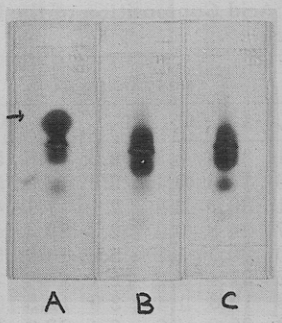

Figure 1. Electrophoretic pattern of (A) bean extract in casein-containing agar gel digested with subtilisin; the marked spot represents the area where the inhibitor protected the casein from digestion; (B) same with lentil extract, negative for subtilisin inhibitor; and (C) control, bean extract in casein-free agar gel; all slides stained with amino black.

of the subtilisin inhibitor against bacterial and fungal attacks in legume plants and seeds.

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Phospholipids of Barley Grain

Fractionation of the phospholipids of two barley (*Hordeum vulgare* L.) varieties, "Kearney" (winter type) and "Prilar" (spring type), by thin-layer chromatography produced ten lipid classes. Phosphorus analysis of each fraction showed that phosphatidylcholine, lysophosphatidylcholine, and phosphatidylethanolamine were present in highest amounts. Lesser amounts of phosphatidylserine, phosphatidylinositol, phosphatidylglycerol, diphosphatidylglycerol, and small amounts of phosphatidic acid and two unknown phospholipids were present. Fatty acids present in the fractions ranged from lauric (12:0) to arachidic (20:0). Linoleic acid (18:2) was the principal fatty acid in seven fractions and palmitic (16:0) was predominant in the other three fractions. This study provides a basis for monitoring of changes in phospholipids during cold acclimation of barley seedlings.

Phospholipids are essential components of the cytoplasmic membranes of vegetative and reproductive tissues, even though they only represent about 0.6% of the dry weight of barley grain (Price and Parsons, 1974). The presence of phospholipids in the structure and function of cell membranes relates to the movement of charged and uncharged molecules, the transport of triglycerides, and levels of enzyme activity.

The composition of phospholipids has been reported for most cereal grains. A few studies have been conducted on the phospholipids of barley (Aylward and Showler, 1962), barley malt (Silberusen and Anthon, 1967), and wort (Perkins, 1969). None of the studies on barley phospholipids have involved fractionation by thin-layer chromatography (TLC), quantification of inorganic phosphorus by colorimetry, or fatty acid analysis by gas–liquid chromatography (GLC).

This study was initiated to obtain detailed information on the phospholipids of the barley (*Hordeum vulgare* L.) varieties "Kearney" (winter type) and "Prilar" (spring type). The information will provide a basis for monitoring changes which occur in barley phospholipids during cold acclimation and the relationship of these changes to winterhardiness.

MATERIALS AND METHODS

Whole grain samples of the barley varieties Kearney and Prilar were ground in a Udy cyclone mill to pass a 0.6-mm screen. Total lipids including the phospholipids were extracted with chloroform–methanol–water (1.0:1.0:0.9) in the modification of the methods (Bligh and Dyer, 1959; Folch et al., 1957; Weber, 1970; Atkinson et al., 1972) and were purified as described previously (Price and Parsons, 1974). The lipids were separated into classes by silicic acid column chromatography (Hirsch and Ahrens, 1958). The phospholipid class was eluted with methanol, and the solvent was removed by a rotary vacuum evaporator at 40 °C. The phospholipids were then transferred to vials, flushed with nitrogen, and stored at −20 °C.

The phospholipids were separated by two-dimensional TLC (Parsons and Patton, 1967) on silica gel HR coated glass plates with the solvent systems of chloroform–methanol–water–28% aqueous ammonia (130:70:7:0.5, v/v) and chloroform–acetone–methanol–acetic acid–water (100:40:20:20:7, v/v). The individual phospholipids, separated by TLC, were identified by cochromatography with authentic reference lipids (Applied Science Laboratories, State College, PA; Supelco, Bellefonte, PA; and Analabs, North Haven, CT) and from published R_f values (Lepage, 1967; Nichols, 1964). Specific sprays were also used to identify phospholipids (Dittmer and Lester, 1964; Stahl, 1969). The phosphorus content of the individual lipids was determined by spraying the developed TLC plates with 50% sulfuric acid, heating the plates for 60 min at 180 °C, and scraping each charred spot into a test tube (Kahovcova and Odavic, 1969). Digestion and color de-

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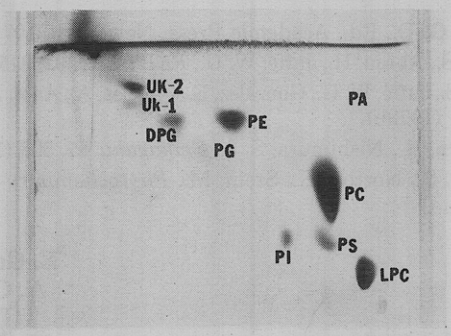

Figure 1. Two-dimensional thin-layer chromatographic separation of phospholipids from Kearney winter barley. Adsorbent: silica gel HR. The spots were identified as follows: LPC, lysophosphatidylcholine; PC, phosphatidylcholine; PS, phosphatidylserine, PI, phosphatidylinositol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; DPG, diphosphatidylglycerol; PA, phosphatidic acid, Uk-1-unknown 1, Uk-2-unknown 2.

Table I. Composition of the Phospholipids of Kearney and Prilar Barley[a]

phospholipid	Kearney mean[b]	Kearney SD	Prilar mean[b]	Prilar SD
lysophosphatidylcholine (LPC)	37.3	2.41	36.8	2.06
phosphatidylcholine (PC)	44.4	2.34	44.3	2.52
phosphatidylserine (PS)	4.8	0.41	5.0	0.45
phosphatidylinositol (PI)	1.3	0.36	1.1	0.43
phosphatidylethanolamine (PE)	8.8	0.33	7.6	0.71
phosphatidylglycerol (PG)	0.2	0.16	0.8	0.54
diphosphatidylglycerol (DPG)	1.5	0.26	1.7	0.32
phosphatidic acid (PA)	Tr		0.2	0.19
unknown 1 (UK_1)	0.5	0.24	0.3	0.36
unknown 2 (UK_2)	1.2	0.68	2.2	0.52

[a] Expressed as percent of total lipid phosphorus. [b] n = 8.

velopment were completed and quantity of each fraction measured according to Morrison's procedure (Morrison, 1964).

The phospholipids were sprayed with 2,7-dichlorofluorescein (Stahl, 1969) and the spots were observed under UV light. Individual spots were scraped into vials containing 3 mL of 5% H_2SO_4 in methanol (v/v) and were transmethylated overnight at 50 °C (Boatman et al., 1969) to produce fatty acid methyl esters for analysis by gas chromatography.

RESULTS

The solvent systems used in two-dimensional TLC produced good separation of the individual lipids in the phospholipid class. Figure 1 is a representative two-dimensional chromatogram of Kearney barley. Chromatograms of the phospholipids of Prilar barley were essentially the same. Ten phosphorus-containing compounds were obtained, and eight of the spots were identified. Unknown spots 1 and 2 both reacted positively to a standard phosphorus-sensitive spray (Dittmer and Lester, 1964), but we could not identify them further.

The phospholipid composition of Kearner and Prilar was quite similar (Table I). Phosphatidylcholine (PC) and lysophosphatidylcholine (LPC) were the predominant phospholipids in these tests, comprising over 80% of this lipid class. Smaller amounts of phosphatidylethanolamine (PE) and phosphatidylserine (PS) were present, along with small quantities of the other six phospholipids. Phosphatidic acid (PA) was present in very small amounts.

The fatty acid composition of the phospholipids was similar for both barley varieties (Tables II and III). Fatty acids of the phospholipids ranged from lauric (12:0) to arachidic (20:0). The major fatty acids detected were

Table II. Fatty Acid Composition of the Phospholipids of Kearney Barley

phospholipid[a]	fatty acid, wt % C_{12}	C_{14}	C_{16}	$C_{16:1}$	C_{18}	$C_{18:1}$	$C_{18:2}$	C_{20}	$C_{18:3}$
LPC	Tr[b]	2.1	36.9	0.3	2.0	5.6	50.2	Tr	2.9
PC	Tr	0.6	17.6	0.4	2.8	19.0	55.2	0.7	3.7
PS	Tr	5.1	30.0	2.8	2.1	7.1	45.0	4.6	3.3
PI	Tr	1.7	24.8	0.2	2.3	22.4	45.6	0.5	2.5
PE	Tr	1.0	20.5	1.1	5.9	14.2	53.9	0.4	3.0
PG		5.2	31.4	1.2	12.1	26.0	20.1	1.3	2.7
DPG		3.8	19.8	1.4	3.9	12.5	56.0		2.6
PA	Tr	7.0	35.0	4.8	6.4	23.0	11.3	8.3	4.2
UK_1	Tr	5.5	36.4	3.0	25.3	9.6	13.6	5.1	1.5
UK_2	Tr	1.6	25.1	2.1	16.1	12.5	40.9	1.1	0.6

[a] Abbreviations identified in Table I. [b] Trace.

Table III. Fatty Acid Composition of the Phospholipids of Prilar Barley

phospholipid[a]	fatty acid, wt % C_{12}	C_{14}	C_{16}	$C_{16:1}$	C_{18}	$C_{18:1}$	$C_{18:2}$	C_{20}	$C_{18:3}$
LPC	Tr[b]	0.3	17.7	0.3	1.5	19.8	56.4		4.0
PC	Tr	0.4	19.5	0.4	1.6	18.8	55.3		4.0
PS		1.5	31.2	0.9	5.1	5.4	52.1	1.5	2.3
PI	Tr	3.5	21.0	1.3	6.4	25.2	42.6		
PE	Tr	0.4	21.1	Tr	6.6	18.8	50.1	1.8	1.2
PG	Tr	4.8	38.7	3.3	10.9	26.7	14.4	0.5	0.7
DPG	Tr	7.9	18.0	2.8	5.3	13.2	51.1	0.5	1.2
PA	Tr	6.5	36.4	1.0	23.9	17.4	12.8	1.2	0.8
UK_1		5.8	37.9		22.6	12.8	20.9		
UK_2	Tr	1.8	28.8	1.0	11.5	7.0	44.9		5.0

[a] Abbreviations identified in Table I. [b] Trace.

linoleic (18:2), palmitic (16:0), and oleic (18.1). Lauric acid was present in most of the fractions, but only in trace amounts. Linoleic acid was the principal fatty acid in all fractions except phosphatidylglycerol (PG), phosphatidic acid (PA), and unknown fraction. In these three, palmitic was the predominant fatty acid.

DISCUSSION

Two-dimensional TLC separated ten phosphorus-containing lipids from the barley grain. Eight of these fractions were identified and two were not (Table I). The presence of *n*-acylphosphatidylethanolamine and *n*-acyllysophosphatidylethanolamine in wheat grain has been reported (Colborne and Laidman, 1975). These compounds had R_f values similar to those of our unknown fractions.

The composition of phospholipids has been reported for corn (Weber, 1970). However, corn contains considerably less phospholipid than does barley (Price and Parsons, 1975) and this could influence the number and weight distribution of the phospholipid fractions. Barley and wheat have comparable distributions of total lipid among the three lipid classes: neutral lipid, glycolipid, and phospholipid (Price and Parsons, 1975). When a lipid extraction system similar to the one in this study was used on wheat (Colborne and Laidman, 1975) the four major phospholipid fractions were PC 28.4%, LPC 30.4%, PE 5.2%, and PS 4.6%. Results from barley (Table I) were PC 44%, LPC 37%, PE 8%, and PS 5%.

Adjustments in technique were required in the steps leading to the preparation of fatty acid methyl esters for GLC analysis. Visualization of the phospholipid fractions on TLC plates was first accomplished with iodine vapor. This vapor is more sensitive than a spray application of 2,7-dichlorofluorescein for revealing phospholipids (Parsons and Patton, 1967). However, we obtained unreliable results in subsequent GLC analyses of the unsaturated

fatty acids with iodine vapor and used 2',7'-dichlorofluorescein thereafter. The error induced by iodine has been noted by others (Nichaman et al., 1963). Also, the presence of small quantities of water from the silica gel HR layer interfered with the boron trifluoride procedure (Metcalf et al., 1966) for methyl ester preparation. The potential problem was overcome by resorting to transmethylation with H_2SO_4 in methanol (Boatman et al., 1969).

The fatty acid composition of the individual phospholipids in barley (Tables II and III) was similar to that in corn (Weber, 1970). Linoleic acid (18:2) was the predominant fatty acid in six fractions, PC, LPC, PE, PS, DPG, and phosphatidylinositol (PI) in the barley phospholipids. Linoleic acid was dominant in corn in the following fractions: PC, LPC, PI, PG, PE, DPG, and PA. Palmitic acid (16:0) was found at concentrations of 30% or more in the PS, PG, and PA fractions of both Kearney and Prilar and also in LPC of Kearney. However, in corn phospholipids only PI and PG were that high.

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Effects of Drying and Duration of Storage on the Extractable Atrazine Content of Soil Samples

The extractable atrazine content of soil samples was reduced by drying at 45 °C for 24 h. Dried samples originally containing 1 ppm atrazine showed no further significant loss of atrazine when stored up to 180 days at room temperature; however, there was significant loss between 180 and 360 days. Dried samples originally containing 10 ppm atrazine showed significant loss after 90 days of storage.

In experiments involving herbicide dissipation in the field it is often necessary to accumulate large numbers of soil samples over a growing season for chemical analysis of residual herbicide. If analytical results are to have any relevance to the field situation, dissipation of the herbicide during storage must be minimal, or at least predictable. Storage at temperatures of −15 to −20 °C is the preferred method, but when a large bulk of samples overtaxes available refrigerated storage, alternative storage procedures must be used. The experiment described in this paper was carried out to determine the effect of drying and subsequent storage at room temperature on the amount of extractable atrazine in soil samples.

MATERIALS AND METHODS

Birganbigil clay loam (van Dijk, 1961), containing 53% clay and 1.7% organic carbon, was fortified with atrazine to a concentration of 1 or 10 ppm. Atrazine was added as a solution of technical grade chemical in acetone. The solvent was allowed to evaporate before the soil was thoroughly mixed. Immediately after mixing, the control samples of each concentration were placed in screw-capped glass jars and stored at −20 °C. A second treatment of each concentration was placed in an oven at 45 °C for 24 h before being transferred to jars and stored in the same manner as the controls.

Water was added to the remaining samples to increase their moisture content from air-dry (8%) to a more typical field moisture (18%). They were then placed in brown paper bags and dried at 45 °C for 24 h, after which one set of samples of each concentration was transferred to glass jars and stored at −20 °C with the earlier samples. The remainder were stored at room temperature in the brown paper bags for 30, 90, 180, or 360 days. At the end of the allotted storage period, samples were transferred to jars and stored at −20 °C. Each sample contained 100 g of soil, and each treatment was replicated three times.

At the end of the experiment the samples were extracted by boiling in acetonitrile–water (9:1), and atrazine content was determined by the gas chromatographic method of Bowmer (1972). Cleanup of the dichloromethane solution was not necessary. Results were corrected for soil moisture content.

RESULTS AND DISCUSSION

Atrazine concentrations of the samples following the drying and storage treatments are given in Table I. The amount of atrazine extractable from the 1-ppm samples

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