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Effect of Jasmonates on Ethylene Biosynthesis and Aroma Volatile Emission in Japanese Apricot Infected by a Pathogen (*Colletotrichum gloeosporioides*)

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ABSTRACT: The effects of the application of the jasmonic acid derivative *n*-propyl dihydrojasmonate (PDJ) on ethylene biosynthesis, volatile compounds, and endogenous jasmonic acid (JA) and methyl jasmonate (MeJA) were examined in Japanese apricot (*Prunus mume* Sieb.) infected by a pathogen (*Colletotrichum gloeosporioides*). The fruit were dipped into 0.4 mM PDJ solution before inoculation with the pathogen and stored at 25 °C for 6 days. The inoculation induced an increase in 1-aminocyclopropane-1-carboxylic acid (ACC), ethylene, JA, and MeJA. In contrast, PDJ application reduced the endogenous JA, MeJA, and ethylene production and expression of the ACC oxidase gene (*PmACO1*) caused by the pathogen infection. The lesion diameter with *C. gloeosporioides* decreased upon PDJ application. The alcohol, ester, ketone, and lactone concentrations and alcohol acyltransferase (AAT) activity increased in the pathogen-infected fruit, but were decreased by PDJ application. These results suggest that PDJ application might influence ethylene production through *PmACO1* and that aroma volatile emissions affected by pathogen infection can be correlated with the ethylene production, which is mediated by the levels of jasmonates.

KEYWORDS: *Colletotrichum gloeosporioides*, ethylene, Japanese apricot, jasmonic acid, *PmACO1*, volatile compounds

INTRODUCTION

Fungal diseases are a cause of major losses of fruit and vegetable crops. *Colletotrichum gloeosporioides* is known to infect a wide variety of hosts and is regularly seen in the field on ripe or overripe fruits. It is the causal agent of anthracnose diseases on fruit crops.[1] In response to fungal pathogen infection, plants induce some signal compounds, including phytohormones, and release several volatile compounds from the damaged sites as a defense mechanism against the pathogens.[2]

Jasmonic acid (JA) and its methyl ester, methyl jasmonate (MeJA), collectively referred to as jasmonates (JAs), are cyclopentanone compounds that are ubiquitous in the plant kingdom. The role of JAs in regulating defense responses against herbivore attack and infection by some pathogens has been well characterized.[3] Exogenously applied or intracellularly generated JA following stress such as wounding or pathogen infection induces the expression of numerous genes,[4] which, in turn, induce resistance against pathogens.[5]

JAs interact with other phytohormones in eliciting biological activity and play a prominent role in signaling plant defenses.[6,7] Ethylene might also function as one of the important components of the signaling pathway regulating pathogenesis in higher plants.[2] It has been shown that there are interactions between JAs and ethylene. In pears, MeJA application was found to enhance ethylene production in preclimacteric fruit but to inhibit it in climacteric and postclimacteric fruit.[8] Although the role of JAs in plant defense against insects and wounding has been widely reported,[4,6,7] the interaction between ethylene and JAs in the defense against pathogenic microorganisms is still unclear. Nevertheless, a clarification of the collaboration between these two substances in plants is considered necessary for the development of defense technology against pathogens in fruit production.

In the present study, we investigated the relationship between JAs, ethylene, and volatile biosynthesis in response to pathogen infection in Japanese apricot (*Prunus mume* Sieb.), a climacteric fruit. These physiological responses might be linked to increased resistance against *C. gloeosporioides*. The synthetic analog of JA, *n*-propyl dihydrojasmonate (PDJ), which is commercially applied at a concentration of 0.4 mmol L^{-1} to apples to promote red color development,[9,10] was applied in this instance to postharvest Japanese apricots. The effect of PDJ on Japanese apricot fruit protection and ripening is also discussed.

MATERIALS AND METHODS

Chemicals. The authentic aroma standards were obtained from the following sources: (*Z*)-3-hexenyl acetate and (*E*)-2-hexeny1 acetate from Bedoukian Research, Inc. (Danbury, CT); 2-hexenal and 1-butanol from Wako Pure Chemical Industries, Ltd. (Osaka, Japan); linalool and damascenone from Sigma-Aldrich Co. (Tokyo, Japan); and others from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). A series of *n*-hydrocarbons (C_6−C_{20}) for Kovats' retention index (RI) was purchased from Kanto Chemical Co., Inc. (Tokyo, Japan); C_{21}−C_{25} were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan).

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Plant Materials and Treatment. "Inazumi" Japanese apricots at a mature green or preclimacteric stage (before the onset of ethylene production) were harvested from trees grafted onto our own rootstock (*Prunus mume* Sieb.) growing in an open field at Chiba University. Apricots of a uniform size (20−23 g fresh weight) and color were selected and rinsed with distilled water prior to the treatments. The fruits were randomly divided into three groups of 120 apricots per treatment (three replications of 40 apricots). In the first and second groups, the fruits were dipped into distilled water containing 0.1% (v/v) surfactant Approach BI (50% polyoxyethylene hexitan fatty acid ester; Kao, Osaka, Japan) for 5 min. In the third group, the fruits were dipped in 0.4 mM PDJ solution (Nippon Zeon Co., Tokyo, Japan) containing 0.1% (v/v) Approach BI. All apricots in each group were then air-dried and wounded (5 mm long, 3 mm deep) with a sharp scalpel. Fruits from the second and third groups were inoculated with *Colletotrichum gloeosporioides*.

Fungal Strain, Culture, and Inoculation. *C. gloeosporioides* 911003-5 (MAFF GeneBank accession no. 239927), originally isolated from an apple fruit in Ibaraki, Japan, was used in this study as the anthracnose pathogen. The fungus was cultured for 7 days at 25 °C on Czapek's agar (CA) (per liter of distilled H_2O): 30 g of sucrose, 2 g of $NaNO_3$, 1 g of K_2HPO_4, 0.5 g of $MgSO_4 \cdot 7H_2O$, 0.5 g of KCl, and 10 mg of $FeSO_4 \cdot 7H_2O$.

A mycelial block (8 mm in diameter) of *C. gloeosporioides* was transferred from CA and placed on the wounded surfaces of the Japanese apricots from the second and third groups, hereafter referred to as $inoc^+PDJ^-$ and $inoc^+PDJ^+$, respectively. A block of CA without fungal mycelial was placed on the wounded surfaces of the control fruits in the first group, hereafter referred to as the control.

All fruits from the three groups were then placed at 25 °C and 95% relative humidity in a controlled room. Thirty fruits (three replications of 10 fruits) were sampled at 1, 2, 4, and 6 days after treatment. The sample [30 fruits (three replications of 10 fruits)] on day 0 was collected immediately after harvest and was left untreated. After lesion diameter and ethylene production had been measured, the fruit pericarp tissues including the skin were sampled and frozen in liquid N_2. The samples were stored at −80 °C to analyze the ACC concentration, ACC oxidase and ACC synthase gene expression, JAs concentration, volatile compounds, and AAT activity.

Ethylene Production and ACC Concentration. Nine fruits from each treatment (three replications of three fruits; each replicate was placed in a different container) were sealed in 250 mL plastic containers and left at 25 °C for 1 h. Then, 1 ml of the headspace gas containing ethylene was measured using a gas chromatograph fitted with a flame ionization detector (FID) [(model GC 2014, Shimadzu, Kyoto, Japan); 2.2 mm i.d. × 2.0 m column (Porapak Q; Waters, Milford, MA); column temperature, 50 °C; He flow rate, 30 mL min^{-1})]. The results were expressed in milliliters of ethylene released per kilogram of tissue per hour (mL kg^{-1} h^{-1}).

The 1-aminocyclopropane-1-carboxylic acid (ACC) concentration was analyzed by GC-FID as described by Kondo et al.[8] The ACC content was estimated from the standard curve.

Jasmonic Acid Analysis. Extraction and quantification of JA and MeJA were performed as previously described by Kondo et al.[11] Five grams of fruit tissue was homogenized with 100 μL of each (±)-2-(2,3-$^2H_2$) JA (100 mg L^{-1}) and (±)-2-(2,3-$^2H_2$) MeJA (100 mg L^{-1}) as internal standards in 10 mL of saturated NaCl solution and 20 mL of diethyl ether containing 0.005% butylated hydroxytoluene (BHT) as an antioxidant. The ether phase was removed after centrifugation at 8000g_n for 15 min. The aqueous layer was extracted a second time with 20 mL of diethyl ether containing 0.005% BHT. The pooled ether extract was dried under warm air. The residue was dissolved in 200 μL of chloroform/isopropylethylamine, 1:1 (v/v), and derivatized at 50 °C for 60 min with pentafluorobenzyl bromide (PFB).

Analysis of PFB-JA and PFB-MeJA was conducted using GC−mass spectroscopy with selected ion monitoring [QP 5000; Shimadzu, Kyoto, Japan; 25 m × 0.25 mm i.d. column (CP-Sil 5 CB; Chrompack, Middelburg, The Netherlands)]. The column temperature gradient was 60 °C for 2 min, 60 to 270 at 10 °C min^{-1}, and 270 °C for 35 min with a 50.2 cm s^{-1} linear He flow and 70 eV electron potential. A relative calibration procedure was used to determine the JA and MeJA contents in the samples.

Volatile Compound Analysis. Volatile compound analysis was performed as described by González-Agüero et al.[12] Two grams of tissue was homogenized in 2 mL of 2 mM sodium fluoride. The homogenate was centrifuged at 8000g_n for 15 min at 4 °C. Two milliliters of supernatant was placed into crimp-seal 5 mL vials containing 0.5 g of NaCl and 10 μL of cyclohexanol (1000 mg L^{-1}) as an internal standard. A polydimethylsiloxane/divinylbenzene (PDMS/DVB; 65-μm thickness) solid-phase microextraction (SPME) fiber (Supelco Co., Bellefonte, PA) was exposed to the headspace for 30 min at 40 °C while the vial contents were stirred.

For quantitation of volatile compounds, the SPME fiber was inserted into the injection port of a gas chromatograph (GC-17A, Shimadzu, Kyoto, Japan). The GC was equipped with a fused silica capillary column coated with a 0.25-μm film of polyethylene glycol (0.25 mm i.d. × 60 m; DB-WAX, J&W Scientific, Folsom, CA) and FID. The temperature of the column was maintained at 40 °C for 10 min, raised to 220 °C at a rate of 3 °C min^{-1}, maintained at 220 °C for 10 min, and then increased to 240 °C at a rate of 10 °C min^{-1}. The injection and detector temperatures were maintained at 230 and 250 °C, respectively. According to Li et al.,[13] the compounds were identified through GC−MS (QP-5000, Shimadzu, Kyoto, Japan) by comparison of the mass spectra with the authentic reference standards and spectra in the National Institute for Standards and Technology (NIST 21 and 27) library. The amount of each compound was calculated by comparison with the peak area of the internal standard (analysis repeated three times).

AAT Activity. AAT was extracted and assayed by the method of Ke et al.[14] with slight modifications. Three grams of tissue was homogenized in 10 mL of 100 mM potassium phosphate buffer (pH 7.5) containing 1 g of polyvinyl polypyrolidone (PVPP). The homogenate was centrifuged at 12000g_n for 25 min at 4 °C. The supernatant was decanted, placed on ice, and used as a crude extract. AAT activity was assayed by mixing 75 mM potassium phosphate buffer (pH 7.5), 1 mM 5,5′-dithiobisnitrobenzoic acid (DTNB), 0.01 mM $MgCl_2$, 0.4 mM *n*-hexanol, 0.1 mL of crude extract, and 0.25 mM acetyl-CoA in the total volume of 1 mL. The increase in absorbance at 412 nm was recorded at 30 °C using a spectrophotometer to detect the production of 2-nitro-5-thiobenzoic acid that was formed by the reaction of DTNB with free CoA. Total protein in the crude extract was assayed according to the method of Bradford,[15] using bovine serum albumin (BSA) as a standard. Enzyme activity is expressed as nanomoles of product formed per minute per milligram of protein.

RNA Extraction and Northern Blot Hybridization. Total RNA was isolated from the fruit tissue using the methods described by Ban et al.[16] Total RNA (5 μg) was electrophoresed on a 1.2% agarose/formaldehyde gel and then blotted onto a nylon membrane by capillary transfer. Partial cDNAs for ripening specific ACC synthase (*PmACS1*) and ACC oxidase (*PmACO1*), which had been isolated previously from "Orihime" with the accession numbers AB499128 and AB499127, respectively, in the DNA data bank of Japan (DDBJ),[17] were labeled with digoxigenin-dUTP (Roche Diagnostics, Mannheim, Germany) using M13 forward and reverse primers by polymerase chain reaction (PCR). The specificity of each probe was confirmed by Southern blot analysis. Prehybridization, hybridization, washing, and detection were carried out according to the manufacturer's instructions and previously reported method of Kondo et al.[8]

Lesion Diameter. The lesion diameter on each fruit wound was determined daily after inoculation. When the visible rot zone beyond the

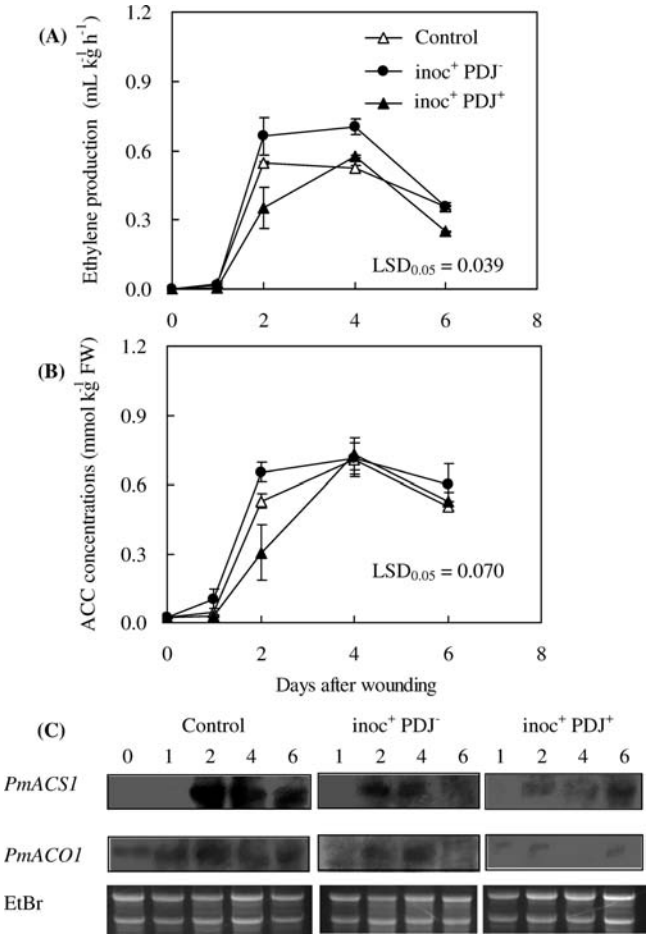

Figure 1. (A) Ethylene production, (B) ACC concentration, and (C) expression level of ethylene biosynthesis pathway genes in Japanese apricots after pathogen inoculation and PDJ treatment. Vertical bars represent SE (n = 3). Ethylene production is the value from three replications of three fruits. Each of the other values is from three replications of 10 fruits. Northern blot was repeated three times. Ethidium bromide staining shows equal loading of rRNA.

wound area on each fruit was more than 5 mm wide, the fruit was counted as infected.

Statistical Analysis. The SAS analysis of variance procedure (SAS Institute, Cary, NC) was used to determine the treatment effects, and the mean separation was analyzed by Fisher's least significant difference ($P \leq 0.05$). The data are presented as the mean values of three replications ± the standard error (SE).

RESULTS

Ethylene Production, ACC Concentrations, and Expression of *PmACS1* and *PmACO1*. Ethylene production in the control fruits increased sharply 2 days after wounding and decreased slightly thereafter (Figure 1A). The ACC concentrations also increased after 2 days and showed a tendency to decrease after 6 days (Figure 1B). The expression of *PmACS1* was not detected before wounding or at 1 day after wounding, but it was increased 2 days after wounding. The transcript levels of *PmACO1* in the control fruits increased from 1 day after wounding (Figure 1C). The inoc^{+}PDJ^{-} fruits showed a marked increase in ethylene production 2 and 4 days after wounding (Figure 1A). This also coincided with an increase in the ACC

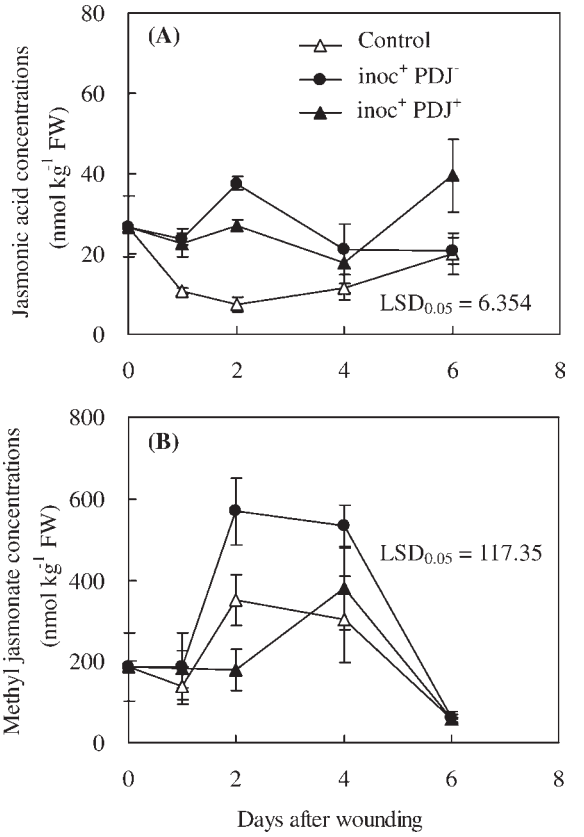

Figure 2. (A) Jasmonic acid and (B) methyl jasmonate concentrations in Japanese apricots after pathogen inoculation and PDJ treatment. Vertical bars represent SE (n = 3). Values are from three replications of 10 fruits.

concentrations 2 days after wounding compared to the control fruits (Figure 1B). The expressions of *PmACS1* and *PmACO1* did not differ between the control and inoc^{+}PDJ^{-} fruits throughout the period during which they were measured. In the inoc^{+}PDJ^{+} fruits, ethylene production and ACC concentration levels were lower than those in the controls and inoc^{+}PDJ^{-} fruits. These findings were associated with a low expression level of *PmACS1* and almost undetectable expression in the *PmACO1* transcripts in the inoc^{+}PDJ^{+} fruit compared to the controls and inoc^{+}PDJ^{-} fruit.

Endogenous Jasmonate Concentrations. The JA concentrations in the control fruits were lowest at 1 and 2 days after wounding (Figure 2A). In contrast, the JA concentrations in the inoc^{+}PDJ^{-} fruits increased 2 days after wounding and declined thereafter. The JA concentration in the inoc^{+}PDJ^{+} fruits increased 6 days after wounding, although it was lower than that in the inoc^{+}PDJ^{-} fruits 2 days after wounding. The MeJA concentrations in the control fruits increased after 2 and 4 days compared to those after 0 and 1 day after wounding (Figure 2B). The inoc^{+}PDJ^{-} fruit showed a significant increase of MeJA 2 days after wounding, and then the concentration diminished. In contrast, the MeJA concentrations in the inoc^{+}PDJ^{+} fruits were lower than those in the inoc^{+}PDJ^{-} fruits 2 days after wounding, but the MeJA concentrations were not different between the inoc^{+}PDJ^{-} and inoc^{+}PDJ^{+} fruits 4 and 6 days after wounding.

Volatile Compounds and AAT Activity. Nineteen primary volatile compounds, including 10 esters, 2 aldehydes, 4 alcohols, 1 lactone, 1 ketone, and 1 acid, were identified in Japanese apricot

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Table 1. Primary Volatile Compounds Identified in Japanese Apricot Different Numbers of Days after Wounding[a]

		relative concentrations (mg kg^{-1})									
		control				inoc$^+$PDJ$^-$			inoc$^+$PDJ$^+$		
RI[b]	compound	0	2	4	6	2	4	6	2	4	6
802	ethyl acetate	ND[c]	ND	0.90 ± 0.5	1.40 ± 1.0	0.80 ± 0.3	5.00 ± 1.9	0.60 ± 0.1	tr[d]	1.00 ± 0.3	0.40 ± 0.1
936	ethyl butanoate	ND	ND	0.70 ± 0.3	1.50 ± 0.2	1.10 ± 0.5	5.30 ± 0.9	1.00 ± 0.1	0.40 ± 0.1	2.20 ± 0.4	0.80 ± 0.2
972	2-methylpropyl acetate	ND	17.7 ± 1.8	43.1 ± 0.4	24.1 ± 3.4	32.6 ± 3.6	69.1 ± 1.3	26.8 ± 1.6	10.4 ± 1.8	27.2 ± 2.6	14.6 ± 2.6
977	hexanal	1.70 ± 0.4	ND	ND	ND	ND	ND	ND	ND	ND	ND
1048	1-butanol	ND	ND	0.40 ± 0.1	0.30 ± 0.1	0.30 ± 0.1	0.30 ± 0.0	0.30 ± 0.1	tr	0.30 ± 0.1	0.30 ± 0.0
1114	2-hexenal	4.35 ± 0.5	0.90 ± 0.5	0.50 ± 0.1	1.10 ± 0.2	1.20 ± 0.1	1.20 ± 0.0	1.10 ± 0.1	2.70 ± 0.3	1.10 ± 0.5	0.70 ± 0.2
1121	butyl butanoate	ND	24.8 ± 5.1	30.5 ± 0.4	22.3 ± 2.9	35.5 ± 3.0	37.3 ± 2.4	30.8 ± 1.5	14.5 ± 0.8	17.7 ± 2.9	27.5 ± 6.5
1135	ethyl hexanoate	ND	0.30 ± 0.1	1.40 ± 0.4	0.30 ± 0.1	1.50 ± 0.6	5.84 ± 2.5	2.80 ± 0.5	0.40 ± 0.1	1.50 ± 0.2	2.40 ± 0.5
1174	hexyl acetate	ND	15.9 ± 1.8	52.2 ± 11	46.8 ± 11	39.2 ± 8.1	100 ± 11	48.2 ± 1.7	7.10 ± 1.0	17.8 ± 1.9	37.7 ± 13
1216	(*Z*)-3-hexenyl acetate	ND	1.70 ± 0.3	6.10 ± 0.5	5.70 ± 1.1	2.80 ± 0.3	6.50 ± 0.1	6.70 ± 0.1	0.70 ± 0.1	3.50 ± 0.2	5.30 ± 1.5
1226	(*E*)-2-hexeny1 acetate	ND	0.20 ± 0.0	3.50 ± 1.1	1.80 ± 0.4	0.40 ± 0.1	5.60 ± 0.3	1.90 ± 0.2	tr	1.70 ± 0.4	1.50 ± 0.4
1255	1-hexanol	ND	0.20 ± 0.0	0.60 ± 0.0	0.50 ± 0.0	0.20 ± 0.0	0.70 ± 0.1	0.50 ± 0.1	tr	0.30 ± 0.0	0.50 ± 0.0
1313	butyl hexanoate	ND	3.80 ± 0.8	7.50 ± 1.6	6.10 ± 0.4	6.60 ± 1.2	12.3 ± 1.2	9.10 ± 1.6	4.60 ± 1.5	5.30 ± 1.3	6.70 ± 2.3
1313	hexyl butanoate	ND	2.60 ± 0.6	6.40 ± 1.1	4.40 ± 0.4	5.20 ± 0.4	7.20 ± 1.1	6.40 ± 0.6	1.20 ± 0.1	3.20 ± 0.7	7.60 ± 0.1
1359	3-hexenoic acid	ND	0.80 ± 0.2	2.30 ± 0.7	1.30 ± 0.1	1.20 ± 0.1	2.90 ± 0.4	1.60 ± 0.3	0.30 ± 0.1	1.50 ± 0.2	1.00 ± 0.2
1390	2-ethylhexanol	0.20 ± 0.0	0.50 ± 0.0	0.70 ± 0.0	1.30 ± 0.1	0.70 ± 0.0	0.80 ± 0.1	0.70 ± 0.3	0.70 ± 0.1	0.70 ± 0.0	1.10 ± 0.1
1446	linalool	0.30 ± 0.0	1.00 ± 0.1	0.90 ± 0.1	1.90 ± 0.1	1.50 ± 0.1	1.90 ± 0.1	1.50 ± 0.5	0.70 ± 0.0	1.10 ± 0.1	1.60 ± 0.6
1730	damascenone	tr	tr	tr	0.30 ± 0.0	0.20 ± 0.0	0.30 ± 0.0	0.30 ± 0.1	tr	tr	0.50 ± 0.1
2057	γ-decalactone	tr	0.90 ± 0.2	2.60 ± 0.1	1.20 ± 0.1	1.33 ± 0.1	4.90 ± 0.6	1.40 ± 0.4	tr	1.10 ± 0.3	0.60 ± 0.1

[a] Data are the means ± SE of three replications. [b] Retention index based on a series of *n*-hydrocarbons. [c] ND = relative concentration less than 0.1 mg kg^{-1}. [d] tr = relative concentration less than 0.2 mg kg^{-1}.

(Table 1). At harvest, aldehydes (2-hexenal and hexanal; 91.6%) were the major group of volatiles, followed by alcohols (7.5%) and ketone (0.4%). After harvest, the composition of volatile compounds varied quantitatively and qualitatively. The relative proportion of aldehydes decreased, but the proportions of other classes increased. The total volatile concentration increased during storage and reached the maximum level 4 days after wounding (Table 1 and Figure 3). This was generally true for both treatments, as well as for the controls (except in the case of the aldehydes and the ketone). The total volatile concentration then remained the same or declined on day 6. Four days after wounding, the relative proportions of esters, acid, alcohols, lactone, aldehydes, and ketone were 94.6%, 2.3%, 1.6%, 1.1%, 0.4%, and 0.1%, respectively. Among the esters identified, hexyl acetate (52.2 mg kg^{-1}), 2-methylpropyl acetate (43.1 mg kg^{-1}), and butyl butanoate (30.5 mg kg^{-1}) were quantitatively the primary compounds of the Japanese apricot (Table 1). The production of volatile alcohols, esters, lactone, and ketone in the inoc$^+$PDJ$^-$ fruits accelerated 4 days after wounding (Figure 3). However, the aldehyde and acid concentrations were not different between the controls and inoc$^+$PDJ$^-$ fruits. Most volatile compounds in the inoc$^+$PDJ$^+$ fruits also increased with time, at least up to 4 days; however, the levels were usually lower than those in controls and in inoc$^+$PDJ$^-$ fruits (especially on day 4).

Changes in the AAT activity in the controls after wounding and in both treatments are shown in Figure 4. The levels of AAT activity increased between 1 and 2 days after wounding. The inoc$^+$PDJ$^-$ fruits showed a rise in AAT activity levels on day 4 compared with the controls but no difference 6 days after wounding. In contrast, the inoc$^+$PDJ$^+$ fruits showed the lowest AAT activities 2 and 4 days after wounding.

Lesion Diameter. The diameters of the lesions of *C. gloeosporioides* in the inoc$^+$PDJ$^-$ or inoc$^+$PDJ$^+$ fruits are shown in Figure 5. Lesion diameters 5−6 days after inoculation were significantly larger in inoc$^+$PDJ$^-$ fruits than in inoc$^+$PDJ$^+$ ones. Thus, the pathogen developed more slowly in the inoc$^+$PDJ$^+$ fruits than in the inoc$^+$PDJ$^-$ fruits.

DISCUSSION

Plant pathogen defense involves a series of signal responses from signal molecules, including JA and MeJA, to induce the expression of defense-related genes and ultimately enhance systemic resistance.[18] In our study, the endogenous JA and MeJA levels sharply increased after the pathogen was inoculated. This result is in agreement with those of previous reports[18,19] showing that JAs often accumulate in response to pathogen attack and that this accumulation occurs as an early event during the resistance response. The large increase in MeJA compared to JA after pathogen infection and the different levels of these compounds between treatments might be due to the volatile nature of MeJA.[4]

It has been shown that the exogenous application of JAs decreases the incidence of decay and induces the defense resistance system against postharvest diseases in several types of fruit.[4,5] Our results showed that PDJ application significantly increased the endogenous JA concentrations 6 days after wounding. Concerning the effect of PDJ on endogenous JA, Yoshikawa et al.[20] suggested that PDJ might increase JA biosynthesis because PDJ affects allene oxide synthase in the octadecanoic pathway. Although the PDJ treatment did not completely regulate fungal decay, it did act to reduce lesion spread on fruit.

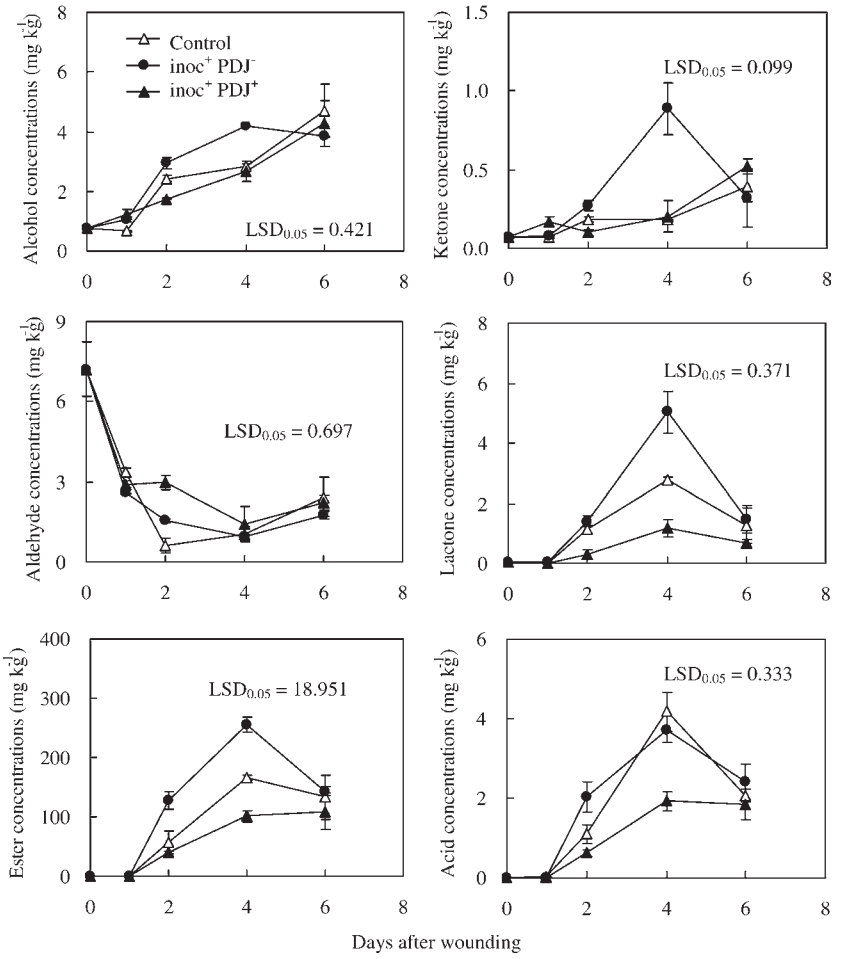

Figure 3. Volatile compounds in Japanese apricots after pathogen inoculation and PDJ treatment. Vertical bars represent SE (n = 3). Values are from three replications of 10 fruits.

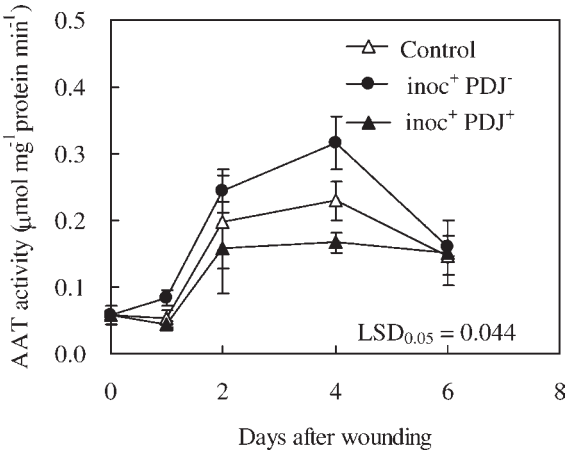

Figure 4. AAT activity in Japanese apricots after pathogen inoculation and PDJ treatment. Vertical bars represent SE (n = 3). Values are from three replications of 10 fruits.

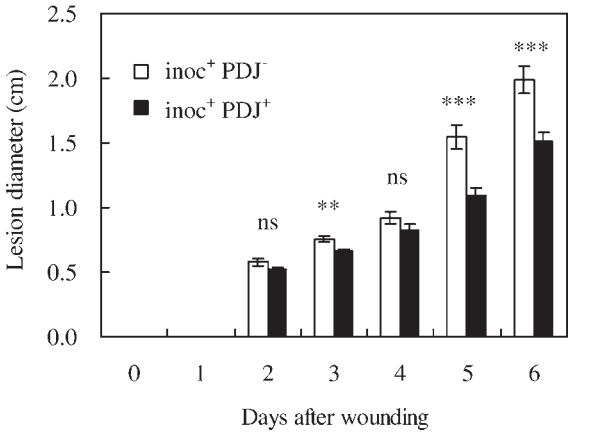

Figure 5. Lesion diameter in Japanese apricots after pathogen inoculation and PDJ treatment. Vertical bars represent SE (n = 3). Values are from three replications of 10 fruits. Asterisks indicate significant differences on each day after treatment (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$).

Yao and Tian[5] found that MeJA could not reduce the disease incidence of brown rot caused by *Monilinia fructicola* in sweet cherry fruit (*Prunus avium* L.), but it induced defense responses such as β-1,3-glucanase and peroxidase activities, resulting in the delay of the disease.

The salicylic acid- (SA-) dependent pathway is a major factor contributing to the resistance.[4] We found an increase of a volatile methyl ester of SA, MeSA, in PDJ$^+$ fruit but not in PDJ$^-$ fruit or in the controls within 1−2 days after wounding (data not

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presented). MeSA is known as an airborne signal that travels from a pathogen-infected plant to neighboring plants, where it activates defense responses and is processed to SA.[21] In general, interactions between SA and JA signaling are antagonistic. SA has been shown to act as a strong suppressor of JA-dependent defense responses, possibly through the inhibition of JA biosynthesis.[19] Therefore, it is possible that PDJ application might induce fruit resistance against pathogens by increasing SA and MeSA, resulting in a decrease of endogenous JAs soon after wounding. Further studies will be necessary to determine the endogenous SA.

JAs and ethylene are considered to act synergistically in response to pathogens.[4] We found an increase of endogenous JA and MeJA along with the production of ethylene in pathogen-inoculated fruit. On the other hand, PDJ applied to Japanese apricots at the preclimacteric stage caused ethylene production to decrease in pathogen-inoculated fruit. In apple fruit (*Malus sylvestris* L.), Kondo et al. [8] suggested that PDJ stimulates the *MdACS1* mRNA expression at the preclimacteric stage when ethylene production is low, but inhibits the same at the climacteric stage when ethylene production is high. In our study, the decreased expression of *PmACO1* might be due to the effect of PDJ in decreasing the production of ethylene incited by the pathogen *C. gloeosporioides*. Yu et al.[18] suggested that ACO activity rather than ACS activity is the key step for controlling ethylene production in response to exogenous stimulation in tomatoes (*Lycopersicon esculentum* Mill.). In melons (*Cucumis melo* L.), the *CmACO1* promoter is positively regulated during senescence and in response to pathogen attack.[22] Thus, the effect of the application of PDJ on *PmACO1* might be associated with a stress reaction. *PmACO1* expression was almost undetectable in the $inoc^{+}PDJ^{+}$ fruits, but these fruits produced as much ethylene as the fruits that underwent other treatments. Further analysis of these issues might lead us to a better understanding of the molecular mechanisms responsible for the activities against pathogens in JAs-ethylene-related phenomena.

We identified esters as the major compounds in the Japanese apricot. Hexyl acetate, with sweet and fruity notes, was found to be the predominant ester in this fruit. This compound has been described as an aroma contributor in several apricot cultivars[23] and as a characteristic aroma component of over-ripe Japanese apricots.[24] 2-Hexenal and hexanal are the most abundant aldehydes in the Japanese apricot and are apparently used as a substrate for alcohol and ester formation. γ-Decalactone and linalool are the most quantitatively important lactone and terpenic alcohol, respectively, and have been associated with the typical apricot aroma.[12,23]

We found clear evidence for cross-talk between the JA and ethylene pathways in the changes in pathogen-induced volatile emissions upon PDJ application. The pathogen-induced ethylene production and the release of aroma volatiles, especially fruity note esters (hexyl acetate, 2-methylpropyl acetate, and butyl butanoate), lactone (γ-decalactone), and terpene alcohol (linalool). This implies that pathogen inoculation promoted fruit senescence. The emission of volatiles from pathogen-infected fruit might serve as a direct defense against pathogen infections. For example, the lipid-derived volatiles 2-hexenal and 1-hexanol, a substrate for hexyl ester (hexyl acetate, hexyl butanoate), are suggested to be important signals involved in plant stresses. The presence of C_6 unsaturated compounds was probably due to lipoxygenase activity and β-oxidation initiated by the disruption of fruit tissues during blending.[12]

The differences in ester concentration among the treatments might be affected by internal ethylene, as well as by aroma-related enzymes.[12,25] In this study, the activity of the enzyme AAT, which catalyzes the last step of ester production, showed a pattern similar to that of ethylene. This result was supported by the results of a previous study by Defilippi et al.,[25] which showed that AAT is regulated by ethylene. In the present study, PDJ application reduced both the magnitude and blend of pathogen-induced volatile emissions during pathogen infection, probably through the regulation of enzymes in volatile biosynthetic pathways by ethylene. Kondo and Mattheis[26] suggested that the effect of JAs on volatile production might be related to the effect of JAs on internal ethylene concentration. Therefore, it is possible that the changes in the aroma volatile compounds induced by the pathogen might be induced by ethylene.

In summary, JAs regulate endogenous ethylene levels in pathogen-infected fruit, but the application of JAs reduces ethylene production and volatile production. These reductions might be associated with the induction of the defense resistance system that occurs with PDJ application. The application of the plant signal molecule JA, which includes the defense resistance system against postharvest diseases, might regulate postharvest decay in practice.

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