

Antianaphylactic and Antipruritic Effects of the Flowers of *Impatiens textori* MiQ.

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The anti-anaphylactic and anti-pruritic activities of a 35% EtOH extract (IT) of the flowers of *Impatiens textori* MiQ. were investigated by *in vivo* assay. IT and apigenin (1), apigenin 7-glucoside (2) and luteolin (3), principal compounds from IT, inhibited compound 48/80 (COM)-induced by blood pressure (BP) decrease, which was an immunoglobulin (Ig)E-independent anaphylaxis-like response. Compounds 1–3 all inhibited BP decrease induced by IgE-dependent anaphylaxis. Furthermore, IT also inhibited the blood flow (BF) decrease induced by antigen-induced anaphylaxis in actively sensitized mice. IT showed a significant inhibitory effect on scratching behavior induced by COM without a central depressant. IT also significantly inhibited platelet activating factor (PAF)- and serotonin (5-HT)-induced scratching behavior and mitigated protease (PA)-induced scratching behavior. These findings showed that the flowers of *I. textori* can be utilized as an anti-anaphylactic and anti-pruritic agent in addition to the traditional applications of this plant.

Key words *Impatiens textori*; apigenin; luteolin; apigenin 7-glucoside; anti-anaphylaxis; anti-pruritus

Whole plants of *Impatiens textori* have been used for detoxication and treatment of carbuncle and contusion in Chinese medicine.¹⁾ Several flavonoid derivatives also been isolated from the whole plants,^{2–4)} however, there has been no report on the anti-allergic effects of the flowers of *I. textori*. In a previous study,⁵⁾ we found that a 35% EtOH extract (IT) of flowers of *I. textori* and isolated compounds significantly suppressed the decrease in blood pressure (BP) induced by exogenous platelet activating factor (PAF), which is a mediator of anaphylaxis, inflammation and pruritus.

Here we report the inhibitory effects of IT and its principal compounds against compound 48/80 (COM)-induced BP decrease, antigen-induced anaphylaxis in actively sensitized mice (antigen-induced anaphylaxis)^{6–10)} and pruritus using our previously reported *in vivo* assay system.¹¹⁾

MATERIALS AND METHODS

Animals Male ddY mice (SPF grade), 5 or 6 weeks old, were obtained from Japan SLC (Shizuoka, Japan) and housed at 24±2 °C. Food and water were available *ad libitum*. All experiments were performed in accordance with the Guidelines for Animal Experiments of Mukogawa Women's University.

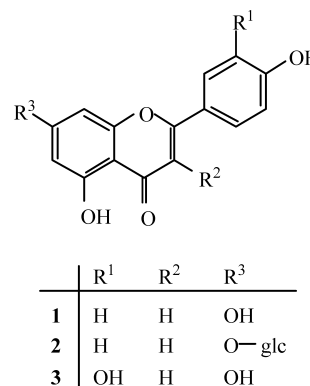
Plant Materials and Extraction The flowers of *I. textori* were collected in Gifu (Japan) in September, 1998. The voucher samples are kept in our university medicinal plant garden. Fresh flowers (2.0 kg) of *I. textori* were extracted twice with 35% EtOH for 1 week at room temperature and filtered. The obtained filtrate was completely evaporated *in vacuo*, giving a residue (designated IT) of 15.5 g.

Materials Apigenin (1), apigenin 7-glucoside (2) and luteolin (3) were isolated from IT as previously reported.⁵⁾ Other agents were obtained as follows: compound 48/80 (COM), Sigma Chemical; disodium cromoglycate (DSCG), Biomol Research Laboratories Inc.; diphenhydramine hydrochloride (DPH), Nacalai Tesque; platelet activating factor (PAF), Funakoshi Co., Ltd.; *rac* 3-(*N*-n-octadecylcarbamoyloxy)-2-methoxypropyl 2-thiazoliethyl phosphate (CV-3988)

and hen egg-white lysozyme (HEL), Wako Pure Chemical Industries, Ltd.; serotonin hydrochloride (5-HT), Tokyo Kasei Kogyo Co., Ltd.; ketanserin tartrate (KS), Research Biochemicals Inc.; protease (PA), Worthington Biochemical Co; diazepam, Takeda Chemical Industries, Ltd. Each test sample or agent used for the present experiments was dissolved in 100 µl water per 10 g body weight in the case of oral administration (*p.o.*), and in 10 µl saline per 10 g body weight in the case of intravenous (*i.v.*), intraperitoneal (*i.p.*) and subcutaneous (*s.c.*) administration before use in the biological experiments.

HEL-Sensitization and Challenge Immunization with HEL was performed as previously described.^{7,10)} Male ddY mice 5 weeks of age were sensitized *i.p.* with 50 µg of HEL on day 0. To provoke IgE-dependent anaphylaxis, on day 9 each HEL-sensitized mouse was challenged by *i.v.* injection into the tail vein with HEL 1 µg/saline 30 µl for BP monitoring⁷⁾ or HEL 10 µg/saline 30 µl for BF monitoring.¹⁰⁾

Blood Pressure (BP) Measurement The BP of the mice was measured using a BP monitoring system as previously reported.^{7,9)} The change in systolic BP of the tail artery of



Principal flavonoid compounds from flowers of *I. textori*

Chart 1

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unanaesthetized mice was measured every 2 min for 20 min immediately after challenge with HEL or COM using a nondirect-type BP monitor (MK-1030, Muromachi Kikai, Japan). Each mouse was placed on a holder in a measuring chamber kept at 36 °C throughout the pre-warming period for 15 min prior to the experiment. The normal BP (90–110 mmHg) of each mouse was measured 10 min before each experiment. The results were expressed as the percentage of normal BP at given times after this injection, because there were individual differences in the BP of normal mice. If the BP decreased below the range (30 mmHg) of the monitor level, we used the value equivalent to 30% of the normal BP.

Blood Flow (BF) Measurement The BF of the mice was measured using a BF monitoring system as previously reported.¹⁰⁾ The change in BF of the tail venous microcirculation of unanaesthetized mouse was measured every 2 min for 20 min immediately after challenge using a noncontact type laser-doppler blood flow meter (FLO-N1, Neoursience). Each mouse was placed on a holder in a measuring chamber kept at 36 °C throughout the pre-warming period for 15 min prior to the experiment. The normal BF of each HEL-sensitized mouse was measured for 10 min before challenge with HEL. The results were expressed as the percentage of normal BF at given times after challenge with HEL, because there were individual differences in the BF of normal mice. If a mouse died due to fatal shock after challenge during measurement, the BF was converted to 0%.

Assay of Inhibitory Effects on COM-Induced BP Decrease COM (1.5 mg/kg) was injected i.v. to provoke decrease of BP. IT (200 mg/kg) and compounds 1–3 (20 mg/kg) were individually administered *p.o.* 1 h before injection with COM. As a positive control, DSCG was administered i.v. at 10 mg/kg 1 h before injection with COM. As a control, water- or saline-treated mice were injected with COM alone. None of IT, compounds 1–3 and DSCG affected the BP in *p.o.* or i.v. administration to normal (non-treated) mice (data not shown).

Assay of Inhibitory Effects on Antigen-Induced Anaphylaxis The inhibitory effect on IgE-dependent anaphylaxis was investigated as previously reported using BP^{7–9)} or BF.¹⁰⁾ IT (200 mg/kg) and compounds 1–3 (20 mg/kg) were individually administered *i.p.* or *p.o.* 1 h before challenge with HEL. As a control, water-treated mice were challenged with HEL alone. None of IT and compounds 1–3 affected the BP or BF in *i.p.* or *p.o.* administration to HEL-sensitized mice without challenge (data not shown).

Measurement of Scratching Behavior The scratching behavior of the mice was measured using a previously reported method.¹¹⁾ The pruritogenic agent was injected *s.c.* into the base of the neck on the back of mice to provoke scratching behavior. The incidences of scratching behavior on the whole body were counted for 20 min. As a control, water-treated or saline-treated mice were injected with the pruritogenic agent alone to examine the incidence of scratching behavior. The normal group was administrated *i.v.* or *p.o.* with saline or water alone without injection of the pruritogenic agents. The results were expressed as the percentage of scratching behavior of the control group.

Assay of Inhibitory Effects on COM-Induced Scratching Behavior COM (3 mg/kg) was injected to provoke scratching behavior. IT (100 mg/kg *p.o.* or *i.v.*), compounds 2

(10 mg/kg *p.o.* or 10 µg/kg *i.v.*) and 3 (10 mg/kg *p.o.*) were individually administered 1 h before injection with COM. As a positive control, DSCG was administered *i.v.* at 10 mg/kg 1 h before injection with COM, and DPH was administered *i.p.* at 10 mg/kg 30 min before injection with COM.

Assay of Inhibitory Effects on PAF-, 5-HT- and PA-Induced Scratching Behavior PAF (10 µg/kg), 5-HT (10 µg/kg) and PA (1 mg/kg) were injected to provoke scratching behavior. IT was administered *i.v.* at 100 mg/kg 1 h before injection with PAF, 5-HT or PA. As a positive control, CV-3988 (10 mg/kg) and KS (2 mg/kg) were individually administered *i.v.* 30 min before injection with PAF or 5-HT.

Assay of Sedative Activity Using Locomotive Behavior Experiments The locomotive behavior of mice was measured as previously reported.¹²⁾ IT (100 mg/kg) and saline (control group) were individually administered *i.v.* 1 h before the experiment. As a positive control, diazepam (5 mg/kg) was administered *s.c.* 30 min before measuring locomotion. Locomotive activity was evaluated as the frequency at which a mouse crossed a line during the 20 min observation period.

Statistical Analysis Statistical analysis was performed with analysis of variance with nested model design, followed Dunnett's multiple comparison test coupled with Bonferroni inequality, which is a special statistical approach used to reduce error risk. Probability (*p*) values less than 0.05 were considered to be statistically significant.

RESULTS

Inhibitory Effects on COM-Induced BP Decrease Figure 1 shows the inhibitory effects of IT, the principal compounds of IT and DSCG on BP decrease induced by COM. IT (Fig. 1A), compounds 1–3 (Fig. 1B) and DSCG,¹³⁾ a classical anti-degranulating agent, (Fig. 1C) all significantly inhibited BP decrease. We have observed that COM also causes BF decrease. This result will be reported in due course together with the application of this assay method.

Inhibitory Effects on Antigen-Induced Anaphylaxis Since the principal compounds of IT significantly inhibited the IgE-independent anaphylaxis-like response, we further examined the inhibitory effects of these compounds on antigen-induced anaphylaxis. Figure 2 shows of inhibitory effects of compounds 1–3 on BP decrease induced by antigen-induced anaphylaxis. These compounds all significantly inhibited BP decrease. Figure 3 shows the inhibitory effects of IT and the principal compounds of IT on BF decrease induced by antigen-induced anaphylaxis. IT significantly inhibited BF decrease (Fig. 3A), but compounds 1–3 did not (Fig. 3B). The results were different for the anti-anaphylactic active compounds in the BP and BF monitoring assays.

Inhibitory Effects on COM-Induced Scratching Behavior The incidence of scratching behavior of the control group for 20 min is 599.9 ± 64.5 counts. Figure 4 shows the inhibitory effects of IT, the principal compounds of IT and the inhibitory agents on COM-induced scratching behavior. Incidence of scratching is shown as a percentage of the control. IT in *i.v.* administration significantly inhibited COM-induced scratching behavior. But IT in *p.o.* administration did not inhibit the scratching behavior. We examined the inhibitory effect of compound 2 in *i.v.* administration at

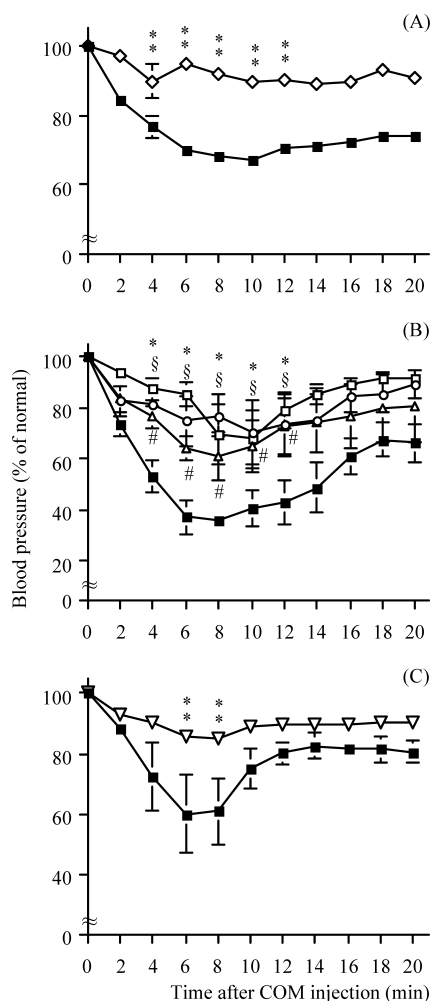


Fig. 1. Inhibitory Effects of IT, Principal Compounds and DSCG on COM-Induced BP Decrease

The mice were injected i.v. with COM without pretreatment (■: control) or following pretreatment *p.o.* with IT (A: ◇) of 200 mg/kg, and compound 1 (B: ○), 2 (B: △) and 3 (B: □) of 20 mg/kg, or following pretreatment i.v. with DSCG (C: ▽) of 10 mg/kg 1 h before injection of COM. All values are mean ± S.E.M. for 5 mice. * $p < 0.05$, ** $p < 0.01$ (IT, 1 or DSCG vs. control). # $p < 0.05$ (2 vs. control). § $p < 0.05$ (3 vs. control).

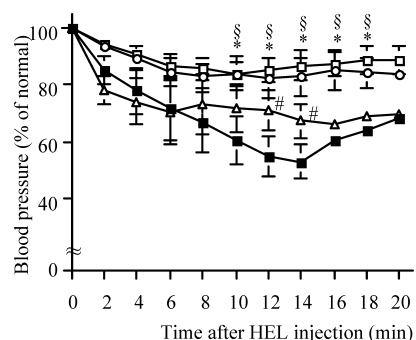


Fig. 2. Inhibitory Effects of Principal Compounds on BP Decrease Induced by Antigen-Induced Anaphylaxis

The HEL-sensitized mice were challenged i.v. with HEL without pretreatment (challenge only) (■: control) or following pretreatment *p.o.* with compound 1 (○), 2 (△) and 3 (□) of 20 mg/kg 1 h before challenge of HEL. All values are mean ± S.E.M. for 5 mice. * $p < 0.05$ (1 vs. control). # $p < 0.05$ (2 vs. control). § $p < 0.05$ (3 vs. control).

10 μ g/kg (the maximum dose considering the solubility in saline). Compound 2 did not show the inhibitory effect (data not shown). Compounds 1 and 3 could not be injected i.v.,

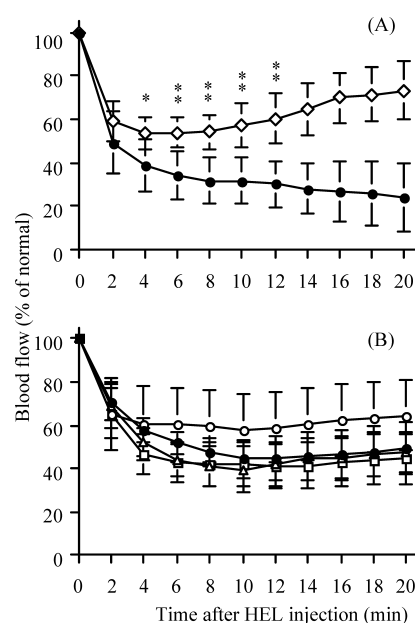


Fig. 3. Inhibitory Effects of IT and Principal Compounds on BF Decrease Induced by Antigen-Induced Anaphylaxis

The HEL-sensitized mice were challenged i.v. with HEL without pretreatment (challenge only) (●: control) or following pretreatment i.p. with IT of 200 mg/kg (A: ◇), or following pretreatment *p.o.* with compound 1 (B: ○), 2 (B: △) and 3 (B: □) of 20 mg/kg 1 h before challenge of HEL. All values are mean ± S.E.M. for 4 or 5 mice. * $p < 0.05$, ** $p < 0.01$ (IT vs. control).

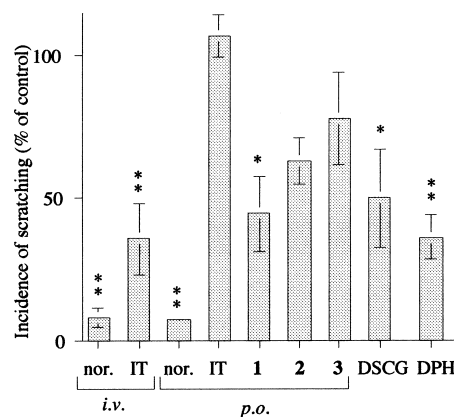


Fig. 4. Inhibitory Effects of IT and Principal Compounds and Agents on COM-Induced Scratching Behavior

The mice were injected s.c. with COM without pretreatment (control) or following pretreatment i.v. or *p.o.* with IT of 100 mg/kg, and compound 1, 2 and 3 of 10 mg/kg 1 h before injection, or following pretreatment i.v. or i.p. with DSCG and DPH of 10 mg/kg 1 h or 30 min before injection of COM. Normal group (nor.) was injected i.v. or *p.o.* with saline or water alone. All values are mean ± S.E.M. for 5 mice. * $p < 0.05$, ** $p < 0.01$ (compared with control). Data of compound 1 cited from previous report.¹²⁾

because they could not be dissolved in saline. We also examined the inhibitory effects of 2 and 3 in *p.o.* administration at dose of 10 mg/kg, because the anti-pruritic effect of 1 in *p.o.* administration was previously reported.¹²⁾ The results showed that 2 and 3 decreased COM-induced scratching behavior, but not significantly.

Inhibitory Effects on PAF-, 5-HT- and PA-Induced Scratching Behavior The incidence of scratching behavior of each control group for 20 min is as follows: PAF; 431.2 ± 99.8 counts, 5-HT; 410.6 ± 93.1 counts, PA; 472.7 ± 157.7 counts. As a normal, the mice were injected i.v. with saline alone. The incidence of scratching behavior of normal

Table 1. Inhibitory Effects of IT and Agents on PAF-, 5-HT- and Protease-Induced Scratching Behavior

Pruritogen (dose)	Treatment	Route	Time of administration before pruritogen (h)	Dose (mg/kg)	Incidence of scratching (% of control)
PAF (1 μ g/kg)	IT	i.v.	1	100	15.4 $\pm$ 4.0**
	1	<i>p.o.</i>	1	10	51.7 $\pm$ 9.6*
	CV-3988	i.v.	0.5	10	14.6 $\pm$ 4.8**
5-HT (10 μ g/kg)	IT	i.v.	1	100	23.1 $\pm$ 6.3*
	1	<i>p.o.</i>	1	10	38.8 $\pm$ 7.2*
	Ketanserin	i.v.	0.5	2	33.3 $\pm$ 12.5*
Protease (1 mg/kg)	IT	i.v.	1	100	49.0 $\pm$ 21.8

The mice were injected s.c. with COM-, PAF, 5-HT or PA without pretreatment (control) or following pretreatment i.v. with IT, CV-3988 and KS. All values are mean $\pm$ S.E.M. for 5 mice. * p <0.05, ** p <0.01 (compared with control). Data of compound **1** cited from previous report.¹²⁾

group for 20 min is 93.6 $\pm$ 9.0 counts. Table 1 shows the inhibitory effects of IT, apigenin (**1**) and the inhibitory agent against PAF-, 5-HT- and PA-induced scratching behavior. Incidence of scratching is shown as a percentage of the control. IT significantly inhibited PAF- and 5-HT-induced scratching behavior. IT decreased PA-induced scratching behavior, but not significantly. Compound **1** in *p.o.* administration showed inhibitory effects on PAF- and 5-HT-induced scratching behavior.¹²⁾

Inhibitory Effects against Locomotive Behavior The locomotive activity of the control group for 20 min was 108.2 $\pm$ 27.2 counts. IT did not inhibit the locomotive behavior of mice. However, diazepam, the positive control, suppressed it (data not shown). This suggested that the sedative action of IT did not involve in the inhibitory mechanism of scratching behavior.

DISCUSSION

IT significantly inhibited COM-induced BP decrease, which is an IgE-independent anaphylaxis-like response (Fig. 1A). The principal compounds **1**–**3** from IT showed the inhibitory effect on COM-induced BP decrease (Fig. 1B). Furthermore, these compounds significantly inhibited BP decrease induced by antigen-induced anaphylaxis (Fig. 2) as well as previously reported^{7–9)} positive controls, DSCG, DPH,¹⁴⁾ and CV-3988,¹⁵⁾ the anti-allergic drug currently used. It has been reported that the main flavones from IT, apigenin (**1**) and luteolin (**3**) have the inhibitory effects of degranulation,¹⁷⁾ histamine release,^{18,19)} and production of Th2-type cytokine (interleukin-4, 5 and 13),²⁰⁾ which plays a role in the enhancement of anaphylaxis²¹⁾ and synthesis of the secondary mediators of anaphylaxis, such as prostaglandins and leukotrienes and PAF, started by the degranulating reaction *in vitro*.^{22,23)} Those compounds also showed a PAF-antagonistic effect *in vivo* as previously reported.⁵⁾ Thus, **1** and **3** were suggested to inhibit anaphylaxis-induced BP decrease by several mechanisms as noted in these reports or findings. Apigenin 7-glucoside (**2**) has anti-inflammatory activity due to its antioxidant effects *in vivo*,²⁴⁾ however, there has been no report on the inhibitory effects of compound **2** against anaphylaxis. In the present experiment, the anti-anaphylactic effects of compound **2** were clarified for the first time.

IT significantly inhibited BF decrease induced by antigen-induced anaphylaxis (Fig. 3A), but compounds **1**–**3** did not show the inhibitory effects of the BF decrease induced by the

anaphylaxis (Fig. 3B). As a positive control, DSCG, CV-3988 and KS¹⁶⁾ of a 5-HT_{2A} antagonistic agent, all showed significant inhibitory effects on BF decrease induced by IgE-dependent anaphylaxis.¹⁰⁾ The mechanism of BP and BF decrease on challenge with HEL has not been completely clarified. We have previously reported that histamine⁸⁾ and PAF⁹⁾ acted as initiators of the BP decrease induced by IgE-dependent anaphylaxis, whereas 5-HT sustained the anaphylaxis. In the case of the BF decrease induced by the anaphylaxis, 5-HT acted as an initiator, but histamine did not affect it at all.¹⁰⁾ On the other hand, PAF was not an initiator, but acted as an important factor in the BF decrease.¹⁰⁾ In a related study, we have observed that the BF of the mouse tends to decrease in response to HEL-sensitization (the stage preceding anaphylaxis) without any change in BP.²⁵⁾ In any case, the present results support a difference in the mechanisms between the BP decrease and the BF decrease induced by anaphylaxis, and are extremely interesting. Further work is needed to search for the principal active compounds that can inhibit anaphylaxis-induced BF decrease and to elucidate the details of the mechanisms involved.

IT in i.v. administration significantly inhibited the scratching behavior (itch-associated response) induced by COM, but the extract in *p.o.* administration did not (Fig. 4). This result suggested that the active compounds may differ among BF decrease, BP decrease and pruritus. More detailed examination including of pharmacokinetical data is necessary.

The anti-pruritic effect of IT dose not seem to be dependent on the central nervous system, because it did not inhibit the locomotive activity of the mice. Instead, it seems to be dependent on the inhibitory effects against PAF and 5-HT, pruritic factors similar to histamine²⁶⁾ (Table 1). In addition, IT decreased the scratching behavior induced by PA, which specifically increases in inflammation or allergy lesion²⁷⁾ (Table 1). IT offers promise as a drug for the treatment of allergic and inflammatory pruritus. Further work is needed on the antagonistic effects of IT on PAF and 5-HT_{2A} receptors.

Compound **1** could not be injected i.v., because it was not soluble in saline. However, in case of the i.v. administration of IT, compound **1** might contribute in the activity by the solubilizing effect of coexisting compound. In *p.o.* administration, compound **1** showed the inhibitory effects on COM, PAF and 5-HT-induced scratching behavior (Fig. 4, Table 1), without a central depressant action.¹²⁾ Kaempferol 3-rhamnosyldigluconide, which was previously isolated from IT, has a significant anti-pruritic effect in i.v. administration.²⁸⁾ Other

active compounds are also being subjected to further study.

To our knowledge, this is the first report of the inhibitory effects of IT against the anaphylaxis and the scratching behavior induced by several pruritogenic agents. Thus, the flowers of *I. textori* should be useful as an anti-anaphylactic and anti-pruritic material in addition to the traditional applications of this plant.

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