Competitive binding of major latex-like proteins (MLPs) to pyrene by amisulbrom and
457 pyrifluquinazon.
458 The 0.2 mg/mL recombinant MLP-PG1 (A) and MLP-GR3 (B) were incubated with 10 μM pyrene
459 and 25 μM or 50 μM amisulbrom and pyrifluquinazon for 1 h at 20°C. The fluorescence of pyrene
460 was measured. –, the incubation without MLPs or pesticides; +, the incubation with 0.2 mg/mL MLPs
461 or 25 μM pesticides; ++, the incubation with 50 μM pesticides. The white, gray, and black bars
462 represent control (no pesticides), + (25 μM pesticides), and ++ (50 μM pesticides), respectively. Error
463 bars indicate standard deviations (n = 3–8). Asterisks indicate significant differences compared to the
464 fluorescence incubated with MLPs without pesticides (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

465

466 Figure 3. Competitive binding of major latex-like proteins (MLPs) to dieldrin by amisulbrom (A) and
467 pyrifluquinazon (B).
468 The 0.1 mg/mL recombinant MLPs were incubated with 0.2 mM amisulbrom (A) and pyrifluquinazon
469 (B) for 2 h at 4°C. The magnetic beads binding 100 mM dieldrin were added and incubated for 4 h at
470 4°C. After the heat elution, samples were subjected to SDS-PAGE, and MLPs were detected by silver
471 staining. C; beads without dieldrin, D; dieldrin-binding beads; –, the incubation with magnetic beads
472 without binding of dieldrin or without pesticides; +, the incubation with magnetic beads binding
473 dieldrin or with 0.2 mM pesticides. Band intensities were quantified by ImageJ.

474

475 Figure 4. Pyrene concentration in xylem sap from zucchini plants under the treatment of Raimei (A)
476 and Colt (B).
477 The *Cucurbita pepo* cultivar 'Magda' was cultivated in the contaminated soil with pyrene (1.25
478 mmol/kg) for 27 days at 26°C under a 16/8 h light/dark cycle. –, the cultivation in the non-
479 contaminated soil with pyrene or the non-treatment of pesticides; +, the cultivation in the contaminated
480 soil with pyrene or the treatment of pesticides at usual dose; +++, the treatment of pesticides at the
481 triple dose. The white, gray, and black bars represent control (no pesticides), + (the treatment of
482 pesticides at usual dose), and +++ (the treatment of pesticides at the triple dose), respectively. Error
483 bars indicate standard deviations (A, n = 5–12, B, n = 6–7). Asterisks indicate significant differences
484 compared to pyrene concentration cultivated in the contaminated soil with pyrene without the
485 treatment of pesticides (*, $p < 0.05$; ***, $p < 0.001$).
486
487 Figure 5. Dieldrin concentration in xylem sap from zucchini plants under the treatment of Raimei and
488 Colt.
489 The *Cucurbita pepo* cultivar 'Raven' was cultivated in the contaminated soil with dieldrin (12.5
490 μmol/kg) for 27 days at 26°C under a 16/8 h light/dark cycle. –, the cultivation in the non-contaminated
491 soil with dieldrin or the non-treatment of pesticides; +, the cultivation in the contaminated soil with
492 dieldrin or the treatment of pesticides at usual dose; +++, the treatment of pesticides at the triple dose.
493 The white, gray, and black bars represent control (no pesticides), + (the treatment of pesticides at usual
494 dose), and +++ (the treatment of pesticides at the triple dose), respectively. Error bars indicate standard
495 deviations (n = 8–19). Asterisks indicate significant differences compared to dieldrin concentration
496 cultivated in the contaminated soil with dieldrin without the treatment of pesticides (*, $p < 0.05$).
497

498 Supplementary Figure 1. Competitive binding of major latex-like proteins (MLPs) to pyrene by
499 amisulbrom and pyrifluquinazon.
500 The 10 μM pyrene and 5 μM or 50 μM amisulbrom and pyrifluquinazon were incubated without
501 recombinant MLPs for 1 h at 20°C (A). The 0.2 mg/mL recombinant MLPs were incubated with 5 μM
502 or 50 μM amisulbrom and pyrifluquinazon without pyrene (B). The fluorescence of pyrene was
503 measured. –, the incubation without pesticides; +, the incubation with 5 μM pesticides; ++, the
504 incubation with 50 μM pesticides. The white, gray, and black bars represent control (no pesticides), +
505 (25 μM pesticides), and ++ (50 μM pesticides), respectively. Error bars indicate standard deviations
506 (n = 3–4).
507
508 Supplementary Figure 2. Competitive binding of major latex-like proteins (MLPs) to dieldrin by
509 amisulbrom (A) and pyrifluquinazon (B).
510 The 0.1 mg/mL recombinant MLPs were incubated with 0.2 mM amisulbrom (A) and pyrifluquinazon
511 (B) for 2 h at 4°C. The magnetic beads binding 100 mM dieldrin were added and incubated for 4 h at
512 4°C. Eluates at the eighth washing step were subjected to SDS-PAGE, and MLPs were detected by
513 silver staining. C; beads without dieldrin, D; dieldrin-binding beads; –, the incubation with magnetic
514 beads without binding of dieldrin or without pesticides; +, the incubation with magnetic beads binding
515 dieldrin or with 0.2 mM pesticides.
516
517 Supplementary Figure 3. Fresh weight of aerial parts of zucchini plants cultivated in the contaminated
518 soil with pyrene under the treatment of Raimei (A) and Colt (B).
519 The *Cucurbita pepo* cultivar 'Magda' was cultivated in the contaminated soil with pyrene (1.25
520 mmol/kg) for 27 days at 26°C under a 16/8 h light/dark cycle. Aerial parts were collected from each
521 plant, and the fresh weight of them was measured. –, the cultivation in the non-contaminated soil with

pyrene or the non-treatment of pesticides; +, the cultivation in the contaminated soil with pyrene or the treatment of pesticides at usual dose; +++, the treatment of pesticides at the triple dose. The white, gray, and black bars represent control (no pesticides), + (the treatment of pesticides at usual dose), and +++ (the treatment of pesticides at the triple dose), respectively. Error bars indicate standard deviations (A, n = 5–12, B, n = 6–7).

Supplementary Figure 4. Fresh weight of aerial parts of zucchini plants cultivated in the contaminated soil with dieldrin under the treatment of Raimei and Colt.

The *Cucurbita pepo* cultivar 'Raven' was cultivated in the contaminated soil with dieldrin (12.5 μmol/kg) for 27 days at 26°C under a 16/8 h light/dark cycle. Aerial parts were collected from each plant, and the fresh weight of them was measured. –, the cultivation in the non–contaminated soil with dieldrin or the non-treatment of pesticides; +, the cultivation in the contaminated soil with dieldrin or the treatment of pesticides at usual dose; +++, the treatment of pesticides at the triple dose. The white, gray, and black bars represent control (no pesticides), + (the treatment of pesticides at usual dose), and +++ (the treatment of pesticides at the triple dose), respectively. Error bars indicate standard deviations (n = 8–19).

Supplementary Figure 5. The change in the amount of major latex-like proteins by the treatment of Colt.

The *Cucurbita pepo* cultivar 'Raven' was cultivated in the contaminated soil with dieldrin (12.5 μmol/kg) for 27 days at 26°C under a 16/8 h light/dark cycle. Roots were collected, and root proteins were extracted. They were subjected to SDS-PAGE, and reacted with anti-MLP-PG1 antibody and anti-MLP-GR3 antibody, respectively. –, the non-treatment of Colt; +++, the treatment of Colt at the triple dose. The white and black bars represent control (no pesticides) and +++ (the treatment of

546 pesticides at the triple dose), respectively. Error bars indicate standard deviations (n = 6). The band

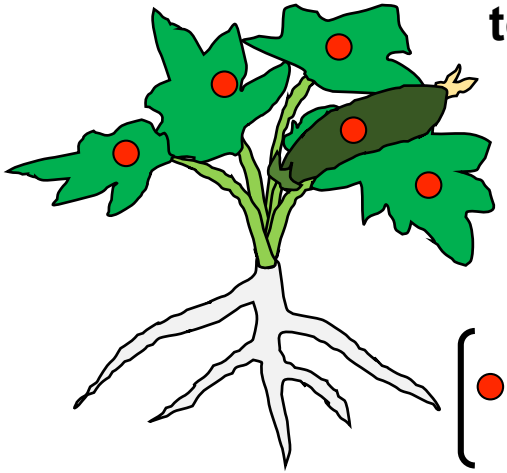
547 intensities were quantified by ImageJ.

Pesticides
(Raimei and Colt)

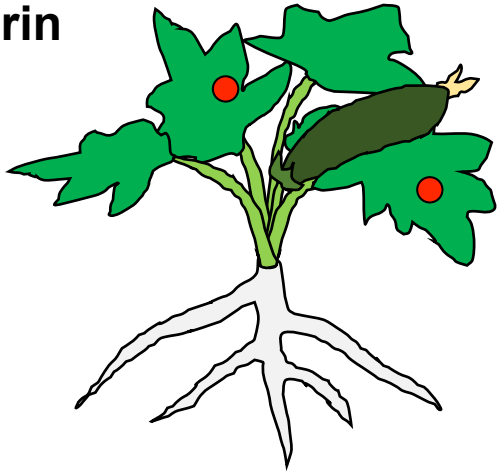
High
toxicity

Less
toxicity

Binding of MLPs
to pyrene and dieldrin

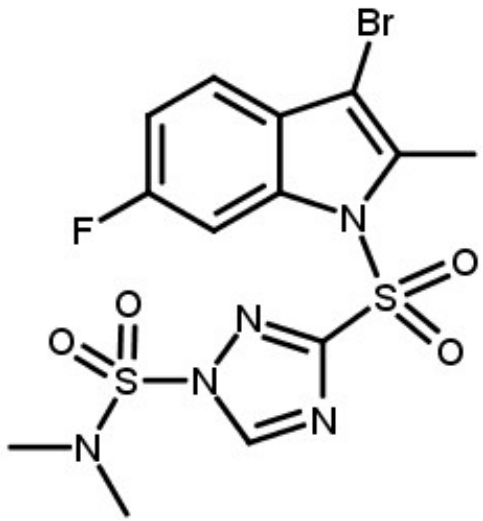


Pyrene
Dieldrin



Zucchini
(Cucurbita pepo)

(A)



(B)

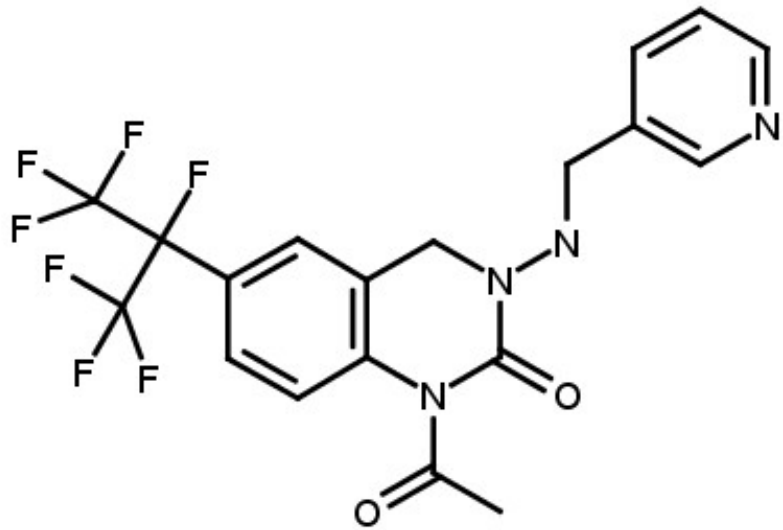


Figure 1

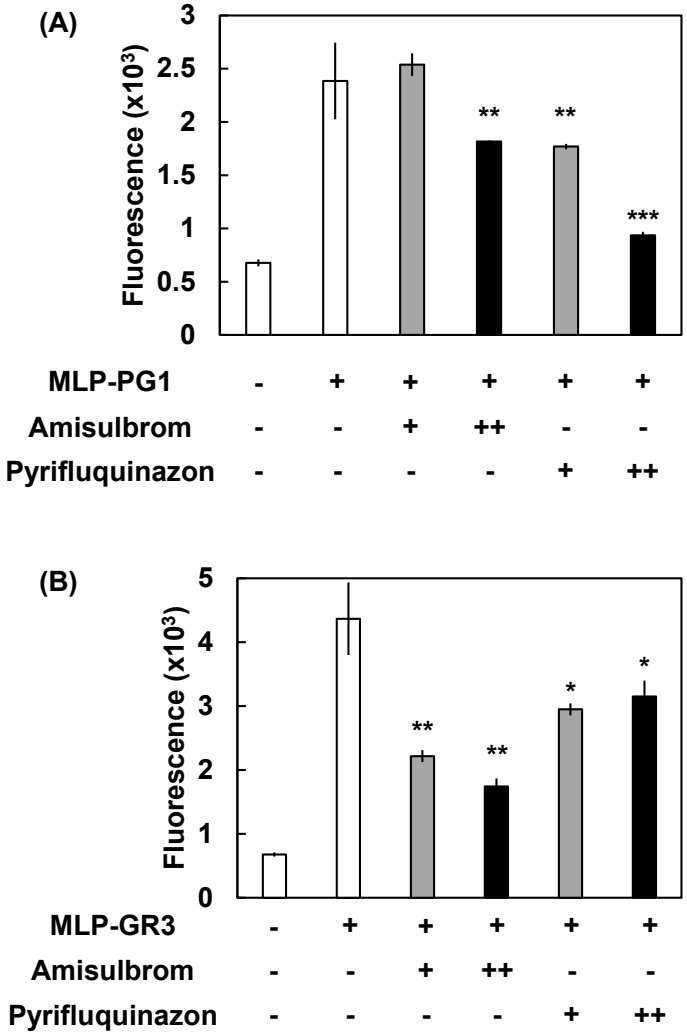

(A)
Fluorescence (x10³)
3
2.5
2
1.5
1
0.5
0
**
**
***
MLP-PG1 - + + + + +
Amisulbrom - - + ++ - -
Pyrifluquinazon - - - - + ++
(B)
Fluorescence (x10³)
5
4
3
2
1
0
**
**
*
*
MLP-GR3 - + + + + +
Amisulbrom - - + ++ - -
Pyrifluquinazon - - - - + ++

Figure 2

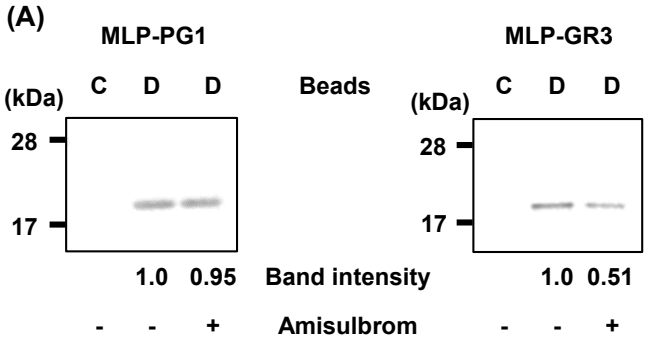
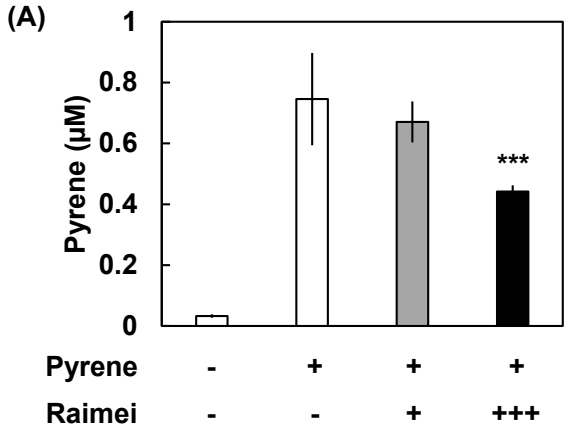

(A)
MLP-PG1
MLP-GR3
(kDa)
C D D
Beads
(kDa)
C D D
28
17
28
17
1.0 0.95
Band intensity
1.0 0.51
- - +
Amisulbrom
- - +
(B)
MLP-PG1
MLP-GR3
(kDa)
C D D
Beads
(kDa)
C D D
28
17
28
17
1.0 0.81
Band intensity
1.0 0.22
- - +
Pyrifluquinazon
- - +

Figure 3


(A)
Pyrene (μM)
1
0.8
0.6
0.4
0.2
0
***
Pyrene - + + +
Raimei - - + +++

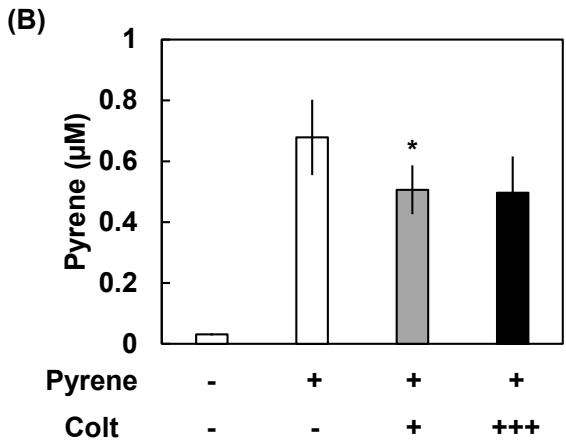

(B)
Pyrene (μM)
1
0.8
0.6
0.4
0.2
0
*
Pyrene - + + +
Colt - - + +++

Figure 4

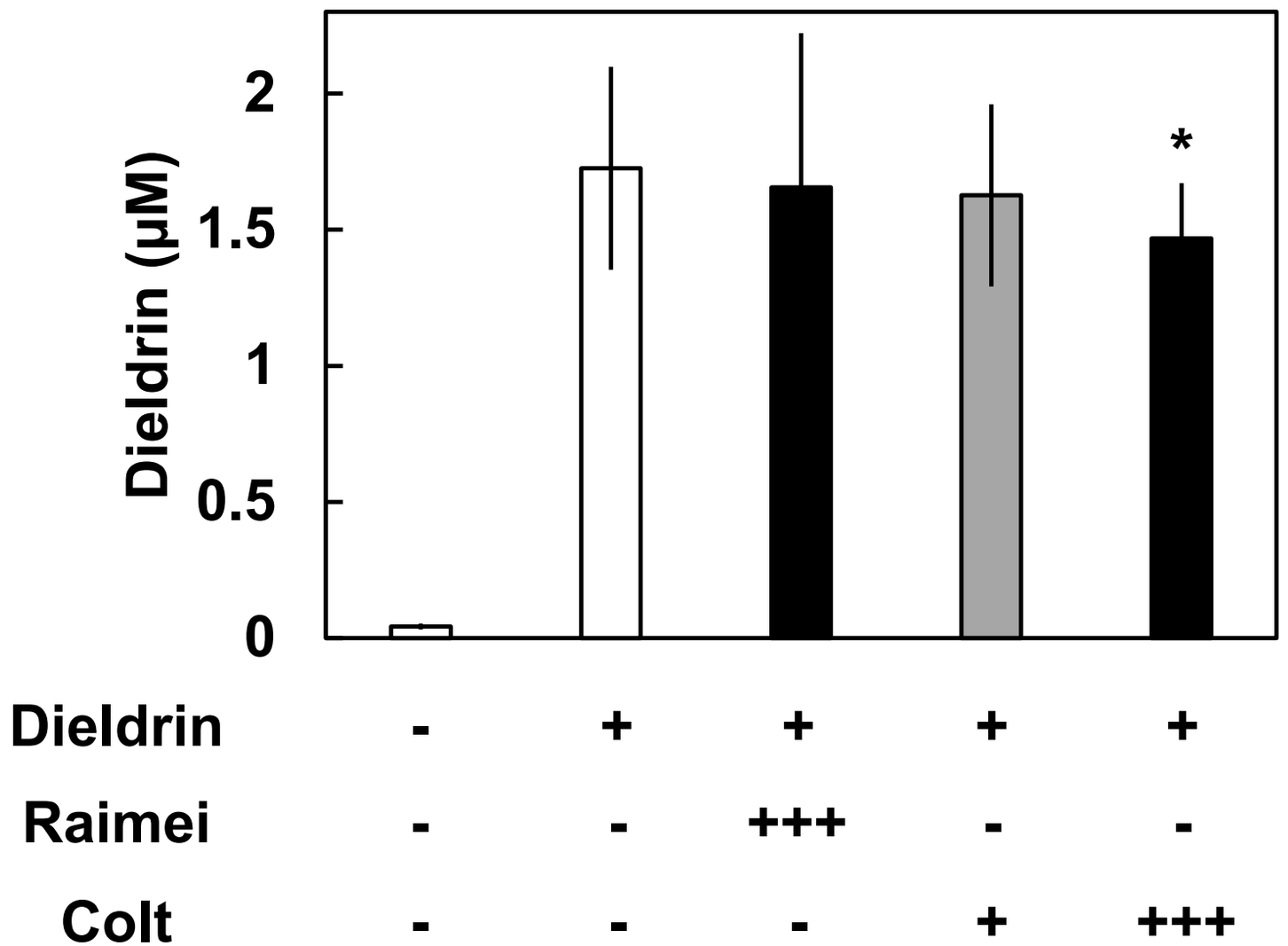


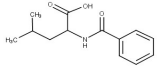
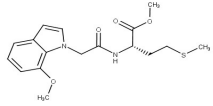
Figure 5

Table 1. MLP-binding compounds in chemical array screening.

MLP	Number of compounds			
	Binding affinity			Total
	+	++	+++	
MLP-PG1	**98**	**48**	**20**	**166**
MLP-GR3	**99**	**55**	**22**	**176**

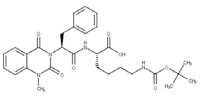
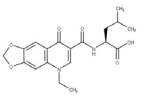
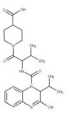
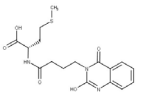
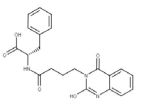
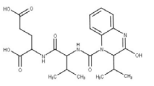
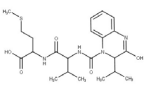
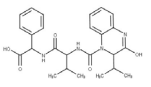
The chemical arrays were incubated with 4 μM of recombinant MLP-PG1 and MLP-GR3, sequentially anti-His antibody, and secondary antibody with Cy5. The fluorescence of Cy5 was detected. For the reference arrays, the chemical arrays were incubated only with anti-His antibody and Cy5-labeled secondary antibody. The Z-score of the difference in fluorescence intensities of each spot between the MLPs-treated arrays and the reference arrays was calculated (Inui et al. 2013). The marks +, ++, and +++ were defined as Z-score>1, >2, and >3, respectively.

Table 2. Compounds with an indole-like structure binding MLPs.

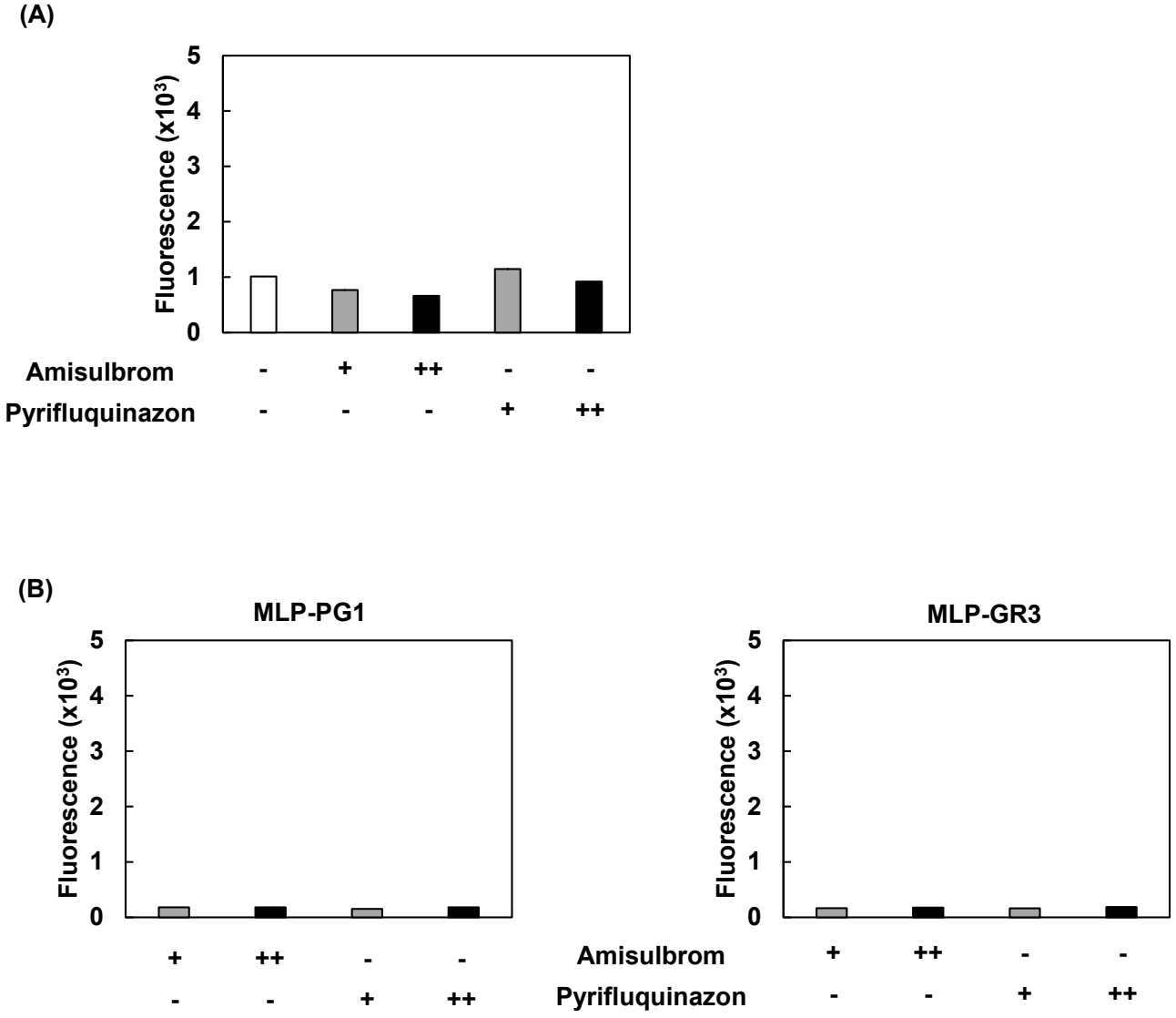
Name	Structure	Binding affinity	
		MLP-PG1	MLP-GR3
NPD9013		+	+
NPD14496		+	+++

By chemical array screening, 242 MLP-binding compound candidates were identified. Publications about selected compounds were searched by SciFinder, and 30 compounds related to plants, such as plant hormones and secondary metabolites, were identified. The marks + and +++ were described in the legend of Table 1.

Table 3. Compounds containing quinazoline-, quinoxaline-, and quinoline-like structures with a high affinity to bind MLPs.

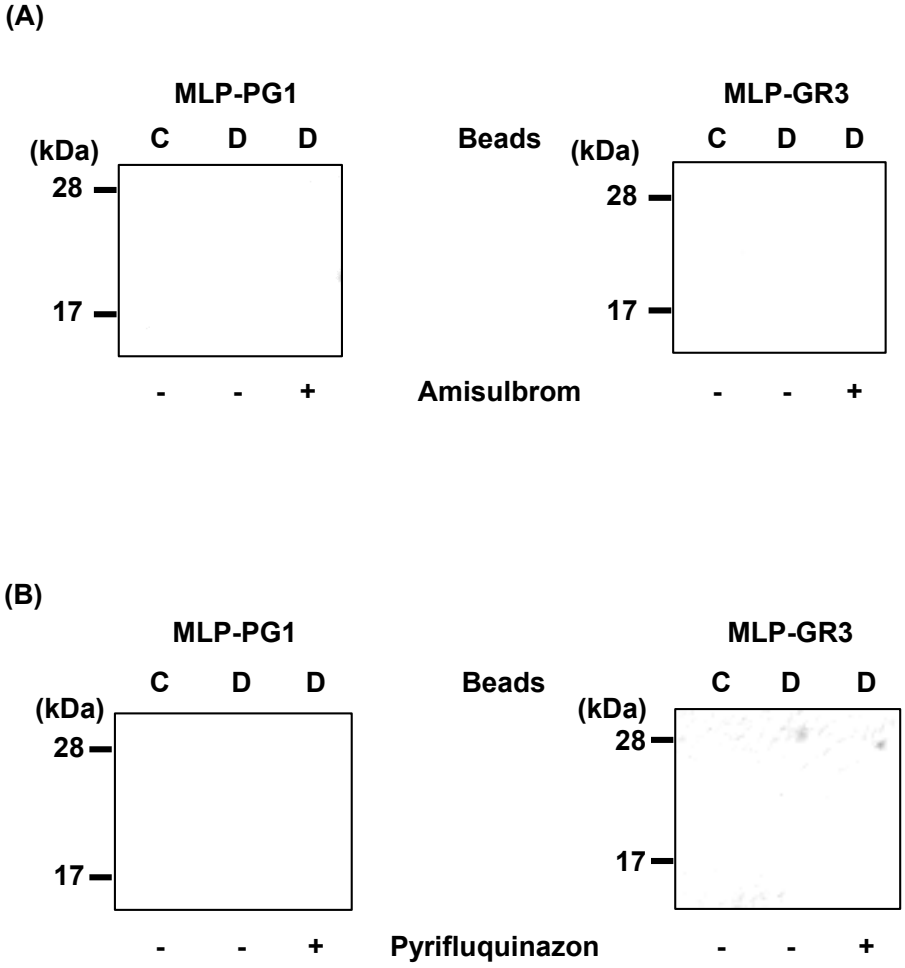
Name	Structure	Binding affinity	
		MLP-PG1	MLP-GR3
NPD8605		++	++
NPD12285		++	++
NPD13695		++	++
NPD13698		+++	++
NPD13699		++	++
NPD13700		+++	+++
NPD13711		+++	++
NPD13717		++	+++
NPD13718		++	+++
NPD13719		+++	+++

By chemical array screening, 242 compounds binding MLPs were identified. The 37 compounds with the high affinity (++ and +++) to bind both MLPs were selected. There were 10 compounds with quinazoline-like, quinoxaline-like, and quinoline-like structures in 37 compounds. The marks ++ and +++ were described in the legend of Table 1.



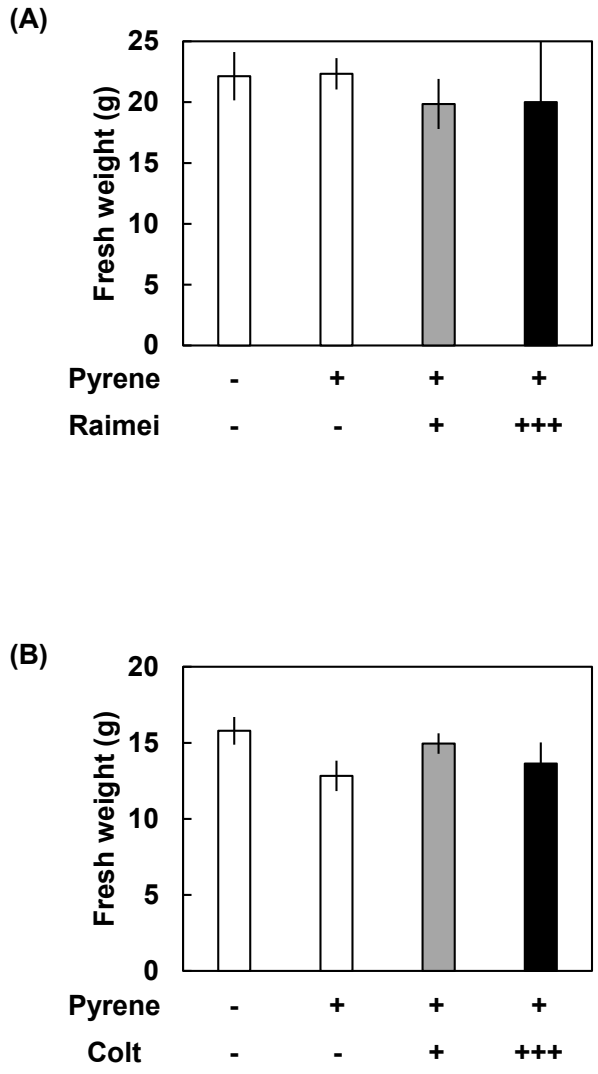
Supplementary Figure 1. Competitive binding of major latex-like proteins (MLPs) to pyrene by amisulbrom and pyrifluquinazon.

The 10 μM pyrene and 5 μM or 50 μM amisulbrom and pyrifluquinazon were incubated without recombinant MLPs for 1 h at 20° C (A). The 0.2 mg/mL recombinant MLPs were incubated with 5 μM or 50 μM amisulbrom and pyrifluquinazon without pyrene (B). The fluorescence of pyrene was measured. –, the incubation without pesticides; +, the incubation with 5 μM pesticides; ++, the incubation with 50 μM pesticides. The white, grey, and black bars represent control (no pesticides), + (25 μM pesticides), and ++ (50 μM pesticides), respectively. Error bars indicate standard deviations (n = 3–4).



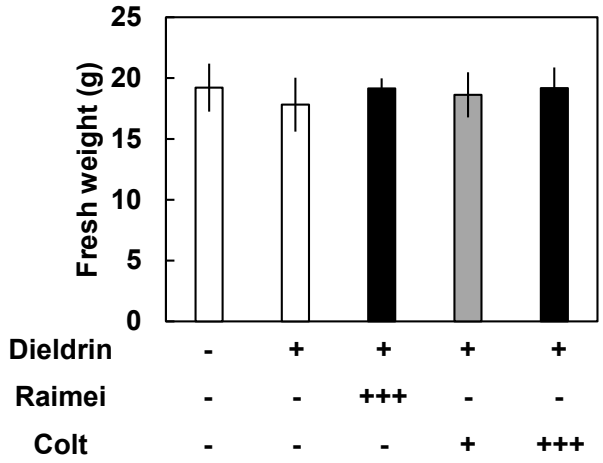
Supplementary Figure 2. Competitive binding of major latex-like proteins (MLPs) to dieldrin by amisulbrom (A) and pyrifluquinazon (B).

The 0.1 mg/mL recombinant MLPs were incubated with 0.2 mM amisulbrom (A) and pyrifluquinazon (B) for 2 h at 4° C. The magnetic beads binding 100 mM dieldrin were added and incubated for 4 h at 4° C. Eluates at the eighth washing step were subjected to SDS-PAGE, and MLPs were detected by silver staining. C; beads without dieldrin, D; dieldrin-binding beads; –, the incubation with magnetic beads without binding of dieldrin or without pesticides; +, the incubation with magnetic beads binding dieldrin or with 0.2 mM pesticides.



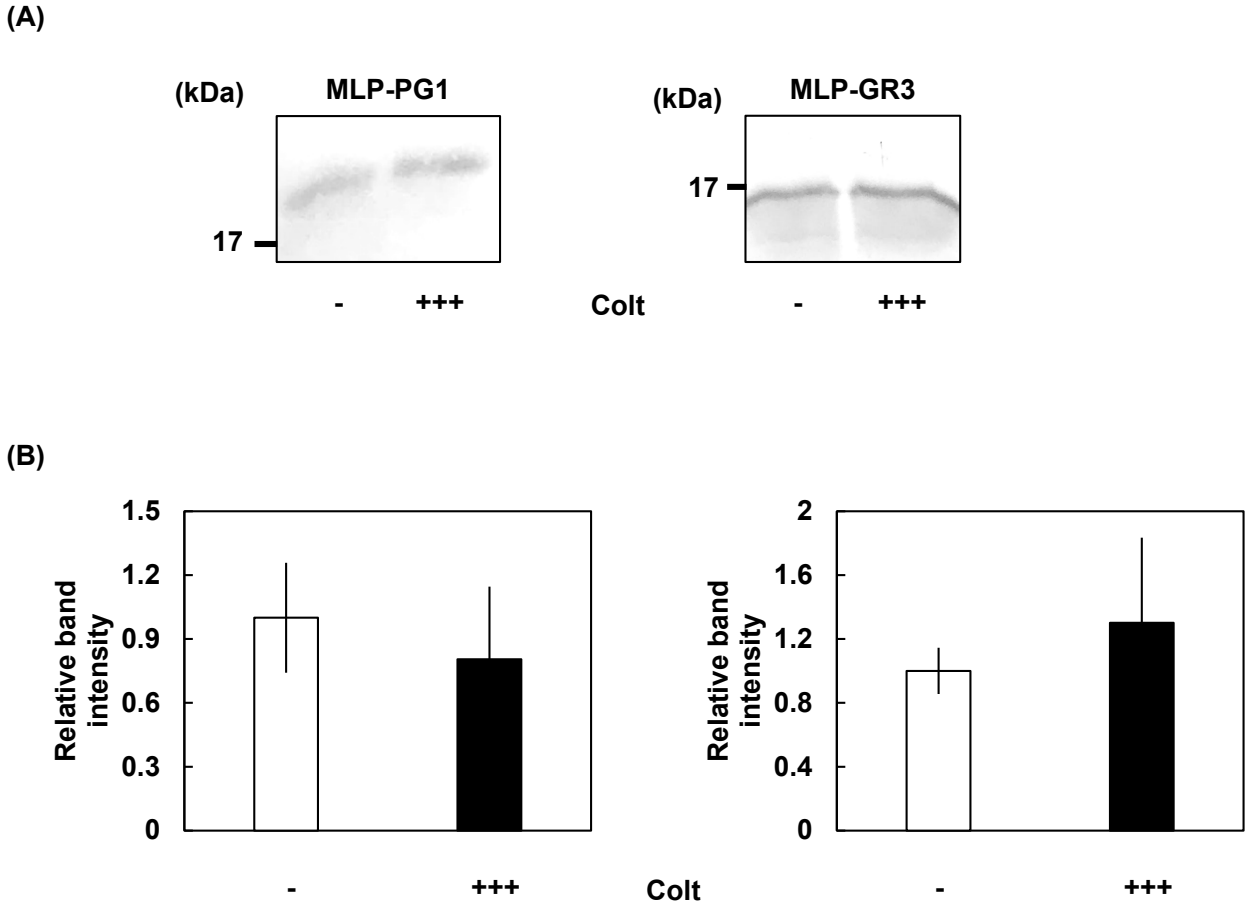
Supplementary Figure 3. Fresh weight of aerial parts of zucchini plants cultivated in the contaminated soil with pyrene under the treatment of Raimei (A) and Colt (B).

The *Cucurbita pepo* cultivar 'Magda' was cultivated in the contaminated soil with pyrene (1.25 mmol/kg) for 27 days at 26° C under a 16/8 h light/dark cycle. Aerial parts were collected from each plant, and the fresh weight of them was measured. –, the cultivation in the non-contaminated soil with pyrene or the non-treatment of pesticides; +, the cultivation in the contaminated soil with pyrene or the treatment of pesticides at usual dose; +++, the treatment of pesticides at the triple dose. The white, grey, and black bars represent control (no pesticides), + (the treatment of pesticides at usual dose), and +++ (the treatment of pesticides at the triple dose), respectively. Error bars indicate standard deviations (A, n = 5–12, B, n = 6–7).



Supplementary Figure 4. Fresh weight of aerial parts of zucchini plants cultivated in the contaminated soil with dieldrin under the treatment of Raimei and Colt.

The *Cucurbita pepo* cultivar 'Raven' was cultivated in the contaminated soil with dieldrin (12.5 μmol/kg) for 27 days at 26° C under a 16/8 h light/dark cycle. Aerial parts were collected from each plant, and the fresh weight of them was measured. –, the cultivation in the non–contaminated soil with dieldrin or the non-treatment of pesticides; +, the cultivation in the contaminated soil with dieldrin or the treatment of pesticides at usual dose; +++, the treatment of pesticides at the triple dose. The white, grey, and black bars represent control (no pesticides), + (the treatment of pesticides at usual dose), and +++ (the treatment of pesticides at the triple dose), respectively. Error bars indicate standard deviations (n = 8–19).



Supplementary Figure 5. The change in the amount of major latex-like proteins by the treatment of Colt. The *Cucurbita pepo* cultivar 'Raven' was cultivated in the contaminated soil with dieldrin (12.5 μmol/kg) for 27 days at 26° C under a 16/8 h light/dark cycle. Roots were collected, and root proteins were extracted. They were subjected to SDS-PAGE, and reacted with anti-MLP-PG1 antibody and anti-MLP-GR3 antibody, respectively. –, the non-treatment of Colt; +++, the treatment of Colt at the triple dose. The white and black bars represent control (no pesticides) and +++ (the treatment of pesticides at the triple dose), respectively. Error bars indicate standard deviations (n = 6). The band intensities were quantified by ImageJ.