Plant Physiol. (1987) 84, 88–92
0032-0889/87/84/0088/05/$01.00/0

# Compositional and Thermal Properties of Thylakoid Polar Lipids of *Nerium oleander* L. in Relation to Chilling Sensitivity

Received for publication October 9, 1986

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## ABSTRACT

**The polar lipid classes from thylakoids of *Nerium oleander* L. were studied with the aim of relating changes in their composition and thermal behavior with reported changes in the transition temperature of their polar lipids and chilling sensitivity of their leaves. With an increase in growth temperature, the transition temperature of phosphatidylglycerol increased from 16°C to 26°C, and for sulfoquinovosyldiacylglycerol from 19°C to 24°C. Transitions in the other lipid classes were below −10°C for plants grown at both growth temperature. The major changes in the molecular species of phosphatidylglycerol, with increasing growth temperature, were an increase in 1-oleoyl-2-palmitoyl phosphatidylglycerol from 21 to 39% and a decrease in 1-oleoyl-2-*trans*-3-hexadecanoic phosphatidylglycerol from 51 to 25%. Although the disaturated species increased from 8 to 23%, the maximum was less than that reported for chilling-sensitive plants. There was no change in the sum of the palmitic, hexadeca-*trans*-3-enoic and stearic acids. Dipalmitoyl sulfoquinovosyldiacylglycerol increased from 12 to 20% and 1-linolenoyl-2-palmitoyl sulfoquinovosyldiacylglycerol decreased from 40 to 30%. It is concluded that the increase in the transition temperature of the polar lipids and the sensitivity of acclimated oleander plants to chilling could not be predicted by the absolute sum of the saturated fatty acids or disaturated molecular species in phosphatidylglycerol. The polar lipid transition appears to be a product of mixing of both high and low melting-point lipids.**

Plants which are sensitive to chilling show symptoms of injury when exposed to low, but nonfreezing temperatures (the chilling range, 15–0°C). The critical temperature below which this injury develops correlates with that of a phase transition in the polar lipids from membranes of these plants (18, 19, 22). The membrane polar lipids of chilling-insensitive plants do not undergo a phase transition above zero (19, 22). Thus, the sensitivity of a plant to chilling injury can be specified in terms of the temperature of this transition which is detectable by several physical techniques (18–20, 23). This avoids the uncertainties inherent in describing the thermal response of plants based on their climatic distribution.

A phase transition, in the chilling range, can be induced in leaf polar lipids of chilling-insensitive plants by the addition of a small amount of a high melting-point, disaturated phospholipid such as DPPG[1] (22). For three plants that showed varying sensitivity to chilling, the temperature of the transition was related to the proportion of lipid that contained dipalmitate (22). Thus, it was postulated that DPPG is a likely initiator of the phase transition in leaf polar lipids (22).

Murata *et al.* found that chilling sensitivity generally correlated with a high proportion of both saturated fatty acids and disaturated molecular species in PG (13, 14). Further surveys (6, 15, 17, 24), involving larger numbers of species, generally supported this correlation though there were a few exceptions. For these surveys (6, 13–15, 17, 24), the classification of a plant as sensitive or resistant to chilling was based on either their climatic distribution or their reported physiological response to a chilling stress. The critical temperature below which injury develops and/or the temperature of the transition in the leaf polar lipids was not determined. Comparisons of the chemical and thermal properties of membrane lipids on the one hand and the susceptibility of plants to chilling on the other, were predicated on the assumption that sensitivity to chilling is directly related to a phase transition in the membrane polar lipids (14). Since the transitions were not determined, these surveys only provide information about the composition of PG from a wide variety of plant species. The lack the details necessary to draw definitive conclusions about the relationship between lipid composition and the sensitivity of the plant to chilling.

As an initial step in understanding the relationship between chilling sensitivity and the properties of membrane lipids, the composition and thermal properties of the thylakoid polar lipids of *Nerium oleander* L. (oleander) were examined. Oleander is a perennial that grows over an extremely wide range of temperatures (2). The transition temperature of the thylakoid polar lipids can be altered by varying the growth temperature such that a clone can be produced with a transition typical of either chilling-sensitive or chilling-insensitive plants (23). Thus, oleander provides a system well suited for studies aimed at determining which lipid components contribute to the transition in the chilling range, without the complexities inherent in comparing different plant species.

## MATERIALS AND METHODS

**Plant material.** Cuttings of *Nerium oleander L.* from a single plant were maintained in controlled growth cabinets at 25°C. For experimental material, the plants were grown at 20°C/15°C (20°-plants) or 45°C/35°C (45°-plants), day/night temperatures, with a 14 h photoperiod for at least 7 d. The light intensity at the surface of the leaves used varied from 400 to 600 μmol photons $m^{-2}$ $s^{-1}$.

**Thylakoid Isolation.** Thylakoids were isolated from mature leaves essentially as described by Raison *et al.* (21). After the first centrifugation at 10,000*g*, thylakoids in the precipitate were resuspended in 5 mM NaCl, loaded onto a cushion of 2 M sucrose, and centrifuged at 10,000*g* for 15 min in a swing-out rotor (HB-

[1] Abbreviations: DPPG, dipalmitoyl phosphatidylglycerol; PG, phosphatidylglycerol; BHT, butylated hydroxytoluene; SQDG, sulfoquinovosyldiacylglycerol; PI, phosphatidylinositol; MGDG, monogalactosyldiacylglycerol; DGDG, digalactosyldiacylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; 16:0, palmitic acid; t16:1, hexadeca-trans-3-enoic acid; 18:0, stearic acid; 18:1, oleic acid; 18:2, linoleic acid; 18:3, linolenic acid.

4, Sorvall, Dupont Instruments). This step was included to reduce starch and mitochondrial contamination. The thylakoids, which formed a tightly packed band at the interface of the sucrose and salt solution, where removed, resuspended in 5 mM NaCl, and centrifuged at 10,000*g* for 20 min.

**Lipid Extraction and Purification.** Lipids were extracted from the thylakoids with three washes of $CHCl_3$:$CH_3OH$ (2:1 v/v) containing BHT (0.01% v/v). The extract was filtered and the non-lipid material removed by washing twice with 0.55 M KCl as described by Folch *et al.* (7). Total lipids were loaded onto an activated silica gel (Biosil A, Bio-Rad Laboratories, Richmond, CA) column at 10 mg lipid/g gel. Components were eluted from the column using 6 ml/g gel of the following solvents: $CHCl_3$ and $CHCl_3$:$CH_3COCH_3$ (85:15 v/v), for the neutral lipids, followed by $CH_3OH$ and $CH_3OH$:$H_2O$ (85:15 v/v), for the polar lipids. The anionic lipids (SQDG, PG, and PI) were separated from the remaining polar lipids (MGDG, DGDG, PC, and PE) essentially as described by Murata *et al.* (14). The lipid classes were separated by preparative TLC on silica gel HR (Merck) plates with $CHCl_3$:$CH_3OH$:$CH_3COOH$:$H_2O$ (85:15:10:3 v/v) containing BHT (0.01 v/v). Lipids were visualized by iodine vapor or primulin. The amount of MGDG, DGDG, and SQDG were estimated by sugar analysis (25) and the amounts of PG, PI, PC, and PE were estimated by phosphorus analysis (1).

**Calorimetry.** Lipids (1–3 mg), dissolved in $CHCl_3$, were loaded into stainless steel pans (70 μl) and the solvent evaporated by a stream of $N_2$ at 30°C and completely removed under vacuum for at least 30 min. Buffer (20 mM Tris/acetate [pH 7.2] containing 2 mM EDTA) was added to give 200% (v/w) excess. The pans were sealed and equilibrated at 37°C for several hours. Thermal behavior was determined by differential scanning calorimetry (model DSC-2, Perkin-Elmer, Norwalk, CT). Thermograms were recorded at scan rates of 5°C/min or 10°C/min against a reference pan containing Sephadex and buffer of similar mass to that of the lipid sample. The instrument was calibrated for temperature and heat capacity using an aqueous dispersion of dimyristoyl phosphatidylcholine, melting-point, 23°C.

**Gas Liquid Chromatography.** Methyl esters of the fatty acids of the lipid classes were formed as described by Morrison and Smith (12). The methyl esters were separated by GLC (model 438A, Packard Instrument Co.) using a capillary (WCOT fused silica, CP-SIL 58, Packard Instrument Co.) column and their relative proportions calculated from the area of the peak by integration (Spectra-Physics SP4270).

**High Performance Liquid Chromatography.** The *p*-methoxybenzoate derivatives of PG and SQDG (1 mg) were produced as described in (5, 9). The derivatives were dissolved in 100 μl acetonitrile and 5 to 10 μl aliquot was injected onto a 150 × 3.9 mm Resolve, reverse phase, stainless steel column (Waters Associates). The molecular species were separated isocratically using acetonitrile:isopropanol 65:35 solvent mixture at a flow rate of 0.5 ml $min^{-1}$ (3). As the methoxybenzoate ring is the only absorbing structure in the molecule at 254 nm, direct quantitation was made by cutting out and weighing each peak (Table III). The molecular species were identified by comparison of retention times with those of standards as well as from a plot of relative retention time as a function of carbon and double bond numbers in acyl pairs (3, 4).

## RESULTS

The polar lipids from the thylakoids of oleander have been shown to undergo a phase transition which increases from about −2 to 7°C with an increase in growth temperature from 20 to 45°C (23).

The thermal response of the classes of the polar lipids are shown in Figure 1. For PG and SQDG, transitions were detected above 0°C. For PG the mean (±SD,*n* = 8) of the temperature of the transition increased from 16 ± 3 to 26 ± 3°C and for SQDG, from 19 ± 3 to 24 ± 3°C, with the increase in growth temperature. For both 20°- and 45°-plants, the variation in the transition temperature was not related to the percentage of any particular saturated or unsaturated fatty acid (data not shown). No transition was evident in DGDG or PC over the temperature range examined (Fig. 1). As hydrated suspensions of MGDG do not form lamellar phase (26) its thermal behavior was not examined.

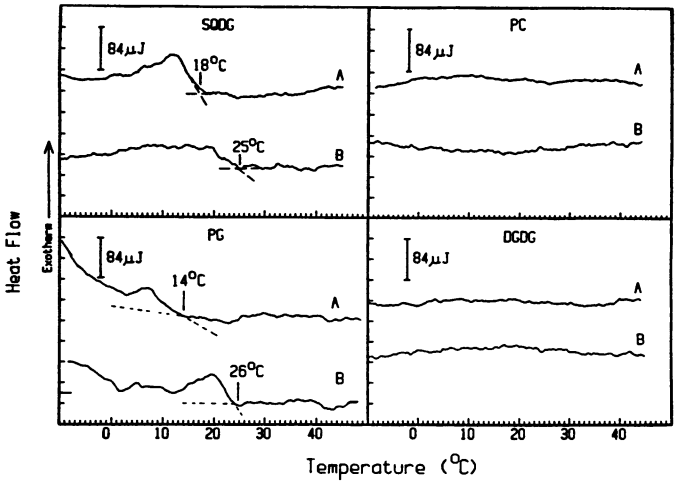


FIG. 1. Thermal response of the PG, SQDG, PC, and DGDG lipid classes of the thylakoid polar lipids isolated from oleander grown at 20°C and 45°C. Cooling rate was 5°K $min^{-1}$ and the sensitivity was 0.42 mJ $s^{-1}$. Traces for lipids from 20°-plants are labeled A while those of 45°-plants are labeled B.

Table I. *Relation between Thylakoid Membrane Lipids and Growth Temperature*

The values are the mean ± SD for *n* samples. For total and polar lipids *n* = 11; for the lipid classes *n* = 3.

| Lipids | Growth Temperature | |
|---|---|---|
| | 20°C | 45°C |
| Total (% w/w of leaf tissue) | 0.57 ± 0.09 | 0.61 ± 0.06 |
| Polar (% w/w of total) | 62 ± 10 | 61 ± 5 |
| MGDG (mol %) | 51 ± 1 | 52 ± 2 |
| DGDG | 29 ± 4 | 30 ± 2 |
| SQDG | 6 ± 1 | 6 ± 2 |
| PG | 6 ± 1 | 5 ± 1 |
| PC | 3 ± 2 | 5 ± 1 |
| PE | 3 ± 2 | 2 ± 1 |
| PI | 1.2 ± 0.1 | 1.3 ± 0.8 |

Table I lists the amount of total and polar lipids as well as the proportion of each lipid class found in the thylakoids of oleander. Apart from the small amount of PE and PI, the polar lipid classes present are typical of thylakoids (8). The results show that there was no significant increase in the amount of total or polar lipid or any of the lipid classes with the increase in growth temperature. PE and PI are not considered constituents of the thylakoid membranes (8) and they do not undergo transitions above −10°C (data not shown) and therefore they were not further investigated.

Table II compares the fatty acid composition of the polar lipid classes from the thylakoid membranes of 20°-plants with those of 45°-plants. With the increase in growth temperature, the percentage of 16:0 in PG increased from about 26 to 42%, with a concomitant decrease in the percentage of *t*16:1 from about 29 to 15%. For SQDG, there was an increase in 16:0 from about 45 to 55% with a concomitant decrease in the percentage of 18:3 from about 31 to 19%. The only significant changes in the fatty acid composition of PC was an increase in the percentage of 18:1

Table II. *Changes in the Proportion of Fatty Acids of Thylakoid Polar Lipids with Growth Temperature*

| Lipid Class | Growth Temperature | Fatty Acid | | | | | |
|---|---|---|---|---|---|---|---|
| | | 16:0 | *t*16:1 | 18:0 | 18:1 | 18:2 | 18:3 |
| | °C | %[a] | | | | | |
| MGDG | 20 | 3.3 ± 0.3 | | 0.6 ± 0.1 | 1.3 ± 0.4 | 7.0 ± 0.6 | 87.4 ± 0.9 |
| | 45 | 6.1 ± 1.3 | | 1.2 ± 0.3 | 4.4 ± 0.7 | 10.7 ± 0.8 | 77.4 ± 4.2 |
| DGDG | 20 | 20.7 ± 0.9 | | 4.7 ± 0.9 | 3.5 ± 1.4 | 7.7 ± 1.8 | 62.8 ± 2.2 |
| | 45 | 23.6 ± 0.8 | | 5.3 ± 0.3 | 4.5 ± 0.5 | 9.1 ± 0.5 | 55.9 ± 1.5 |
| SQDG | 20 | 44.5 ± 1.1 | | 3.9 ± 0.7 | 11.9 ± 0.6 | 8.0 ± 1.4 | 31.3 ± 2.7 |
| | 45 | 55.4 ± 5.6 | | 5.1 ± 1.2 | 14.6 ± 2.3 | 6.1 ± 1.8 | 18.7 ± 5.4 |
| PG | 20 | 25.9 ± 5.7 | 28.5 ± 4.4 | 2.8 ± 0.4 | 33.1 ± 3.4 | 7.1 ± 2.0 | 3.4 ± 1.2 |
| | 45 | 41.6 ± 2.4 | 15.1 ± 2.9 | 3.8 ± 0.6 | 32.7 ± 2.4 | 3.6 ± 0.5 | 2.7 ± 0.6 |
| PC | 20 | 30.1 ± 6.0 | | 4.5 ± 0.5 | 12.0 ± 3.1 | 29.7 ± 3.2 | 21.9 ± 6.0 |
| | 45 | 33.6 ± 3.3 | | 4.6 ± 0.8 | 25.6 ± 1.3 | 15.0 ± 1.8 | 20.9 ± 1.8 |

[a] Percentages might not add to 100% as values less than 0.5% have not been included. The values are the mean ± SD for five samples.

and a decrease in the percentage of 18:2 with an increase in growth temperature. For MGDG, the percentage of 18:3 decreased with growth at high temperature with a concomitant increase in the percentage of all other fatty acids. There were only minor changes in the fatty acid composition of DGDG.

Table III shows the molecular species analysis of PG and SQDG from thylakoids of 20°- and 45°-plants. For PG, there was an increase in 1-oleoyl-2-palmitoyl PG from about 21 to 39% and a decrease in 1-oleoyl-2-*trans*-3-hexadecanoyl PG from about 51 to 25%, with an increase in growth temperature. There was an increase in the proportion of all the desaturated molecular species with dipalmitoyl PG increasing to the greatest extent from about 2.6 to 11%. The sum of the desaturated molecular species in PG increased from 8 to 23%. For SQDG, the major changes in molecular species was an increase in the proportion of dipalmitoyl SQDG from 12 to 20% and a decrease in that of linolenoyl-palmitoyl SQDG from 40 to 30%.

The positional distribution of the fatty acids of SQDG was not determined as the HPLC system used to analyze the molecular species of SQDG and PG does not separate positional isomers. However, for PG from thylakoid membranes, it has been established (14) that C18 fatty acids are esterified exclusively to the C-1 position of the glycerol moiety while *t*16:1 is esterified exclusively to the C-2 position and 16:0 can be esterified to both the C-1 and C-2 positions. Thus, the molecular species of PG are limited to the positional distribution shown in Table III except for the small amount of 18:1/18:0 which could also be 18:0/18:1 with respect to the C-1 and C-2 positions.

## DISCUSSION

As *Nerium oleander* is a desert perennial which is able to acclimate to different growth temperatures (2), it would not

Table III. *Changes in the Proportion of the Molecular Species of Phosphatidylglycerol and Sulfoquinovosyldiacylglycerol with Growth Temperature*

The values are the mean ± SD of two determinations of two to three plants for PG and four plants for SQDG.

| Molecular Species C1/C2 | Molecular Species | | | |
|---|---|---|---|---|
| | PG growth temperature | | SQDG growth temperature | |
| | 20°C | 45°C | 20°C | 45°C |
| | % | | | |
| 18:3/18:3 | –[a] | – | 3.2 ± 1.0 | 2.4 ± 0.4 |
| 18:3/18:2 | – | – | 3.0 ± 1.2 | 2.2 ± 0.3 |
| 18:2/18:2 | – | – | +[b] | + |
| 18:3/18:1 | – | – | 3.6 ± 0.7 | 3.3 ± 0.4 |
| 18:3/t16:1 | 2.2 ± 0.1 | 1.2 ± 0.5 | – | – |
| 18:3/16:0 | 2.2 ± 0.3 | 2.2 ± 0.9 | 40.2 ± 4.9 | 30.2 ± 0.3 |
| 18:2/18:1 | – | – | 1.4 ± 0.4 | 2.1 ± 0.5 |
| 18:2/t16:1 | 7.9 ± 1.1 | 3.0 ± 0.6 | – | – |
| 18:2/16:0,18:0/18:3[c] | 13.0 ± 1.2 | 12.9 ± 1.7 | 5.7 ± 0.1 | 3.6 ± 0.9 |
| 18:1/t16:1 | 50.8 ± 1.3 | 24.7 ± 1.0 | – | – |
| 18:0/18:2,18:1/18:1 | – | – | + | 1.5 ± 0.4 |
| 16:0/t16:1 | 4.6 ± 2.3 | 4.6 ± 0.9 | – | – |
| 18:1/16:0 | 21.4 ± 2.4 | 39.2 ± 1.8 | 18.3 ± 3.6 | 21.9 ± 4.3 |
| 16:0/16:0 | 2.6 ± 0.4 | 11.1 ± 2.1 | 11.8 ± 3.0 | 19.8 ± 3.6 |
| 18:1/18:0 | 2.1 ± 0.2 | 2.2 ± 0.3 | 1.1 ± 0.4 | + |
| 18:0/t16:1 | + | 1.4 ± 0.9 | – | – |
| 18:0/16:0 | 0.7 ± 0.1 | 4.1 ± 0.8 | + | 1.2 ± 0.7 |
| 18:0/18:0 | + | 1.9 ± 1.1 | 1.8 ± 0.8 | 1.6 ± 0.7 |

[a] Not detectable. [b] <0.5% of molecular species. [c] For PG, % represents that of 18:2/16:0 only.

normally be considered a chilling sensitive plant. However, when oleander is grown under a 45°C/37°C day/night regime, the polar lipids undergo a phase transition at about 7°C which is typical of a chilling-sensitive plant (20, 23). As well, measurements of the photosynthetic capacity of 45°-plants show that marked inhibition occurs when leaf tissue is illuminated at temperatures below about 8°C (10). This physiological response is typical of a chilling-sensitive plant (10). Thus, in respect of the thermal properties of their polar lipids and the physiological response of their leaf tissue, 45°-plants behave similarly to chilling-sensitive plants. Thylakoids from plants grown at 20°C/15°C do not show a polar lipid transition above zero nor do they show any inhibition of photosynthetic capacity in the chilling range (10). Thus, 20°-plants behave similar to chilling-insensitive plants. Since, for oleander, there is a good correlation between the transition temperature and the response of the tissue to chilling (10), thylakoids from the two temperature groups provide ideal material for investigating the relation between the composition and phase behavior of the membrane polar lipids and the susceptibility of the plants to chilling. This avoids the complexities inherent in comparisons of plants from different species.

The polar lipids of plant membranes are heterogeneous with respect to lipid class and these classes contain many molecular species (8). It follows that the transition in the thylakoid polar lipids, in the chilling range, could well be the result of the mixing of a number of particular lipids of varying thermal properties. Therefore, an initial step in understanding the factors which regulate the shift of the transition is to examine the relation between the magnitude of the shift in the transition and factors such as changes in the quantity of total and polar lipids as well as the thermal behavior and molecular species composition of the lipid classes. The shift of about 10 C degrees in the transition temperature of the polar lipids of oleander, with change in growth temperature (23), cannot be attributed to changes in either the quantity of lipid or the percentage of any lipid class (Table I). With regard to the thermal behavior of the lipid classes, PG and SQDG (Fig. 1) shifted their transition in the same direction as that of the polar lipids (23) suggesting that PG and SQDG play a role in regulating the temperature of this transition. For SQDG and PG, the transition temperature is between 18°C and 30°C (Fig. 1), well above 10°C, the maximum temperature for the transition in the polar lipids (23). Therefore, the SQDG and/or PG must mix with lower melting-point lipids to produce the transition in the polar lipids at temperatures in the chilling range. Thus, neither PG nor SQDG, alone, could be the determinant of the transition temperature. The lower melting-point lipids, are probably derived from the DGDG, MGDG, and PC classes which undergo transitions well below zero (Fig. 1). This conclusion is supported by reconstitution experiments which showed that, for mung bean (*Vigna radiata*), all the constituent lipid classes need to be present to produce a transition at the same temperature as that of the original polar lipids (22). Thus, in studies aimed at determining how the composition and thermal properties of the membrane polar lipids might relate to the sensitivity of plants to chilling, it is important to consider all of the lipids since they could all influence the temperature of the transition.

The thermal behavior of a lipid class is governed, primarily, by the melting point and positional distribution of its constituent fatty acids (11). It has been recently found that dipalmitoyl SQDG undergoes a transition at 42°C (4). Thus, the increase in the transition temperature of the SQDG (Fig. 1), with an increase in growth temperature, is consistent with the increase in the proportion of this desaturated molecular species from 12 ± 3% to 20 ± 3% (Table III). The presence of a significant proportion of this high melting-point disaturated molecular species (Table III) also explains the high transition temperature observed for this lipid (Fig. 1). Thus, SQDG could be the main lipid component involved in the phase transition in the thylakoid polar lipids in the chilling range, a conclusion contrary to that of Murata and Hoshi (16) which was based on the results of an analysis of a number of plant species.

For similar comparisons between the fatty acid composition of PG and its transition temperature, consideration must be given to the change in the amount of *t*16:1 present in PG. Due to the physical constraints imposed by the *trans* double bond and its position in the acyl chain, it is probable that *t*16:1 has similar thermal properties to 16:0 (14). Murata *et al.* (14) postulated that the PG from leaf polar lipids of plants considered susceptible to chilling injury, contain a higher proportion of 16:0 plus *t*16:1 fatty acids than those considered insensitive to chilling. Roughan (24) extended this proposition to include 18:0 and found that there were several exceptions in comparisons between sensitive and insensitive species from different genera. The increase in the transition for the PG, with an increase in growth temperature (Fig. 1), correlates with the increase in the percentage of 16:0 (Table II). The proportion of *t*16:1 decreased under the same conditions and 18:0 remained unchanged (Table II). Thus, even though the 20°- and 45°-plants are genetically the same, but differ in their sensitivity to chilling, there is no significant change in the sum of the saturated fatty acids in PG; that is 56 ± 3% and 61 ± 4% (mean ± SD), respectively. In addition, for a comparison of two populations of mangroves, *Avicennia germinans*, which vary in their tolerance to chilling (17), the sum of the saturated fatty acids in PG was similar. Thus, knowing the fatty acid composition of the lipid classes would not necessarily allow prediction of the transition temperature of the polar lipids and thus the sensitivity of the plants to chilling injury.

Murata (13) found that the proportion of dipalmitoyl plus 1-palmitoyl-2-*trans*-3-hexadecanoyl in PG, was higher for chilling-sensitive plants, ranging from about 25 to 65%, than for chilling-insensitive plants in which they made up only about 3 to 19% of the PG molecular species. On this premise, both the 20°C- and 45°C-plants would be classified as chilling-insensitive as their PG contains only about 7 and 16%, respectively, of these molecular species. Similarly, Norman *et al.* (17) found that even though there was a high proportion of saturated molecular species in the PG from a chilling-sensitive population of mangrove, the high absolute value for the sum of the saturated molecular species of the PG from a chilling-insensitive population of the species, would have also classified it as a chilling-sensitive plant (13). Thus, the view that sensitivity to chilling is related to the absolute level of disaturated molecular species in PG, as proposed by Murata (13), is not substantiated in the comparisons of species which acclimate in their susceptibility to chilling.

It is important to note that in only one study was the composition of PG related to its transition temperature (15). Furthermore, in none of these studies was the sensitivity of the plants to chilling categorized in terms of the temperature of the phase transition in the membrane polar lipids. For oleander, the relationship between the temperature of the transition in the thylakoid polar lipids and the molecular species composition of PG is only in partial agreement with Murata's hypothesis (13–15). Furthermore, to produce a transition in the polar lipids at the temperatures usually associated with chilling injury (0–15°C), it would be necessary for the PG to mix with some low melting-point lipid component. Thus, until it is known how the high and low melting-point lipids combine to produce the transition in the chilling range, it would be inappropriate to attempt to predict sensitivity to chilling of a plant from the proportion of saturated fatty acids or disaturated molecular species in PG or in any other lipid class.

*Acknowledgment*—The authors wish to express their deep appreciation of the

assistance given by the late David Bishop in the analysis of the molecular species of PG and SQDG.

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