Effects of Ozone and Peroxyacetyl Nitrate on Polar Lipids and Fatty Acids in Leaves of Morning Glory and Kidney Bean

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ABSTRACT
To compare the effects of ozone and peroxyacetyl nitrate (PAN) on leaf lipids, fatty acids and malondialdehyde (MDA), morning glory (Pharbitis nil Choisy cv Scarlet O’Hara) and kidney bean (Phaseolus vulgaris L. cv Gintebo) plants were exposed to either ozone (0.15 microliter per liter for 8 hours) or PAN (0.10 microliter per liter for up to 8 hours). Ozone increased phospholipids in morning glory and decreased in kidney bean at the initial stage (2-4 hours) of exposure, while it scarcely changed glycolipids, the unsaturated fatty acids, and MDA in both plants. A large reduction of glycolipids occurred 1 day after ozone exposure in both plants. PAN caused marked drops in phospholipids and glycolipids in kidney bean at relatively late stage (6-8 hours) of exposure, while it increased phosphatidic acid and decreased the unsaturated fatty acids, an increase which was accompanied by a large increase in MDA. These results suggest that ozone may not directly oxidize unsaturated fatty acids at the initial stage of exposure, but may alter polar lipid metabolism, particularly phospholipids. On the other hand, PAN may abruptly and considerably degrade phospholipids and glycolipids by peroxidation or hydrolysis at the late stage of exposure. The present study shows that ozone and PAN affect polar lipids in different manners.

Ozone and PAN\(^1\) are the main phytotoxic components of photochemical air pollutants. In Japan, atmospheric oxidant (mostly ozone) concentrations have often been recorded above 0.12 \(\mu\)L/L, the standard value for issue of photochemical oxidant warning, in urban areas (16). Atmospheric PAN concentrations averaged 0.001 to 0.002 \(\mu\)L/L or less, with occasional peaks of between 0.01 and 0.03 \(\mu\)L/L (17). Ozone and PAN cause visible injuries to many susceptible plant species in Japan. Ozone causes bleached spots, pigmentation stippling, and necrosis on the upper surface of mature leaves, while PAN causes damage such as silvering, metallic glazing, and bronzing on the lower surface of young expanding leaves. The development of visible injury symptoms results from biochemical and physiological changes. The initial effects of oxidants are to alter the membrane permeability, allowing leakage of the cell contents into the extracellular spaces and leading to an osmotic or ionic imbalance within the cell (7).

It has been considered that membrane lipids may be one of the primary sites of ozone injury (7), since ozone in vitro rapidly reacts with double bonds leading to the production of oxidation products (7). Indeed, Pauls and Thompson (19, 20) have reported that chemical and physical changes in membrane lipids such as the breakdown of phospholipids, the loss of sterols from the membrane, and the formation of gel phase lipid membrane were induced by treating the isolated microsomal membrane from bean leaves with ozone in solution. However, the results obtained from isolated systems have not been confirmed in plant tissues.

PAN reacts with the sulphydryl group to form either disulfide or S-acetyl group and also reacts with olefine to form epoxides (14). Such reactions could affect the proteins and the lipids of membranes. Ozone not only destroys the double bond in unsaturated fatty acids, but also affects lipid biosynthesis (12, 13). Lipid synthesis from acetate and acetyl-CoA (12) and the incorporation of galactose from UDP-galactose into galactolipids (13) were inhibited by bubbling ozone through a chloroplast suspension. PAN in vitro also inhibited the synthesis of fatty acids from acetate (11). Thus, exposure to ozone and PAN seems to alter the membrane lipids in leaf tissues.

The present study was initiated to compare the effects of ozone and PAN on the leaf lipids, fatty acids, and MDA of two different plants. Morning glory and 6 to 7 d old kidney bean plants were exposed to either ozone (0.15 \(\mu\)L/L for 8 h) or PAN (0.10 \(\mu\)L/L for 8 h) and then examined for changes in their fatty acids, polar lipids, and MDA that occurred immediately after or 24 h after the exposure periods.

Morning glory and kidney bean are plants susceptible to ozone, and morning glory in particular has been used as an indicator plant of photochemical oxidants (ozone) in Japan (16). Kidney bean is also susceptible to PAN; for example, it was injured when exposed to 0.030 to 0.045 \(\mu\)L PAN/L for 4 h (18). Therefore, these two plants were selected as the testing materials. There are several reports of the effects of ozone on lipids and fatty acids in leaves exposed to very high concentrations such as 0.30 to 0.50 \(\mu\)L ozone/L (6, 23, 26, 28), but no reports of exposure to low ozone concentrations. Therefore, 0.15 \(\mu\)L ozone/L was chosen in this study. On the other hand, there are no reports of effects of PAN on the leaf lipids and fatty acids in leaves. Concentration of 0.10 \(\mu\)L PAN/L was chosen because it produced injury to kidney bean from the moderate to the severe within 8 h exposure.

MATERIAL AND METHODS

Plant Materials. Two seeds of morning glory, Pharbitis nil Choisy cv Scarlet O’Hara, were planted in each plastic pot (soil surface area: 200 cm\(^2\), pot height: 19 cm) and one seed of kidney bean, Phaseolus vulgaris L. cv Gintebo, was planted per plastic pot (soil surface area: 100 cm\(^2\), pot height: 6 cm) containing a commercial culture soil (Kureha Chemical Co. Ltd). Morning

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\(^1\) Abbreviations: PAN, peroxyacetyl nitrate; MGDG, monogalactosyldiglyceride; DGDG, digalactosyldiglyceride; SQDG, sulfoquinovosyldiglyceride; PC, phosphatidyl choline; PE, phosphatidyl ethanolamine; PG, phosphatidyl glycerol; PI, phosphatidyl inositol; PA, phosphatidic acid; MDA, malondialdehyde.
glory and kidney bean plants were grown in a naturally lit, environment-controlled glass chamber (2 m [d] × 2 m [w] × 1.8 m [h]) equipped with activated charcoal filters. The conditions in the chamber were maintained at 27/22°C and 70/80% day/night for temperature and relative humidity, respectively. Morning glory plants with 12 to 14 leaves about 5 weeks after germination were used for ozone exposure, and kidney bean plants with primary leaves expanded fully at 6 to 7 d after germination were used for ozone and PAN exposure.

Ozone and PAN Exposure Procedures. Ozone was generated by passing air through a high voltage discharging tube and was introduced into the naturally lit, environment-controlled glass chamber in which the plants were placed. Environmental conditions during ozone exposure were the same as those for the growing periods. The concentration of ozone in the glass chamber was maintained at 0.15 ± 0.01 μL/L and monitored with a UV absorption ozone analyzer (Dashihi, model 1003AH) which was dynamically calibrated with a 1% neutral buffered KI method.

Synthesis of PAN and determination of PAN concentration were by the methods described previously (18). PAN exposure was made in an exposure chamber (0.6 m [d] × 1.0 m [w] × 1.1 m [h]) made of acryl resin. The chamber was installed in a larger environment-controlled chamber (1.3 m [d] × 1.0 m [w] × 1.1 m [h]) equipped with 9 metal halide lamps (Yoko Lamp 400 W: Toshiba). The light intensity was about 400 μE m⁻² s⁻¹ at the center of the exposure chamber. Temperature in the exposure chamber was kept at 27°C, but relative humidity varied from 60 to 80% by transpiration from leaves. The volume of incoming air which contained PAN was approximately 15 L min⁻¹, and the air velocity in the chamber was controlled at 0.5 ms⁻¹ by two propellers. PAN concentration in the exposure chamber was monitored by using a gas chromatograph with an electron capture detector (Yanagimoto Co. Ltd, PAN monitor, model GPH-10A).

A teflon tube, 450 mm long and 3 mm in diameter, and packed with 5% polyethylene glycol on Chromosorb W (AW DMCS, 60–80 mesh) was used for the GC column.

Lipid Analysis. Twenty leaf discs (10 mm in diameter), cut from interveinal areas of leaves, were excised either immediately after or 24 h after the end of a given exposure. These leaf discs were immersed 3 min in boiling water to inactivate lipases. Lipids were extracted according to the method of Bligh and Dyer (3), dissolved in 8 ml of chloroform containing 0.05% (w/v) α-tocopherol, and stored under N₂ at −20°C. The crude lipid solution concentrated under N₂ was spotted on a silica gel plate (Merck; 5717 Silica Gel 60F₂₅₄). The plate was developed in the first direction with chloroform-methanol-water (65:25:4, v/v) and in the second direction with chloroform-methanol-10% ammonia (65:35:5, v/v) (1). The chromatogram was viewed under UV after spraying with 0.01% (w/v) primuline in 80% acetone. Individual polar lipids were identified by co-chromatography with authentic standards and by their reaction with specific spray reagents of anthrone sulfuric acid, Dittmer, Dragendorff, ninhydrin, and iron (III) chloride specific to glycolipid, phospholipid, the choline group, the amino group, and sterol, respectively. Each fluorescent lipid area was scraped from the plate and subjected to methanolysis with 10% (w/v) sulfuric acid in methanol at 40°C overnight with a given amount of heptadecanoic acid (Sigma) as an internal standard. A small amount of the lipid extract before TLC separation was also subjected to methanolysis to determine the total fatty acid content.

The fatty acid methyl esters were extracted with n-hexane and chromatographed on a GLC (Shimadzu GC-9A) equipped with a hydrogen flame ionization detector and an integrator (Shimadzu, Chromatopac C-R1B). Separations were carried out at 200°C using a glass column (5 mm × 2 m) packed with 5% Thermo-X-1000 (Wako Pure Chem.) on Chromosorb W (AW DMCS, 80–100 mesh). The fatty acids were identified by their retention time relative to that of standard methyl ester mixtures (Applied Science Laboratories). Quantitative analyses of the fatty acids and their parent lipid were made according to Allen and Good (1).

MDA Content. MDA content in leaves was assayed according to the method of Heath and Packer (8). The discs excised from leaves were homogenized in distilled water. Three ml of leaf homogenate in distilled water were mixed with 5 ml of 0.5% thiobarbituric acid in 20% trichloroacetic acid. The mixture was incubated in a boiling water bath for 30 min. After centrifugation, the MDA content in the supernatants was determined by using the difference absorbance coefficient of 155 mm⁻¹ cm⁻¹ at 532 and 600 nm (8).

RESULTS

Effects of Ozone on Fatty Acid Content and Composition in Morning Glory Leaves. Morning glory plants were exposed to 0.15 μL ozone/L for 0, 2, 4, 6, and 8 h under natural light. In morning glory plants exposed to 0.15 μL ozone/L, water-soaked spots appeared on the upper surface of leaves at 6 h after the beginning of exposure and then spread over the interveinal area. At 1 d after exposure, numerous small bleached spots had appeared on leaves exposed to ozone for 4, 6, and 8 h, and the injured area was 40 to 60% of the leaf area. For the lipid assay, the most sensitive leaves that were third or fourth from the bottom were used.

Figure 1 shows the changes in the total fatty acid content and composition in morning glory leaves immediately after and 24 h after the end of 0.15 μL ozone/L exposure. The total fatty acid content and composition in control leaves stayed almost constant during the course of the experiment. The total fatty acid content and composition in morning glory leaves exposed to ozone were almost the same when compared to those of control until 6 h of exposure, and then significantly decreased to 86% of the control level at 8 h. At 8 h the decreased fatty acids, linoleic acid (C18:2) and linolenic acid (C18:3), reached 82 and 72% of the control level, respectively. Twenty-four h after exposure, the contents of total fatty acids and C18:3 were similar to those immediately after the end of exposure. But a slight increase in content of palmitoleic acid (C16:1) in 2 to 8-h-exposed leaves and a reversion to control level of C18:2 in 8-h-exposed leaves were observed.

Effects of Ozone on Fatty Acid Content and Composition in Kidney Bean Leaves. Ozone exposure and assays of fatty acids and polar lipids of kidney bean leaves were carried out in the same way as with morning glory. Obvious water-soaked spots did not appear on kidney bean leaves during exposure. However, when plants were left 1 d after the end of each period of exposure, injury occurred for every period of exposure. Numerous bleached spots appeared at 2, 4, and 6 h, and the percentage of foliar injury was 20 to 30% for each. The percentage of foliar injury at 8 h was about 50%, and injury symptoms were mixed with bleached spots and reddish brown stipple on the upper surface of the leaves.

The diurnal changes in the total fatty acid content and composition in control leaves of 6 to 7-old kidney bean were compared to the results obtained immediately after and 24 h after the end of exposure (Fig. 2). The total fatty acid content remained approximately unchanged during the daytime under natural light, but its components varied; C18:2 began to increase accompanied by a decrease of C18:3 from the morning to the evening. During exposure to ozone, total fatty acid content and composition were nearly equal when compared to those of control. Twenty-four h after the end of exposure, the total fatty acid content also remained approximately unchanged, but a slight increase in content of C16:0 was observed in 6 to 8-h-exposed leaves.
Effects of PAN on Fatty Acid Content and Composition in Primary Leaves of Kidney Bean. Kidney bean plants were exposed to 0.10 μl PAN/L for 0, 1, 2, 4, 6, and 8 h under artificial light. Kidney bean leaves showed water-soaked lesions on the upper surface at 6 h. When the plants were left 1 d after the exposure, foliar injury occurred even in those given 1 h exposure. Leaves exposed to 0.10 μl PAN/L for 1 h showed bronzing over about 70% of their lower surface area, those exposed for 2 h showed bronzing over 100% of their lower surface area and about 20% bifacial bleached necrosis, and those exposed for 4 to 8 h showed about 90 to 95% bifacial bleached necrosis, remaining green at the large veins.

The total fatty acid content and composition in the primary leaves of kidney bean plants during exposure to PAN and 1 d after exposure for either 2 h (bronzing symptoms) or 8 h (bifacial bleached necrosis) are shown in Figure 3. The fatty acid content and composition in control leaves stayed almost constant during the course of experiment. The fatty acid content during 8 h of exposure to PAN showed little or no change till 4 h and then began to decrease at 6 h (appearance of water-soaked lesions) and reached 75% at 8 h. The fatty acid composition during PAN exposure also showed little or no change compared with control till 4 h. After this, both C18:2 and C18:3 began to decrease at 6 h and reached 77 and 66% of the control level at 8 h, respectively. Twenty-four h after the end of exposure, the content of total fatty acids, C18:2 and C18:3, decreased more than the levels during the exposure. C16:0 and C16:1 showed little or no change after exposure to PAN.

Effects of Ozone and PAN on MDA Content in Leaves of Morning Glory and Kidney Bean. Changes in the MDA content in leaves of morning glory and kidney bean plants during ozone or PAN exposure are shown in Figure 4. During ozone exposure, MDA content in morning glory remained unchanged till 6 h, and then slightly increased to 134% of the initial level at 8 h, while little or no change was observed in kidney bean plants. The MDA content in kidney bean leaves during PAN exposure began to increase to 240 and 296% of the initial level at 6 and 8 h, respectively. These increases of MDA content occurred only after water-soaked symptoms had appeared.

Effects of Ozone on Polar Lipids in Morning Glory Leaves. Changes in the glycolipid and phospholipid content during ozone exposure are shown in Figure 5. A and B, respectively. There were significant changes in the phospholipid content during exposure. PC, PG, PE, and PI rapidly increased at an initial exposure (2–4 h) and decreased at a late stage of exposure (6–8 h). The maximum PC, PG, and PE content reached about 175, 180, and 200% of the control levels, respectively, at 4 h. In contrast, the glycolipid content remained unchanged or slightly decreased during ozone exposure.

Changes in the glycolipid and phospholipid content 24 h after the end of the exposure are shown in Figure 6. A and B, respectively. When plants were left 1 d to permit symptoms to develop, the contents of MGDG, DGDG, and SQDG showed a large drop. The phospholipid contents at 24 h after the exposure were almost the same (PE, PI, and PA) or decreased (PC and PG) when compared to the content immediately after exposure, but all the levels were higher than those of the control.

Effects of Ozone on Polar Lipids in Primary Leaves of Kidney Bean. Changes in the glycolipid and phospholipid content during exposure are shown in Figure 7. A and B, respectively. The change in patterns of MGDG, DGDG, and SQDG were similar to those in morning glory. That is, MGDG and SQDG decreased...
Fig. 2. Changes in total fatty acid content and composition of kidney bean leaves immediately after and 24 h after the end of 0.15 μl ozone/L. Conditions as described in Figure 1.

Fig. 3. Changes in total fatty acid content and composition of kidney bean leaves immediately after and 24 h after the end of 0.10 μl PAN/L. Conditions as described in Figure 1.
almost disappeared and phospholipids were reduced remarkably. But PA increased even in necrotic leaves.

**DISCUSSION**

The fatty acid content and composition in kidney bean leaves exposed to ozone showed little or no change during 8 h exposure to a low concentration of ozone. This conforms to the results of Tomlinson and Rich (28), Swanson et al. (26), and Fong and Heath (6). In addition, MDA, which is formed both from ozonolysis and lipid peroxidation of unsaturated fatty acids (14), also remained unchanged during ozone exposure. On the other hand, fatty acid content in morning glory leaves significantly decreased only after an 8 h exposure to ozone and was accompanied by a concomitant slight accumulation of MDA (Fig. 4). Fatty acids whose levels decreased were linoleic and linolenic acid (Fig. 1). These results suggest that ozone or oxy-radical cause oxidation of the unsaturated fatty acids in morning glory at the late stage of exposure. Oxy-radical, which is formed by ozone decompositon in aqueous solution, enzymically, or the oxygen reduction in the chloroplasts (27), can cause lipid peroxidation and lipid deesterification (9). Peters and Mudd (21) pointed out that oxidation of unsaturated fatty acids did not occur, presumably because double bonds of the fatty acid are embedded in the bilayer and protected by reduced glutathione or by proteins more susceptible to oxidation.

Although only the C18:2 and C18:3 showed the significant difference among fatty acids upon the exposure of morning glory to ozone for 8 h, a slight increase in the content of C16:1 in morning glory and C16:0 in kidney bean were observed 24 h after the end of exposure. These results may be explained by alterations in lipid metabolism by the secondary damage.

The phospholipid and glycolipid content altered markedly with ozone exposure in the leaves of morning glory and kidney bean. Phospholipid content such as PC, PE, PG, and PI increased remarkably in morning glory and decreased in kidney bean at a 2- to 4-h-period after the start of exposure (Figs. 5B and 7B), while unsaturated fatty acids in morning glory decreased only at 8 h (Fig. 1). A slight decrease also occurred in MGDG in both the leaves of morning glory and kidney bean during ozone exposure (Figs. 5A and 7A). These results suggested that 0.15 μL L⁻¹ ozone may not directly oxidize unsaturated fatty acids of polar lipids by ozonolysis or lipid peroxidation at the initial stage, but it may affect enzymes relating to the lipid metabolism in leaves.

In kidney bean plants exposed to ozone, the decreases in phospholipid and MGDG content may be due to either the inhibition of lipid synthesis, the increase of hydrolysis, or a combination of these. It is well known that ozone inhibits lipid biosynthesis in spinach chloroplast suspensions or mitochondrial or mesosomal fractions from rat lung by way of oxidation of sulfhydryl enzymes (12, 13, 21). Moreover, Mudd et al. (13) reported that glycolipid biosynthesis was inhibited in chloroplasts treated with ozone and the formation of DGDG was more strongly inhibited than the formation of MGDG. However, the present study showed that MGDG was more sensitive to ozone than was DGDG. It is possible that ozone may enhance polar lipid hydrolysis. The predominant loss of MGDG compared with DGDG in both leaves of morning glory and kidney bean might be caused by ozone-induced activation of galactolipase, which is known to be more hydrolytic with MGDG than with DGDG (2).

In morning glory exposed to ozone, the contents of the phospholipid components increased at 2 h, reached their maximum levels at 4 h, and then decreased gradually until 8 h (Fig. 5A). The large increase in the contents of phospholipid components would suggest the synthesis of membranes and thereafter the degradation of membranes. Thus, increases of phospholipid content at the initial stage as seen in morning glory exposed to ozone

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**Fig. 4.** Changes in MDA content in leaves of morning glory and kidney bean with exposure of 0.15 μL ozone/L or 0.10 μL PAN/L. (○), Kidney bean for ozone; (●), kidney bean for PAN; (Δ), morning glory for ozone.

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a little, while DGDG remained unchanged. On the other hand, changes in the phospholipid content differed remarkably from those of morning glory. PC, PG, PE, and PI decreased after an initial 2 h exposure, but PG, PE, and PI returned to control levels at 4 to 6 h. At 8 h exposure, PE and PI had remarkably increased and reached about 150% of the control levels, while PC and PG were the same as control levels. One day after exposure, MGDG rapidly decreased with the increase of exposure duration, while DGDG and SQDG remained almost unchanged compared with control level (Fig. 8A).

In phospholipids, PG decreased at 6 to 8 h, but PC, PE, PI, and PA increased at 4 to 8 h (Fig. 8B). The increase of PE and PI content was particularly remarkable.

**Effects of PAN on Polar Lipids of Primary Leaves of Kidney Bean Leaves.** Changes in the glycolipid and phospholipid content during PAN exposure are shown in Figure 9, A and B, respectively. All polar lipids showed little change until 4 h (at which time there was no occurrence of water-soaking symptoms), while there was a marked drop in MGDG, DGDG, PC, PE, and PI at 6 h. PA began to increase rapidly at 6 h and reached about 6 times the level of control levels at 8 h. Figure 10, A and B, show the content of polar lipids in both mildly injured leaves (1 h exposure) and severely injured leaves (8 h exposure) in plants which were left 1 d after exposure to PAN. Glycolipid, MGDG and DGDG, levels decreased in leaves showing bronzing symptoms, while the phospholipid levels remained stable. As for the polar lipid content in bifacial bleached necrosis leaves, glycolipids...
FOLIAR LIPIDS WITH O₃ AND PAN

Fig. 5. Changes in polar lipid content of morning glory leaves during exposure to 0.15 μL ozone/L. A, Glycolipids and sulpholipids; B, phospholipids. Straight lines: ozone treatment; dashed lines: control (air treatment). Each symbol is the mean of three samples, and the vertical bar represents a standard deviation.

Fig. 6. Changes in polar lipid content of morning glory leaves 24 h after the end of exposure to 0.15 μL ozone/L. A, Glycolipids and sulpholipids; B, phospholipids. Legend as described in Figure 5.
have not been reported in other plant species previously (6, 23, 26), but increases in the phospholipid content have been observed as an adaptation to water stress in wheat and barley (4), to salt stress in cotton (29), and to rust infection (10). In the present study, it is impossible to explain the mechanism by which the contents of individual phospholipid component increased uniformly during ozone exposure; such changes may be a species-specific injury response to ozone of morning glory alone.

MGDG, DGDG, and SQDG in morning glory and MGDG in kidney bean leaves decreased markedly 1 d after exposure to ozone. MGDG and DGDG are located in both the envelope membrane and thylakoid membranes of chloroplasts and they comprise about 80% of nonpigment lipids in the chloroplast of higher plants (30). Therefore, a large reduction of these glycolipids in ozone-exposed leaves (1 d after exposure) suggested that chloroplast membranes had collapsed. In fact, using morning glory leaves 1 d after exposure to 0.15 μl ozone/L, Nouchi et al. (15) observed by electron microscopy that with an increasing duration of ozone exposure the thylakoid membranes of chloroplasts collapsed, and the thylakoid membranes in leaves exposed for 8 h almost disappeared. Large reductions of glycolipids occurred after ceasing ozone exposure, suggesting that the large destruction of thylakoid membranes was not directly caused by low concentrations of ozone, but that their destruction may result from secondary damage of membrane permeability.

Compositional changes in phospholipids in leaves of morning glory and kidney bean, especially the considerable increase of PE and PI, were observed 24 h after the end of ozone exposure (Figs. 6B and 8B). These changes of PE and PI and the preferential loss of MGDG rather than DGDG resemble the pattern of changes in senescing leaves (5). Therefore, such lipid changes may be a generalized feature of cell deterioration.

Swanson et al. (26) showed by TLC that the amounts of the glycolipids and phospholipids of tobacco leaf did not change significantly immediately after 2 h exposure to 0.30 μl ozone/L. Similarly, Fong and Heath (6) reported that exposure of 15-d-old bean leaves to 0.30 or 0.50 μl ozone/L for 1 h caused small alterations in PE, PG, and MGDG/DGDG molar ratio only in severely injured leaves (0.50 μl ozone/L) several hours or 24 h after exposure. On the other hand, Sakaki et al. (22, 23) reported that the exposure of spinach to 0.50 μl ozone/L for 8 h produced a marked decrease in MGDG, DGDG, and PC within 4 to 6 h. The present results at low concentrations of ozone (0.15 μl/L) were different from the findings of Swanson et al. (26), Fong and Heath (6), and Sakaki et al. (23). This could be due either to the different plant species, ages, or ozone dose or to a combination of these factors.

PAN can oxidize NADPH and prevent the incorporation of acetate into long chain fatty acids (11), but little is known about the effect of PAN on lipids or fatty acids. The present study indicated that the exposure of kidney bean leaves to 0.10 μl PAN/L caused a marked drop in phospholipids, glycolipids, and total fatty acids. Little or no change occurred in phospholipids and glycolipids till 4 h from the beginning of PAN exposure. Thereafter, the content of PC, PG, PE, PI, MGDG, DGDG, and unsaturated fatty acids decreased remarkably, while the content of PA and MDA showed a sharp increase, as water-soaking and wilting began to be observed 6 h after exposure (Fig. 9, A and B). Since PA is a common breakdown product of phospholipids by phospholipase D, the large increase of PA suggested

**Fig. 7.** Changes in polar lipid content of kidney bean leaves during exposure to 0.15 μl ozone/L. A, Glycolipids and sulpholipids; B, phospholipids. Legend as described in Figure 5.
Fig. 8. Changes in polar lipid content of kidney bean leaves 24 h after the end of exposure to 0.15 μL ozone/L. A, Glycolipids and sulpholipids; B, phospholipids. Legend as described in Figure 5.

Fig. 9. Changes in polar lipid content of kidney bean leaves during exposure to 0.10 μL PAN/L. A, Glycolipids and sulpholipids; B, phospholipids. Straight lines: PAN treatment; dashed lines: control (air treatment). Each symbol is the mean of three samples, and the vertical bar represents a standard deviation.
that PAN may enhance the hydrolytic activity of phospholipase D. In addition, a large reduction of unsaturated fatty acids such as C18:2 and C18:3 occurred at 8 h during exposure to PAN (Fig. 3), which was accompanied by a concomitant large accumulation of MDA (Fig. 4). These results suggested that PAN may cause lipid peroxidation of unsaturated fatty acids at the late stage of PAN exposure.

PAN produced singlet oxygen when decomposed with alkalisolvent (25). Shimazaki et al. (24) have reported that singlet oxygen plays a dominant role in the lipid peroxidation of SO2-exposed leaves. Therefore, PAN might break down polar lipids and fatty acids through its oxidizing property as a peroxyde or the toxicity of active oxygen. The massive decomposition of lipid components, particularly of glycolipids such as MGDG and DGDG by PAN, proceeded faster than that by ozone. It was assumed that the PAN molecule itself or its breakdown product may directly and strongly attack the thylakoid membranes of chloroplasts and induce an abrupt collapse of the membrane structure. Although the reason the effects of ozone and PAN on polar lipids and fatty acids were very different is not known, these results suggested that the attack of ozone and PAN on polar lipids differed from each other.

The present study revealed that both ozone and PAN altered the content and composition of the polar lipids of membranes. In addition, there is a distinct difference in the mode of action of the effect on lipids between ozone and PAN. The content and composition of phospholipids was altered rapidly by ozone prior to any visible injury appearing, while that of phospholipids and glycolipids decreased markedly by PAN, which was concomitant with the appearance of symptoms of water-soaking. Even if such polar lipid changes are not the cause of initial damage, alterations in the content and components of polar lipids by ozone and PAN could affect the functions associated with membranes such as the control of permeability, fluidity, and membrane-bound enzyme activities.

![Diagram of lipid content changes](image-url)

**Fig. 10.** Changes in polar lipid content of kidney bean leaves 24 h after the end of exposure to 0.10 μl PAN/L. A, Glycolipids and sulpholipids; B, phospholipids. Each symbol is the mean of three samples, and the vertical bar represents a standard deviation.

**LITERATURE CITED**