Preformed Antifungal Compounds of Lemon Fruit: Citral and Its Relation to Disease Resistance

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The young mature-green lemon fruit manifests a significantly lower level of postharvest decay as compared to the older yellow fruit. Inoculation with *Penicillium digitatum* Sacc. demonstrated that the resistance of young fruit to decay is related to a factor localized in the oil glands of the flavedo. The main antifungal compound of lemon flavedo was identified as the monoterpene aldehyde citral. The flavedo of green lemon contained 1.5–2 times higher levels of citral as compared to the yellow fruit. In parallel with citral decline, the flavedo extracts of yellow lemons exhibited an increased level of the monoterpene ester neryl acetate, which exerted practically no inhibitory activity against *P. digitatum* and, in concentrations below 500 ppm, even stimulated development of the pathogen. During long-term storage of lemon fruit, citral concentration decreased in parallel with the decline of antifungal activity in the peel and with an increase of decay incidence. It is suggested that the level of citral in the flavedo is related to the resistance of lemon fruit to postharvest decay.

Keywords: *Lemon; postharvest; disease resistance; citral; antifungal compounds*

INTRODUCTION

The biological role of the fruit, among others, is related to protection of developing seeds from various abiotic and biotic hazards. Accordingly, the young fruit has a highly active system of natural resistance against pathogens, comprised of several preformed and induced defensive mechanisms. With completion of seed development and fruit maturation, disease resistance usually declines, especially during the postharvest period when the fruit is detached from the mother plant (Brady, 1987; Ben-Yehoshua et al., 1988, 1990). As a result, methods to control postharvest decay of fresh agricultural produce must be employed, such as synthetic fungicides and cooling. These methods are not always environmentally friendly or effective. A better understanding of the endogenous mechanisms of disease resistance may help in the development of new suitable biological and biotechnological approaches to reduce fruit decay.

With citrus fruit, including lemon, the green mold disease caused by *Penicillium digitatum* Sacc. is a main factor of postharvest decay. The presence of a peel wound is usually a prerequisite for *Penicillium* invasion (Nadel-Schiffmann and Littauer, 1956). Lemons picked green are known to have longer storage life as compared to fruit harvested fully colored (Erickson, 1968).

In our previous work, the monoterpene aldehyde citral (3,7-dimethyl-2,6-octadienal), a natural mixture of the geometric isomers geranial and neral, was found to be one of the preformed antifungal materials of lemon peel exerting high inhibitory activity against *P. digitatum* (Ben-Yehoshua et al., 1992). However, the physiological role of citral in fruit disease resistance is not completely clear since this material, like all of the compounds of citrus essential oils, is not distributed uniformly throughout the fruit surface but is located inside oil glands (cavities) in the flavedo layer of the peel (Shaw, 1977). Additional evidence is necessary to determine whether citral is capable of inhibiting the pathogen in situ. Moreover, according to the early findings of Nadel-Schiffmann and Littauer (1956) later repeated by Homma and Arimoto (1988), a fully mature citrus fruit, including lemon, is easily infected by *Penicillium* when the oil glands are artificially inoculated.

Although the antifungal activity of citral is well documented in many works (Tripathi et al., 1984; Asthana et al., 1988; Onawunmi, 1989), other publications (French et al., 1978) report that it can stimulate, under certain conditions, spore germination of *P. digitatum* and *Penicillium italicum*.

The objective of this work was to further assess the involvement of citral in the natural defensive system of lemon fruit.

MATERIALS AND METHODS

General. The work was conducted with lemon fruit (*Citrus limon* L. Burm.) of Eureka variety. Young (mature-green, approximately 6–7 months after anthesis) and old (yellow, approximately 10–11 months after anthesis) lemons were harvested simultaneously from the same trees. The fruit were obtained either directly from the orchard or from the packing house before any postharvest treatment had been applied. The fruit were stored at 17 °C and 85% relative humidity (RH).

Inoculations. The inoculation was done according to the procedure of Nadel-Schiffmann and Littauer (1956) by piercing the flavedo to a depth of 1.5 mm with a three-needle tool. The tool was immersed in a suspension of *P. digitatum* spores (10^6 spores/mL) prior to each piercing operation. Spore suspension was prepared as described previously (Kim et al., 1991). The inoculated fruit was kept at 17 °C and 85% RH. Pathogen development was characterized by the percentage of infected fruit (decay percentage) and by lesion diameter (centimeters).

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To check the role of oil glands in fruit/pathogen interaction, the fruit was inoculated by spore injection. Twenty microliters of spore suspension (10^6 spores/mL) was injected with a syringe in the flavedo to the depth of 1.5 mm, either into or between oil glands. Filtered fresh orange juice (1%) was added to the spore suspension. A droplet of injected spore suspension stayed on the fruit surface above the wound. Five neighboring oil glands, or five places between oil glands, were injected within each inoculation site of 7–8 mm in diameter.

Analytical. Flavedo tissues were extracted with petroleum ether as described by Kim et al. (1991) and Ben-Yehoshua et al. (1992). The separation of petroleum ether extracts included preliminary fractioning by flash chromatography on a silica gel column, further separation by thin-layer chromatography (TLC), and final purification of active fractions by high-performance liquid chromatography (HPLC). The details of separation techniques used are described in our previous publication (Ben-Yehoshua et al., 1992).

Combined gas chromatography and mass spectrometry (GC–MS) was used for identification of detected materials. The analysis was carried out on a HP 5988A mass spectrometer (Hewlett-Packard, Palo Alto, CA) equipped with a HP-5 column and a quadropole detector. The initial column temperature of 70 °C was kept for 1 min and then increased to 230 °C at 10 °C min^{-1} and held at 230 °C for an additional 1 min. The structures of the isolated compounds were confirmed by the ^1H NMR spectrum at 360 MHz in $CDCl_3$ on a Bruker WM-360 spectrometer as described by Ben-Yehoshua et al. (1992).

Concentrations of monoterpenes in flavedo extracts were quantified by gas chromatography (GC) using the method of external standardization. The authentic samples of citral (64% geranial and 32% neral, Sigma Chemical Co., St. Louis, MO), geranyl acetate, and neryl acetate (both from Aldrich Chemical Co., Milwaukee, WI) were used as external standards. The GC analysis was carried out on a Vega 6000 GC instrument (Carlo Erba Strumentazione, Milano, Italy) equipped with a flame ionization detector and fitted with an OV-17, Chrom W-HP steel column (6 ft length and 1/4 in. o.d.). Nitrogen was used as carrier gas; flow rate was 80 mL min^{-1}. The initial temperature of 105 °C was held for 5 min, and then the temperature was increased to 130 °C at 1 °C min^{-1} and held at 130 °C for an additional 5 min. The temperature of the injector was 250 °C and of the detector 220 °C.

Bioassays. The direct bioassay of antifungal activity on TLC plates (Kim et al., 1991) was used for preliminary detection of inhibitory materials in the crude extract. *Cladosporium cladosporioides* G. A. De Vries was used as the test organism because of its ability to grow and to sporulate on TLC plates.

Antifungal activity was evaluated quantitatively by percent inhibition of *P. digitatum* spore germination and germ-tube elongation. The procedures were carried out according to those of Kim et al. (1991). Additionally, the effect of tested materials on mycelial growth of *P. digitatum* was checked in Petri dishes using an agar diffusion bioassay (Asthana et al., 1988; Kim, 1992).

All experiments and analyses were carried out at least in triplicate. Data were analyzed by ANOVA and means were separated by Duncan's multiple-range test.

RESULTS

Effect of Fruit Age on Decay Susceptibility. During storage of noninoculated fruit, the young mature-green lemon (approximately 6–7 months after anthesis) exhibited significantly lower decay incidence than the older yellow fruit (approximately 10–11 months after anthesis) (Figure 1). The decay of an old fruit started during the first month of storage, while the young lemon at that period had practically no rot. Later, the decay percentage of yellow fruit was approximately twice as high as that of the green.

Also, when inoculated artificially with *P. digitatum* spores (10^6 spores/mL), the green lemon demonstrated a markedly slower rate of pathogen development, as compared to the yellow fruit (Figure 2), in terms of both lesion diameter and percentage of infected fruit.

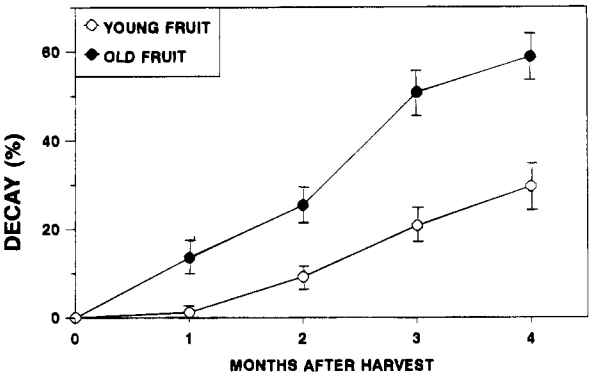

Figure 1. Percentage of fruit infected during storage of young and old noninoculated lemons. Young (mature-green, approximately 6 months after anthesis) and old (yellow, approximately 11 months after anthesis) fruit were harvested at the same time and stored at 17 °C and 85% RH. Bars indicate standard errors.

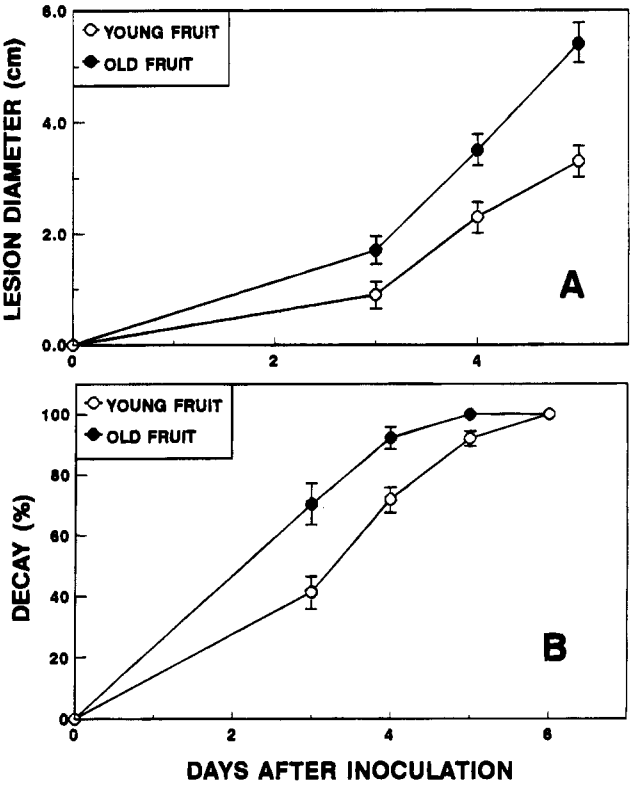

Figure 2. Relationship between fruit age and decay of lemons inoculated with *P. digitatum*. Young and old fruit were inoculated with a spore suspension of *P. digitatum* (10^6 spores/mL) immediately after harvest and kept for 6 days at 17 °C and 85% RH. (A) Lesion diameter (cm); (B) percentage of infected fruit. Bars indicate standard errors.

Role of Oil Glands in Decay Susceptibility of Lemon Fruit. To check the involvement of oil gland content in the fruit–pathogen interaction, young and old lemons were inoculated by injecting spore suspensions of *P. digitatum* either into the oil glands or in the tissues between the glands (Figure 3). With yellow fruit, more than 80% of inoculation sites rotted when inoculated into the oil glands. However, inoculation of young green lemons into oil glands caused no decay development. No significant difference in disease susceptibility between young and old fruit was observed when the inoculation was done between oil glands. Low decay incidence in those cases could not be attributed to the lack of germination-stimulating materials since

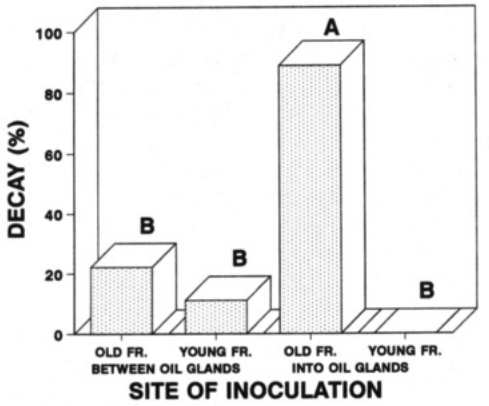

Figure 3. Role of oil glands in the susceptibility of lemon fruit to *P. digitatum*. Ten microliters of spore suspension (10^6 spores/mL) containing filtered fresh orange juice (1% v/v) was injected in the flavedo either into or between oil glands. The decay percentage was examined 4 days after inoculation. Different letters indicate significantly different values as determined by Duncan's multiple-range test ($p = 0.05$).

1% orange juice was added to the spore suspension. Trials without addition of orange juice gave similar results. These findings indicated that the difference in decay susceptibility between young and old fruit might be related to the changes in the composition of oil gland content.

Preformed Antifungal Compounds of Lemon Fruit. The TLC bioassay revealed the presence of several antifungal materials in lemon flavedo (Figure 4). In accordance with our previous data, the largest inhibitory spot on Figure 4 was attributed to citral (3,7-dimethyl-2,6-octadienal) on the basis of GC–MS and ^{1}H NMR analysis. Some of the other inhibitory spots belonged to the previously described preformed antifungal materials of coumarin or furanocoumarin nature such as 5-geranoxy-7-methoxycoumarin, limettin (5,7-dimethoxycoumarin), and isopimpinellin (5,8-dimethoxypsoralen). The identification of other antifungal compounds of lemon flavedo is in progress.

The inhibitory activity of identified compounds toward *P. digitatum* was checked by their effect on spore germination and germ-tube elongation of the pathogen. On the basis of germ-tube elongation, the median effective dose (ED_{50}) of citral was determined as 170 ppm, varying, in different trials, from 100 to 215 ppm. The activity of preformed antifungal materials of coumarin nature was markedly lower: ED_{50} = 1578 and 886 ppm for 5-geranoxy-7-methoxycoumarin and limettin, respectively. Citral also inhibited the mycelial growth of *P. digitatum* in Petri dishes (data not shown).

Effect of Fruit Age on Citral and Neryl Acetate Levels. The flavedo extracts of green lemons contained 1.5–2 times more citral as compared to yellow fruit (800–900 and 400–600 μg/g fresh flavedo, respectively). Along with citral decline, the extract of yellow lemon exhibited an increased level of another compound identified using GC–MS as the monoterpene ester neryl acetate. As can be judged from Figure 5, the neryl acetate in yellow fruit was the predominant oxygenated monoterpene. In contrast to neryl acetate, its *trans* isomer, geranyl acetate, was present in lemon flavedo in low concentration and did not vary significantly in fruit of different age.

Relative Activity of Citral and Monoterpene Esters. Figure 6 compares the effect of citral (mixture of 66% geranial and 34% neral, from Sigma) and the corresponding esters, geranyl acetate and neryl acetate (both from Sigma), on the germ-tube elongation of *P. digitatum* spores. This effect depended strongly on the concentration of compounds tested, changing from stimulation with low doses of terpenoids to inhibition at higher concentrations. Monoterpene esters, especially neryl acetate, exerted very poor inhibitory activity

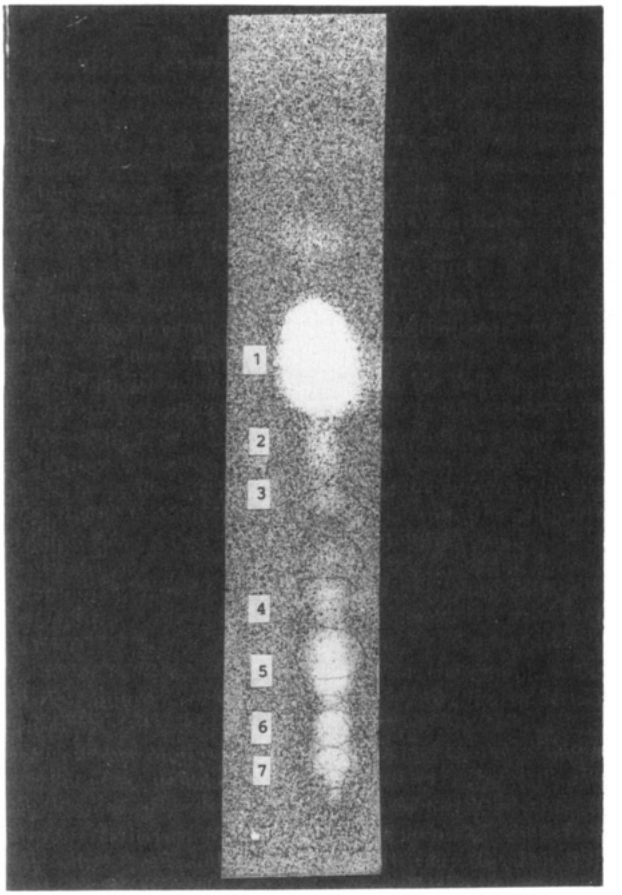

Figure 4. Detection of antifungal compounds in lemon essential oil. The essential oil was separated by thin-layer chromatography (TLC) using *n*-hexane–ethyl acetate (85:15) as the developing solvent. The developed plate was sprayed with a spore suspension of test organism (*C. cladosporioides*) and incubated at 24 °C and saturated RH for 3 days. The zones of fungal growth inhibition indicate the presence of antifungal compounds: 1, citral; 3, 5-geranoxy-7-methoxycoumarin; 4, isopimpinellin; 5, limettin; 6, unidentified psoralen derivative; 2 and 7, unidentified.

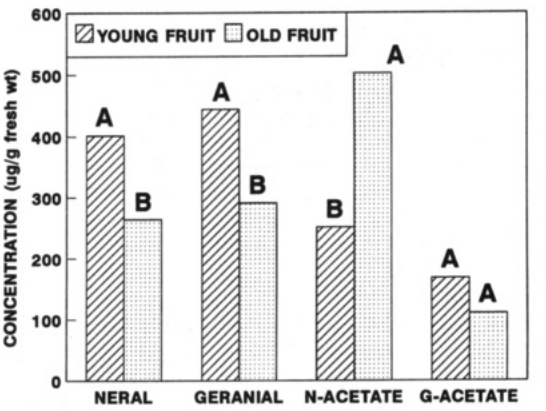

Figure 5. Effect of fruit age on concentration of citral isomers (geranial and neral) and corresponding esters (geranyl acetate and neryl acetate) in lemon flavedo. The petroleum ether flavedo extracts from young and old fruit were analyzed by gas chromatography (GC), and the concentration of monoterpenes was determined using authentic samples as external standards. Different letters indicate the statistically significant difference between young and old fruit as determined by Duncan's multiple-range test ($p = 0.05$).

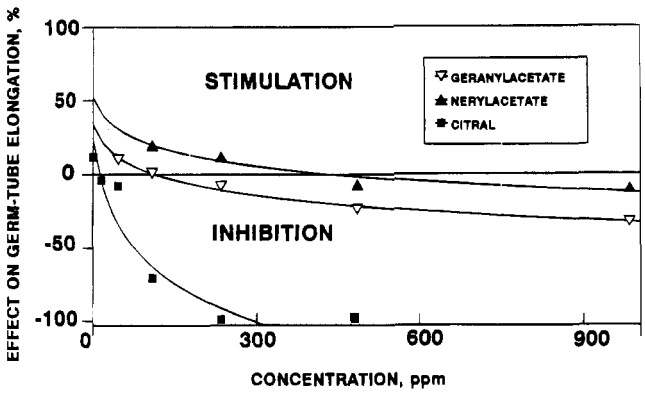

Figure 6. Effect of citral, geranyl acetate, and neryl acetate on germ-tube elongation of *P. digitatum* (logarithmic regression). Spore suspensions of *P. digitatum* (2.5×10^4 spores/mL) containing 0.5% sucrose and 0.5% fresh filtered orange juice were incubated for 20 h at 17 °C and saturated RH in the presence of different concentrations of authentic citral, geranyl acetate, and neryl acetate. The effects of materials were expressed as percentage of inhibition or stimulation of germ-tube elongation as compared with the control incubated without monoterpenes.

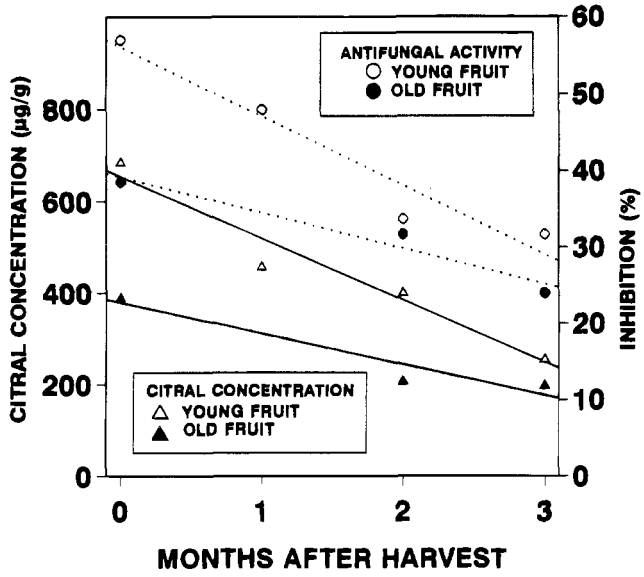

Figure 7. Effect of prolonged fruit storage on citral content (solid line) and antifungal activity (dotted line) in lemon flavedo. Young and old lemon fruit were stored at 17 °C and 85% RH. Citral content was measured after different storage periods as described in Figure 5. The antifungal activity of flavedo extracts was evaluated by inhibition of germ-tube elongation of *P. digitatum* as described in Figure 6.

toward *P. digitatum*. Moreover, in concentrations below 500 ppm, neryl acetate stimulated germ-tube elongation (Figure 6) and germination (data not shown) of the pathogen.

The integral antifungal activity of the flavedo extract from yellow fruit was almost twice as low as that of green fruit (25–38 and 48–57%, respectively).

Effect of Prolonged Storage on the Preformed Defensive System of Lemon. During the prolonged storage of lemons, the content of citral declined in parallel with the reduction of antifungal activity in flavedo extracts (Figure 7) and with the increase of decay incidence. Citral decline was more rapid in young fruit, so that after 3 months in storage the concentration of citral in young fruit was only slightly higher (by about 20%) than in the old ones (Figure 7). Of two citral isomers, geranial demonstrated faster decrease during fruit storage, as compared to neral (data not shown).

DISCUSSION

The results of this research suggest that a high level of citral in the oil glands of young lemon is related to its relative resistance to postharvest decay. In inoculation experiments, the compounds located in the oil glands of young lemon were capable of inhibiting the pathogen in situ, while the gland content of old fruit was not active and, possibly, even stimulated disease development, as described previously (Nadel-Schiffmann and Littauer, 1956). In flavedo tissue between oil glands, the resistance against pathogen invasion was related to a factor other than citral. This factor did not change significantly with fruit maturity and might be related either to the chemical composition of this tissue or to its anatomical features.

In nature, the penetration of *P. digitatum* is confined, at its early stages, to rind wounds of citrus fruit (Nadel-Schiffmann and Littauer, 1956). On the other hand, mechanical wounding was shown to cause rupture of citrus oil glands and consequent spillage of essential oil into the intercellular space of subepidermal tissue (Shomer and Erner, 1989). Considering the high density of oil glands in citrus rind, even small mechanical wounds should injure and rupture several oil glands. Consequently, the content of essential oil glands may contact a penetrating wound pathogen, such as *P. digitatum*. This suggestion is in agreement with the statement of Mansfield (1983) that most preformed antifungal materials are more effective in the inhibition of fungal development following pathogen penetration than on the plant surface.

Changes of citral concentration in lemon flavedo corresponded to the level of fruit disease susceptibility. It is accordingly proposed that citral may be one of the oil-gland-localized factors determining the resistance of lemon against pathogens. However, the contrast between young and old lemons cannot be attributed only to different citral levels. Recently, we have detected in the peel of green lemon another highly active but labile antifungal material, presumably of terpenoid nature, which is practically absent in fully colored fruit (Rodov and Ben-Yehoshua, unpublished data). The identification of this compound is currently in progress. Green and yellow lemons differ also in their capability to produce induced antifungal materials, such as the phytoalexin scoparone (Kim, 1992).

The reduced citral level in yellow lemon was accompanied by a high concentration of neryl acetate. The citral isomers, geranial and neral, and the two prenyl esters, geranyl acetate and neryl acetate, have the same acyclic monoterpene skeleton and close biogenetic origin, both being derived from the corresponding primary alcohols, respectively, geraniol and nerol (Akhila, 1985; Perez, 1988; Claon and Akoh, 1993). Similar to citral, geraniol and nerol were shown to inhibit strongly the growth of various microorganisms (Tripathi et al., 1984; Bard et al., 1988). However, the antimicrobial activity practically disappears with acetylation of the primary alcohol in the monoterpene molecule.

The increase of neryl acetate concentration in parallel with decline of citral in senescent lemon might be a result of conversion of citral into terpenyl acetates, via the corresponding alcohols. In our previous work the reduction of exogenous citral into geraniol and nerol was shown to take place in cell cultures of various plants (Rodov et al., 1988), including lemon (Rodov, unpublished data). An alternative explanation of the changing aldehyde/ester ratio might be redistribution of a common precursor in favor of neryl acetate production,

combined with ongoing degradation of citral. The biosynthesis of terpenoids in the flavedo of immature and mature citrus fruit differs at both ultrastructural and enzymatic levels (Perez, 1988; Gershenzon and Croteau, 1990).

The dosage of monoterpenes significantly affected pathogen spore viability. This observation may partly explain the conflicting reports on citral both inhibiting (Asthana et al., 1988; Onawunmi, 1989; Ben-Yehoshua et al., 1992) and stimulating (French et al., 1978) fungal development. A similar concentration-dependent shift from stimulation to inhibition of phytopathogenic fungi has been described for other natural materials such as chlorogenic acid [see Lattanzio et al. (1994)]. Additionally, the stimulative influence of citral and some aliphatic aldehydes on germination of *Penicillium* spores was observed on water agar in the absence of any other organic materials (French et al., 1978), but addition of nutrients caused these aldehydes to be inhibitory. We believe that the latter conditions more closely simulate the situation inside the rind wound of citrus fruit.

The effect of fruit storage on the level of antifungal compound in Satsuma mandarin was studied by Homma et al. (1989). These authors found in the calyx of just-harvested mature fruit an antifungal hesperidin-like substance which disappeared by the end of a 3-month storage period. The increase of stem-end rot during prolonged mandarin storage was attributed to a decline of antifungal activity in the fruit's extract. In our experiments, a decline in citral concentration was also correlated with an increase in decay of stored lemons.

Thus, the shift from relative resistance to high disease susceptibility of citrus fruit after completion of its protective biological function can be related to the changes in the amount and ratio of citral in the oil glands. These changes may be part of the mechanism determining the natural life span of citrus fruit. Extension of citrus storage life by inhibiting the decline of preformed antifungal materials is the subject of a separate publication.

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LITERATURE CITED

Akhila, A. Biosynthetic relationship of citral-*trans* and citral-*cis* in *Cymbopogon flexuosus* (Lemongrass). *Phytochemistry* **1985**, *24*, 2585–2587.

Asthana, A.; Dixit, K.; Kishore, N. Toxicity of citral and eugenol against some storage fungi. *Arch. Phytopathol. Pflanzenschutz.* **1988**, *24*, 17–421.

Bard, M.; Albrecht, M. R.; Gupta, N.; Guynn, C. J.; Stillwell, W. Geraniol interferes with membrane functions in strains of *Candida* and *Saccaromyces*. *Lipids* **1988**, *23*, 534–538.

Ben-Yehoshua, S.; Shapiro, B.; Kim, J. J.; Sharoni, J.; Carmeli, S.; Kashman, Y. Resistance of citrus fruit to pathogen and its enhancement by curing. In *Proceedings, 6th International Citrus Congress, Tel Aviv, Israel*; Goren, R., Mendel, K., Eds.; Balaban Publishing: Rehovot, Israel, 1988; pp 1371–1379.

Ben-Yehoshua, S.; Kim, J. J.; Shapiro, B.; Sharoni, J.; Henis, Y. Effect of age on resistance of mature citrus fruit against pathogens. In *Proceedings, 23rd International Horticultural Congress, Firenze, Italy*; Lo Scarabes Edizion Scientifiche: Bologna, 1990; Abstr. 2504, p 683.

Ben-Yehoshua, S.; Rodov, V.; Kim, J. J.; Carmeli, S. Preformed and induced antifungal materials of citrus fruits in relation to enhancement of decay resistance by heat and UV treatment. *J. Agric. Food Chem.* **1992**, *40*, 1217–1221.

Brady, C. J. Fruit ripening. *Annu. Rev. Plant Physiol.* **1987**, *38*, 155–178.

Claon, P. A.; Akoh, C. C. Enzymatic synthesis of geraniol and citronellol esters by direct esterification in n-hexane. *Biotechnol. Lett.* **1993**, *15*, 1211–1216.

Erickson, L. C. The generaly physiology of citrus. In *The Citrus Industry*; University of California: Berkeley, 1968; Vol. II, pp 86–126.

French, R. C.; Long, R. K.; Latterell, F. M.; Graham, C. L.; Smoot, J. J.; Shaw, P. E. Effect of nonanal, citral and citrus oils on germination of conidia of *Penicillium digitatum* and *Penicillium italicum*. *Phytopathology* **1978**, *68*, 877–882.

Gershenzon, J.; Croteau, R. Regulation of monoterpene biosynthesis in higher plants. In *Biochemistry of the Mevalonic Acid Pathway to Terpenoids*; Towers, G. H. N., Stafford, H. A., Eds.; Plenum Press: New York, 1990; pp 99–160.

Homma, A.; Arimoto, Y. Studies on citrus melanose and citrus stem-end rot by *Diaporthe citri* (Faw.) Wolf. Part 8. Distribution of pathogen inhibitory substances in citrus tissues. *Ann. Phytopath. Soc. Jpn.* **1988**, *54*, 1–8.

Homma, Y.; Takahashi, H.; Arimoto, Y. Studies on citrus melanose and citrus stem-end rot by *Diaporthe citri* (Faw.) Wolf. Part 10. Hyphal elongation of *Diaporthe citri* from citrus fruit pedicel to stem-end and behavior of inhibitins. *Ann. Phytopath. Soc. Jpn.* **1989**, *55*, 131–139.

Kim, J. J. Involvement of antifungal compounds in natural and induced resistance of citrus fruits to fungal pathogens. Ph.D. Thesis, Hebrew University of Jerusalem, 1992.

Kim, J. J.; Ben-Yehoshua, S.; Shapiro, B.; Henis, Y.; Carmeli, S. Accumulation of scoparone in heat-treated lemon fruit inoculated with *Penicillium digitatum* Sacc. *Plant Physiol.* **1991**, *97*, 880–885.

Lattanzio, V.; Cardinali, A.; Palmeri, S. The role of phenolics in the postharvest physiology of fruits and vegetables: browning reactions and fungal diseases. *Ital. J. Food Sci.* **1994**, *6*, 3–22.

Mansfield, J. W. Antimicrobial compounds. In *Biochemical Plant Pathology*; Callow, J. A., Eds.; Wiley: New York, 1983; pp 237–265.

Nadel-Schiffmann, M.; Littauer, F. Mode of infection of *Penicillium digitatum* and *P. italicum* in citrus fruits. *Ktavim* (Records of the Agricultural Research station, Rehovot, Israel) **1956**, *6*, 61–67.

Onawunmi, G. O. Evaluation of the antimicrobial activity of citral. *Lett. Appl. Microbiol.* **1989**, *9*, 105–108.

Perez, L. M. Subcellular distribution of enzymes of mono- and sesquiterpene biosynthesis in plant tissues. *Arch. Biol. Med. Exp.* **1988**, *21*, 51–58.

Rodov, V.; Bugorskii, P.; Butenko, R.; Popov, A. Capacity of higher plant tissue cultures for biotransformation of monoterpenes. *Sov. Plant Physiol.* **1988**, *35*, 413–418.

Shaw, P. E. Essential oils. In *Citrus Science and Technology*; Nagy, S., Shaw, P. E., Veldhuis, M. K., Eds.; AVI: Westport, CT, 1977; Vol. 1, pp 427–462.

Shomer, I.; Erner, Y. The nature of oleocellosis in citrus fruits. *Bot. Gaz.* **1989**, *150*, 281–288.

Tripathi, N. N.; Asthana, A.; Dixit, S. N. Toxicity of some terpenoids against fungi infesting fruits and seeds of *Capsicum annuum* L. during storage. *Phytopathol. Z.* **1984**, *110*, 328–335.

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