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Fluidity and lipid composition of oat and rye shoot plasma membrane: effect of sterol perturbation by xenobiotics

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Oat and rye plants were treated with either tetcyclacis (an experimental plant growth regulator), nuarimol (a fungicide) or γ-ketotriazole (an experimental herbicide). These treatments reduced shoot growth and changed the lipid composition of the shoot plasma membranes. In oat, both tetcyclacis and nuarimol treatments increased plasma membrane cholesterol and increased the phosphatidylethanolamine/phosphatidylcholine (PE/PC) ratio, whereas γ-ketotriazole treatment reduced cholesterol and the PE/PC ratio. In rye, all treatments reduced the PE/PC ratio. Generally, the sterol/phospholipid ratio was less in oat than in rye but the cholesterol/phospholipid ratio was greater. With all treatments in oat and rye, increases were observed in unsaturation of the phospholipid acyl chains. The fluidity of membranes was measured by steady-state fluorescence polarisation of the probe diphenylhexatriene; oat membranes were more fluid than rye. Membrane fluidity was greater in plasma membranes from plants treated with the xenobiotics than the controls. The results are discussed in the context of the effect of plasma membrane lipid composition on membrane fluidity, and it is concluded that there appears to be no overall simple relationship between membrane lipid composition and fluidity that holds for all treatments in both species.

Introduction

The relationship between membrane fluidity (or order parameter) and lipid composition is of considerable interest because of the connection between these parameters and acclimation or adaptation of plants to stress. Correlations between fluidity and lipid composition have been frequently sought in connection with temperature acclimation or injury; such work has been reviewed by Steponkus [1]. Changes in membrane lipids and fluidity have been observed in wheat subjected to drought stress [2] and in citrus cultivars susceptible to salt stress [3].

A number of different membrane systems of stressed plants have been studied, including mitochondria [4], chloroplasts [5], plasma membranes [6] and isolated protoplasts [7], as well as phospholipid vesicles made from lipid extracts of wheat seedlings [8]. However, attempts to study a relationship between the composition and physical state of a membrane by stressing the system may not provide reliable information, because there may be unsuspected interactions between the products of stress metabolism and membranes which involve components other than lipids. Also the results from isolated synthetic systems such as liposomes, may not reflect accurately those from native membranes.

Therefore, in this present work it was decided to treat whole oat and rye plants with a range of xenobiotics with known and reproducible effects on lipid composition. They were, 5-(4-chlorophenyl)-3,4,5,9,10-pentaazatetracyclo-5,4,1,$O^{2,6}$,$O^{8,11}$-dodeca-3,9-diene (tetcyclacis), an experimental plant growth retardant, which dramatically alters oat plasma-membrane sterol composition [9]; α-(2-chlorophenyl)-α-(4-fluorophenyl)-

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Abbreviations: PE, phosphatidylethanolamine; PC, phosphatidylcholine; RH, relative humidity; PAR, photosynthetically active radiation; DTT, dithiothreitol; PVP, poly(vinylpyrrolidone); PEG, poly(ethyleneglycol); DPH, 1,6-diphenyl-1,3,5-hexatriene; THF, tetrahydrofuran.

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5-pyrimidine methanol (Nuarimol), a fungicide which has been found to inhibit C14-demethylation in oat and rye sterol biosynthesis [10] and 1-[2-methoxyphenyl]-4,4-dimethyl-1-(1,2,4-triazol-1-yl)-1-penten-3-one (γ-ketotriazole), an experimental herbicide which also inhibits C14-demethylation [11]. The changes in lipid composition and fluidity (as measured by steady state fluorescence polarisation of the probe diphenylhexatriene) were measured in the shoot plasma membranes of treated and untreated plants.

Materials and Methods

Plant culture

Plants were grown as described by Cooke and Burden [12]. Winter oats (cv. Peniarth) or winter rye (cv. Rheidol) were sown in vermiculite and supplied with a balanced nutrient regime comprising ($mg \cdot l^{-1}$) K-156, N-140, Ca-40, P-31, Mg-18 and Fe-5 plus micronutrients. The plants were grown in a controlled environment of 16 h day at 20°C, 8 h night at 16°C, relative humidity (RH) 70% and photosynthetically active radiation (PAR) 350 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Treated plants were supplied with either tetcyclacis (1 μM), nuarimol (3 μM) or γ-ketotriazole (1 μM) at the time of sowing and then twice a week, by root drench after emergence. After 14 d the shoots of both treated and untreated plants were harvested and the plasma membrane extracted immediately.

Isolation and purification of plasma membranes

The aqueous two-phase polymer technique of Larsson et al. [13] was used to isolate and purify the plasma membranes as follows.

Shoot material, 30 g, was chopped finely and vacuum-infiltrated with 90 ml 50 mM Hepes and 0.5 M sucrose, made to pH 7.5 with NaOH; plus 1 mM DTT, 5 mM ascorbic acid and 0.6% (w/v) PVP. This and all subsequent operations were done at 4°C. The buffer-saturated material was homogenised, filtered through a 240 μm nylon cloth and centrifuged for 15 min at $10000 \times g$. The supernatant was then centrifuged for 30 min at $100000 \times g$ to yield a microsomal pellet which was resuspended in 4 ml 0.33 M sucrose/5 mM phosphate buffer (pH 7.8). For oat, 3 ml of the suspension was added to 9 g phase mixture to produce a 12 g two-phase system with a final composition of 6.3% (w/w) Dextran T500, 6.3% (w/w) PEG 3350, 3 mM KCl, 5 mM phosphate buffer (pH 7.8) and 0.33 M sucrose. For rye, 3 ml of suspension was added to a similar volume of phase mixture to give a 12 g system composed of 6.4% (w/w) Dextran T500, 6.4% (w/w) PEG 3350, 5 mM KCl, 5 mM phosphate buffer (pH 7.8) and 0.33 M sucrose. The phase system was mixed and centrifuged in a swing-out rotor for 3 min at $4000 \times g$. The resulting plasma membranes (upper-phase) were purified using a batch procedure [13] and the third upper-phase was diluted with phosphate buffer (pH 7.8) and centrifuged at $100000 \times g$ for 30 min. The resulting pellet was resuspended in 1.5 ml 5 mM Mes-Tris (pH 6.5)/0.33 M sucrose and aliquots taken for sterol, phospholipid and protein analysis, and steady-state fluorescence polarisation determinations.

Sterol analysis

Sterols were analysed using the method previously described by Cooke et al. [12]. Resuspended plasma membrane (0.5 ml) plus 15–25 μl of β-cholestanol (0.1 $mg \cdot ml^{-1}$) as an internal standard, were added to 5% KOH in 80% ethanol (2.5 ml). The mixture was warmed to 80°C, cooled, 10 ml hexane added along with 5 ml water and shaken. The hexane layer was removed and retained and a further 10 ml hexane was added to the original extract. The mixture was shaken and the hexane layer was removed. The hexane extracts were pooled, dried under a vacuum, and acetylated for 1 h with 50 μl pyridine and 50 μl acetic anhydride. The solvent was removed under nitrogen and the residue dissolved in ethyl acetate (about 50 μl). The sterol acetates were analysed by gas-chromatography (GC) using an SE-52 bonded capillary column (25 m × 0.25 mm i.d.) with hydrogen ($0.75\ kg \cdot cm^{-2}$) as the carrier gas and a temperature programme of 120–260°C at $10\,C^{\circ} \cdot min^{-1}$. The identities of all sterols were confirmed by comparison with authentic compounds under identical conditions using a GC mass-selective detector.

Phospholipid analysis

This was done using the method previously described by Cooke et al. [12]. Plasma membrane suspension (0.5 ml) was added to 0.75 ml chloroform/methanol (1 : 2) in an Eppendorf tube and the mixture was shaken, after which a further 0.25 ml chloroform was added, followed by more shaking and centrifugation at $10000 \times g$ for 6 min. The chloroform layer was removed, the solvent evaporated under nitrogen, and 0.2 ml propan-2-ol: hexane/water (54 : 40 : 6 (v/v)) added. Phosphatidylethanolamine (PE) and phosphatidylcholine (PC) were separated by HPLC using the method of Patton et al. [14]. The PE and PC fractions were collected, 50 μl methyl heptadecanoate added as internal standard and the solvent evaporated under nitrogen. 2 ml of sodium methoxide (0.5 M) containing 5% (v/v) 2,2'-dimethoxypropane was added to the residue and heated for 10 min at 40°C. After cooling the solution, 2 ml hexane and 1 ml water were added, the mixture shaken and the hexane layer, containing the methyl esters of the fatty acids from the hydrolysed phospholipids, transferred to another tube. The hexane was evaporated under nitrogen and the residue dissolved in 25 μl ethyl acetate. The resultant solution was analysed for fatty acids by GC using an RSL 500 BP-bonded capillary column (25

TABLE I

Sterol composition (μg/mg protein) of oat (cv. Peniarth) shoot plasma membranes treated with tetcyclacis, nuarimol or γ-ketotriazole or of untreated controls (± S.E.)

	Control	Tetcyclacis	Nuarimol	γ-Ketotriazole
Cholesterol	4.37 (0.31)	10.89 (2.0) *	10.27 (1.18) *	3.35 (0.46)
14α-Methyl-Δ^8-cholestenol	–	–	6.81 (0.99)	5.49 (0.72)
Campesterol	0.89 (0.08)	0.66 (0.12)	0.76 (0.18)	0.65 (0.12)
14-α-Methyl-Δ^8-ergostenol	–	–	0.29 (0.13)	0.33 (0.03)
Stigmasterol	3.59 (0.35)	2.46 (0.38) *	1.33 (0.16) *	1.88 (0.15) *
Obtusifoliol	–	–	0.92 (0.17)	1.51 (0.12)
Dihydroobtusifoliol	–	–	0.51 (0.10)	0.51 (0.10)
Sitosterol	1.42 (0.19)	0.92 (0.20)	0.61 (0.81) *	0.84 (0.05) *
Cycloartenol	4.86 (0.39)	4.91 (0.73)	2.89 (0.29) *	4.21 (0.67)
	($n = 9$)	($n = 6$)	($n = 6$)	($n = 6$)

* Significant at the 95% level of confidence or better (unpaired *t*-tests), compared with controls.

m × 0.25 mm i.d.) with helium as the carrier gas (0.75 $kg \cdot cm^{-2}$) and a temperature programme of 170–200°C at 2 $C° \cdot min^{-1}$. The phospholipid content was expressed as the amount of fatty acid found in the PE and PC fractions and the PE/PC ratio was calculated from these data.

Fluorescence polarisation determinations

Steady-state fluorescence polarisation measurements were made using a Perkin Elmer LS5 luminescence spectrometer fitted with a polarisation accessory. The determinations were made at 25°C using the resuspended membranes, diluted with an assay medium of 40 mM Hepes/KOH (pH 7.0), 100 mM KCl, 5 mM $MgSO_4$ and 0.1 mM EGTA, to give a protein concentration of 150 $\mu g \cdot ml^{-1}$. Samples contained 0.5 μl 1 mM DPH in THF. Individual fluorescence intensities were corrected for light scattering by scattering emission values obtained from the same membranes samples without the fluorescent probe. The steady-state fluorescence polarisation (P) was calculated according to the relationship

$$P = \frac{I_{VV} - I_{VH}G}{I_{VV} + I_{VH}G}$$

where I refers to the fluorescence intensity through polarisers orientated vertically (V) and horizontally (H) with respect to the plane of polarisation of the excitation beam. G (the grating correction factor) is described by the ratio I_{HV}/I_{HH}. The wavelength of the excitation light was 360 nm and the slit width 5 nm. The emission wavelength was 430 nm with the slit width 10 nm.

Protein determinations

Protein concentration was estimated by the method of Bradford [15] using Bio-Rad reagents with thyroglobulin as the standard.

Data analysis

All experiments were repeated at least three times and the data were analysed using unpaired *t*-tests.

TABLE II

Sterol composition (μg/mg protein) of rye (cv. Rheidol) shoot plasma membranes treated with tetcyclacis, nuarimol or γ-ketotriazole, or of untreated controls (± S.E.)

	Control	Tetcyclacis	Nuarimol	γ-Ketotriazole
Cholesterol	1.57 (0.12)	1.81 (0.46)	0.88 (0.24)	0.66 (0.18) *
Campesterol	23.77 (1.10)	18.11 (5.05)	13.29 (2.31) *	6.06 (1.50) *
14α-Methyl-Δ^8-ergostenol	–	–	9.77 (2.90)	14.93 (4.33)
Stigmasterol	5.55 (0.82)	2.26 (0.67) *	1.37 (0.24) *	0.83 (0.32) *
Obtusifoliol	–	–	4.73 (1.35)	5.87 (1.81)
Dihydroobtusifoliol	–	–	8.79 (3.24)	15.05 (4.67)
Sitosterol	54.02 (4.49)	47.62 (15.52)	19.47 (4.19) *	8.39 (2.97) *
Cycloartenol	–	–	1.69 (0.74)	2.01 (0.65)
	($n = 3$)	($n = 3$)	($n = 3$)	($n = 3$)

* Significant at the 95% level of confidence or better (unpaired *t*-tests), compared with controls.

Results

Shoot growth was reduced by up to 50% following tetcyclacis and γ-ketotriazole treatment in oat and rye. Nuarimol reduced shoot growth by about 30% in both species. Since such dilute solutions of xenobiotics were used, the results indicated very effective mechanisms for their absorption and transport.

The results of the sterol analyses in oat and rye are shown in Tables I and II, and indicate that the major oat plasma membrane sterols of control plants were cholesterol, stigmasterol and cycloartenol (the common precursor of the other sterols), whereas in rye, campesterol and sitosterol comprised the greatest constituents.

With tetcyclacis treatment in oat (Table I), cholesterol was increased significantly, without the formation of 14α-methylsterols seen with nuarimol treatment, which also caused an increase in cholesterol. A significant decrease in stigmasterol was common to all treatments, and with nuarimol and γ-ketotriazole a significant reduction in sitosterol occurred along with the formation of 14α-methylsterols, particularly 14α-methyl-Δ^8-cholestenol.

In rye (Table II), the only effect of tetcyclacis was a decrease in stigmasterol. For nuarimol and γ-ketotriazole treatments, the effects were similar to those found in oat, as they also resulted in significant decreases in sitosterol and campesterol accompanied by the formation of 14α-methyl sterols as well as small amounts of cycloartenol. A significant reduction in cholesterol was observed with γ-ketotriazole (Table II).

The PE/PC ratios in the plasma membranes of oat and rye are shown in Table III. In oat, both tetcyclacis and nuarimol treatment resulted in a significant increase in the PE/PC ratios (Table III), compared with control. This was caused by an increase in PE (Table IV). However, with γ-ketotriazole treatment a reduction in the PE/PC ratio was observed, due to a decrease in PE and an increase in PC (Table IV), although this was not significant. A significant increase in the PE/PC ratio was found with all treatments in rye plants (compared with controls, Table III). Again, this was due to a reduction in PE and increase in PC, compared with the control plants (Table IV).

The fatty acid composition of PE and PC in oat and rye plasma membranes was found to be essentially the same in terms of acyl chain length, irrespective of phospholipid, treatment or species (Tables V, VI, VII and VIII). However, increases in the bond index (BI), which represents the degree of unsaturation of the fatty acids (a greater BI indicates more unsaturation), were found in the acyl chains of treated oat plasma membrane PE and PC (Tables V and VI). These increases were greater in PE than in PC and also for γ-ketotriazole-treated material. In rye, increases in BI were observed for the fatty acids of PE and PC from nuarimol and γ-ketotriazole-treated plant plasma membranes, but not tetcyclacis treatment (Tables VII and VIII). Like oat, the degree of unsaturation was greater in PE than in PC.

The mole percentage total sterol of the total lipid (sterol and phospholipid) for oat and rye (assuming an average molecular weight of 390 for sterols and 750 for phospholipids) is shown in Table IX. These data indicated that there was no difference in the total sterol to

TABLE III

Ratio of phosphatidylethanolamine (PE) to phosphatidylcholine (PC) in the plasma membranes of oat and rye shoots treated with tetcyclacis (1 μM), nuarimol (3 μM), γ-ketotriazole (1 μM) or in untreated controls (± S.E.)

	PE/PC	
	oat	rye
Control	0.91 (0.06)	1.45 (0.15)
Tetcyclacis	1.64 (0.06) *	0.87 (0.08) *
Nuarimol	1.47 (0.06) *	0.81 (0.05) *
γ-Ketotriazole	0.69 (0.09)	0.87 (0.06) *
	($n = 3$)	($n = 3$)

* Significant at the 95% level of confidence or better (unpaired *t*-tests), compared with controls.

TABLE IV

Phosphatidylethanolamine (PE) and phosphatidylcholine (PC) content (μg/mg protein) in the plasma membrane from oat and rye shoots treated with tetcyclacis (1 μM), nuarimol (3 μM) or γ-ketotriazole (1 μM) or from untreated controls (average of three determinations)

	Oat		Rye	
	PE	PC	PE	PC
Control	70.3	77.3	109.7	73.5
Tetcyclacis	121.9	72.9	77.1	89.9
Nuarimol	100.0	69.1	90.3	112.1
γ-Ketotriazole	62.3	91.9	69.6	81.9

TABLE V

Fatty acid composition (%) and bond index (BI) of phosphatidylethanolamine from plasma membranes of oat shoots treated with tetcyclacis (1 μM), nuarimol (3 μM) or γ-ketotriazole (1 μM) or from untreated controls (± S.E.)

Fatty acid	Control	Tetcyclacis	Nuarimol	γ-Ketotriazole
16:0	41.1(1.7)	35.6(2.4)	37.0(5.2)	32.5(0.7)
16:1	5.2(3.0)	5.3(2.9)	5.5(3.3)	2.6(0.3)
18:0	2.3(0.8)	2.2(0.8)	3.1(1.4)	3.3(0.5)
18:1	3.2(0.8)	4.0(1.4)	3.9(1.2)	3.8(0.5)
18:2	27.2(2.9)	33.6(4.4)	32.1(5.7)	39.9(1.4)
18:3	20.0(3.5)	19.3(2.8)	18.5(5.2)	17.9(1.7)
Bond index	122.8	134.4	129.1	139.9 ($n = 3$)

TABLE VI

The fatty acid composition (%) and bond index (BI) of phosphatidylcholine from plasma membranes of oat shoots treated with tetcyclacis (1 μM), nuarimol (3 μM) or γ-ketotriazole (1 μM) or from untreated controls (± S.E.)

Fatty acid	Control	Tetcyclacis	Nuarimol	γ-Ketotriazole
16:0	40.5(6.2)	38.5(3.6)	37.9(4.0)	33.7(3.1)
16:1	7.6(2.5)	6.5(2.4)	6.4(2.4)	4.5(2.2)
18:0	5.3(1.1)	5.7(0.6)	5.1(0.7)	4.3(1.4)
18:1	5.5(0.3)	5.5(0.5)	4.9(0.3)	3.9(0.7)
18:2	23.6(4.8)	26.2(1.2)	30.0(2.3)	34.4(5.0)
18:3	17.5(5.2)	17.6(2.4)	15.7(1.5)	19.2(1.9)
Bond index	112.8	117.2	118.4	134.8 ($n = 3$)

TABLE VII

The fatty acid composition (%) and bond index (BI) of phosphatidylethanolamine from plasma membranes of rye shoots treated with tetcyclacis (1 μM), nuarimol (3 μM) or γ-ketotriazole (1 μM) or from untreated controls (± S.E.)

Fatty acid	Control	Tetcyclacis	Nuarimol	γ-Ketotriazole
16:0	37.8(2.3)	38.0(3.2)	32.5(1.8)	35.3(2.3)
16:1	4.0(1.1)	5.9(0.6)	4.3(0.9)	3.9(0.9)
18:0	2.4(0.5)	3.2(0.8)	2.6(0.1)	2.0(0.3)
18:1	3.3(0.3)	3.4(0.5)	2.8(0.5)	2.2(0.5)
18:2	31.0(1.2)	24.9(1.7)	33.4(3.9)	34.9(1.4)
18:3	21.5(2.4)	24.6(2.8)	24.4(3.3)	21.7(2.5)
Bond index	133.8	132.9	147.1	141.0 ($n = 3$)

TABLE VIII

Fatty acid composition (%) and bond index (BI) of phosphatidylcholine from plasma membranes of rye shoots treated with tetcyclacis (1 μM), nuarimol (3 μM) or γ-ketotriazole (1 μM) or from untreated controls (± S.E.)

Fatty acid	Control	Tetcyclacis	Nuarimol	γ-Ketotriazole
16:0	38.1(4.3)	40.1(3.8)	37.1(0.9)	31.8(3.9)
16:1	6.9(1.8)	5.9(0.6)	3.8(0.7)	4.5(1.0)
18:0	4.5(1.1)	3.5(1.3)	3.2(0.2)	4.5(0.5)
18:1	4.6(0.3)	3.3(0.6)	3.3(0.3)	4.1(0.5)
18:2	25.7(3.1)	21.1(2.1)	28.5(1.0)	33.5(4.6)
18:3	20.2(1.9)	26.1(2.9)	24.1(1.5)	22.1(1.5)
Bond index	123.5	129.7	136.4	141.9 ($n = 3$)

phospholipid ratio between control and treated plants for oat and rye. However, the ratio was greater in rye, compared with oat, for all treatments, and was due to the difference in sterol content between the two species (Tables I and II). The mole percentage cholesterol of total lipid is also shown in Table IX. With oat, tetcyclacis and nuarimol treatments produced significant increases in this ratio compared with the controls. This was due to the changes in cholesterol which resulted from these treatments (Table I). Similarly, for rye, changes in cholesterol with treatment, i.e., a trend towards an increase with tetcyclacis and decreases with nuarimol and γ-ketotriazole compared with control (Table II) were reflected in the mole percentage cholesterol of total lipid (Table IX). Comparison of this parameter between oat and rye, showed that it was much greater in oat than in rye, a reverse of the case with the mole percentage total sterol (Table IX).

The results of the fluidity measurements, as determined by steady-state fluorescence polarisation, are shown in Table X. Significant increases in fluidity (indi-

TABLE IX

Mole percentage total sterol, and cholesterol of total lipid (sterol and phospholipid) in the plasma membranes of oat and rye shoots treated with tetcyclacis (1 μM), nuarimol (3 μM) or γ-ketotriazole (1 μM) or of untreated controls (± S.E.)

Assume average M_r sterol = 390 and phospholipid = 750; M_r cholesterol = 386.7.

	Oat (mol%)		Rye (Mol%)	
	total	cholesterol	total	cholesterol
Control	22.6 (1.2)	6.2 (0.9)	46.9 (1.5)	0.93 (0.19)
Tetcyclacis	25.2 (1.1)	13.9 (0.7)	48.2 (3.6)	1.27 (0.37)
Nuarimol	24.1 (0.9)	10.2 (1.1)	38.5 (0.9)	0.59 (0.14)
γ-Ketotriazole	27.9 (2.8)	4.9 (1.0)	44.9 (4.1)	0.57 (0.06) $n = 3$

TABLE X

Fluidity as determined by steady-state fluorescence polarisation (P) at 25°C, of plasma membranes from oat and rye shoots treated with tetcyclacis (1 μM), nuarimol (3 μM), γ-ketotriazole (1 μM) or from untreated controls (± S.E.)

	P	
	oat	rye
Control	0.298 (0.002) ($n = 5$)	0.325 (0.003) ($n = 4$)
Tetcyclacis	0.230 (0.004) * ($n = 3$)	0.293 (0.008) * ($n = 3$)
Nuarimol	0.266 (0.001) * ($n = 3$)	0.285 (0.006) * ($n = 3$)
γ-Ketotriazole	0.259 (0.008) * ($n = 3$)	0.292 (0.010) * ($n = 3$)

* Significant at the 95% level of confidence or better (unpaired *t*-tests), compared with controls.

TABLE XI

The protein content ($mg \cdot cm^{-3}$) of oat and rye shoot plasma membranes, treated with tetcyclacis (1 μM), nuarimol (3 μM) or γ-ketotriazole (1 μM) or of untreated controls (± S.E.)

	Control	Tetcyclacis	Nuarimol	γ-Ketotriazole
Oat	0.72 (0.05)	0.76 (0.10)	0.93 (0.10)	0.93 (0.09)
Rye	0.69 (0.07)	0.52 (0.07)	0.55 (0.08)	0.70 (0.07) ($n = 6$)

cated by lower P values) were found for all treatments compared with control, in both oat and rye. Oat was found to be more fluid than rye, control for control and treatment for treatment (Table X).

The total protein content of the plasma membranes of both oat and rye were found to be unaffected by treatment (Table XI).

Discussion

A comparison of the mole percentage total sterol of total lipid (sterol + phospholipid) and fluidity between oat and rye, irrespective of treatment (Tables IX and X), confirmed the known relationship between these parameters, [16,17], i.e., fluidity increased as the sterol to phospholipid ratio decreased. However, there are also differences in fluidity between control and treated plants with oat and rye, although the total sterol to phospholipid ratios (expressed as mole percentage sterol of sterol + phospholipid) are essentially the same within each species (Table IX). This suggests that the relationship between membrane lipid composition and fluidity may be more complex than previously appreciated, a fact recognised recently by Vincent and Gallay [18] and Lee et al. [19]. Thus, although differences in fluidity between oat and rye can be explained in simple terms, the differences between control and treated membranes, for each species, may be more complex and a simple interpretation of the results of our experiments, based on a unique sterol–phospholipid interaction that applies to both oat and rye, may not be possible.

No changes were found in total membrane-protein content with treatment (Table XI). Although changes in membrane-bound enzyme activity have been observed after xenobiotic treatment [12], these were related to changes in lipid composition and no evidence for a direct protein–xenobiotic interaction was found.

With oat, both the tetcyclacis and nuarimol treatments resulted in large increases in membrane fluidity (Table X). It has been shown, with lipid extracts from natural membranes, that fluidisation of the membrane will occur when the mole percentage of cholesterol is between 7 and 50 [20]. However, it has been demonstrated, using liposomes, that increases in cholesterol only fluidise membranes composed of phospholipids with saturated acyl chains [21]. Tetcyclacis and nuarimol treatments resulted in a membrane cholesterol content of 14 and 10 mol%, respectively (Table IX). At these levels, cholesterol increases membrane fluidity in the presence of phospholipids with saturated acyl chains. However, when only unsaturated acyl chain phospholipids are present, cholesterol makes the membrane less fluid [21].

These results give no indication of the role played by the other plant sterols in modulating plasma membrane fluidity, although it is interesting to note that the increase in fluidity with tetcyclacis was significantly greater in oat, than with any other treatment (Table X). In this case no 14α-methylsterols were produced (Table I), and the mole percentage of cholesterol was greatest (13.9%; Table IX). Also, the value for P (0.23; Table IX) found with this treatment is almost exactly the same as that found for liposomes made from phospholipids, extracted from rat liver plasma membranes, with 14 mol% cholesterol added [21]. With the other treatments, cholesterol is lower (Table IX) and P greater (Table X).

There was also an increase in the PE/PC ratio (Table III), caused by an increase in PE (Table IV) and it has been argued that PE reduces membrane fluidity, while PC increases it [22]. Differences have been found in the mobilities of PE and PC which have led to the suggestion that PE may decrease membrane fluidity, either locally or in general, but that this may be compensated for by their carrying more unsaturated (fluid) fatty acids [23]. The results of the analyses of the fatty acids associated with the phospholipids of the oat plasma membranes showed increases in unsaturation (expressed as BI) of the PE and PC acyl chains in tetcyclacis- and nuarimol-treated plants, compared with controls (Tables V and VI).

Thus, the increase in fluidity observed in oat shoot plasma membranes with higher cholesterol levels might have been modulated by the increased unsaturation of PE and PC acyl chains or vice versa.

This idea is supported by results from the γ-ketotriazole treatment, where increased fluidity was accompanied by a reduced cholesterol content and PE/PC ratio (Tables I and III), the latter effect being the consequence of an increase in PC (Table IV). There was also a larger increase in BI for both PE and PC with γ-ketotriazole treatment of oat, than with either of the other two treatments indicating more unsaturation (Tables V and VI). This factor may also have influenced membrane fluidity in this case.

However, the rôle of cholesterol in modulating membrane fluidity is difficult to define from these data as the analytical technique used to assess the phospholipids and their associated fatty acids did not distinguish which acyl chains were attached to which phospholipid. It was therefore impossible to determine the presence of a phospholipid such as DPPC, which exerts a similar membrane-ordering effect to cholesterol [21]. Therefore, interpretation of the data in terms of individual phospholipid species has not been attempted.

The results from rye plasma membranes do not support the notion of cholesterol-phospholipid mediated membrane fluidity. Like oat, rye plasma membrane fluidity was increased by all treatments (Table X), along with decreases in the PE/PC ratio (Table III), which were due to increases in PC (Table IV). Also the BI for PE and PC in both the nuarimol and γ-ketotriazole plant plasma membranes were increased compared with

controls (Tables VII and VIII), indicating greater unsaturation of the acyl chains. These changes might explain the increases in fluidity found in the nuarimol and γ-ketotriazole treated plant plasma membranes, but in the tetcyclacis treated plants there was only an increase in the BI of PC (Table VIII).

Unlike oat plasma membranes, the cholesterol content of rye membranes was almost an order of magnitude less and was little affected by tetcyclacis or nuarimol treatment, although it was reduced by γ-ketotriazole (Tables I and II). Therefore it is hard to see what rôle, if any, is played by cholesterol in moderating membrane fluidity in rye, as our results indicated that there was no obvious balance between PE, PC and cholesterol. Instead, the changes in membrane fluidity could be explained by the increases in PC along with a possible effect of increased unsaturation of the phospholipid acyl chains. Again, it was impossible to determine which fatty acyl chains were associated with each phospholipid and so we were unable to assess whether a phospholipid such as DPPC directly influenced membrane fluidity.

These results seem to imply that our interpretation of the oat data is wrong or, that the two species are different in respect of their lipid modulation of membrane fluidity. The large differences in plasma membrane sterol composition between oat and rye (Tables I and II) suggest they may have different lipid requirements. Also the rôle played by one sterol may vary from one species to another. It, therefore, has to be concluded that it may not be possible to make direct comparisons of changes in fluidity and lipid composition between different plant species.

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