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Effect of Temperature and BASF 13 338 on the Lipid Composition and Respiration of Wheat Roots

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ABSTRACT

The fatty acid composition of wheat seedling roots changed in response to temperature. As temperature declined, the level of linolenic acid increased and the level of linoleic acid decreased. The distribution of phospholipid classes was not influenced by temperature. Phosphatidyl choline and phosphatidyl ethanolamine were the predominant phospholipids isolated and comprised 85% of the total lipid phosphorus. Smaller quantities of phosphatidyl glycerol, phosphatidyl inositol, phosphatidic acid, and phosphatidyl serine were isolated. The fatty acid composition of phosphatidyl choline and phosphatidyl ethanolamine were the same and temperature affected the fatty acid composition of both phospholipids in the same manner.

Growth in the presence of the substituted pyridazinone, BASF 13 338 (4-chloro-5-dimethylamino-2-phenyl-3(2*H*)pyridazinone), reduced the level of linolenic acid and increased the level of linoleic acid in the phosphatidyl choline, phosphatidyl ethanolamine, and total polar lipid fractions. BASF 13 338 did not affect the levels of palmitate, stearate, and oleate or the distribution of phospholipid classes.

Respiration rates of wheat root tips were measured over a range of temperatures. The respiration rate declined as the temperature decreased. Neither the temperature at which the tissue was grown nor BASF 13 338 treatment influenced the ability of root tips to respire at any temperature from 4 to 30 C. The results indicated that the relative proportion of linolenic acid to linoleic acid did not influence the plants ability to grow and respire over the range of temperatures tested.

As the temperature declines in the fall of the year, winter wheat undergoes physiological changes which lead to increased freezing resistance. Considerable research has focused on changes in membrane lipid composition during temperature acclimation. When wheat plants are exposed to cold temperatures, the level of linolenic acid increases and the level of linoleic acid decreases (4, 9, 27). Fatty acid unsaturation increases to the same extent in cultivars varying widely in resistance to freezing (6, 27).

Recent studies demonstrated that the substituted pyridazinone BASF 13 338 simultaneously inhibits the accumulation of linolenic acid and the acquisition of winter hardiness (22, 23, 26). It was proposed that an increase in linolenic acid would increase the fluidity of membranes at low temperatures. This should enable wheat to function normally at low temperatures and perform the necessary steps involved in the acquisition of freezing resistance.

Our objectives were to characterize the effect of temperature and pyridazinone treatment on the phospholipid and fatty acid composition of wheat roots and to determine the relation between lipid composition and the ability of wheat plants to grow and respire at low temperatures.

MATERIALS AND METHODS

Growth of Wheat Seedlings. Winter wheat (*Triticum aestivum* L. 'Arthur') seeds were germinated in paper seed towels moistened with either distilled H_2O or 100 μM BASF 13 338 (4-chloro-5-dimethylamino-2-phenyl-3(2*H*)pyridazinone). Seeds were germinated in the dark in laboratory incubators at either 25, 20, 15, or 10 C for various lengths of time. Root growth of seedlings was measured by fresh weight. Under these conditions, seedlings grown 5 days at 25 or 20 C, 8 days at 15 C, and 12 days at 10 C were morphologically equivalent and used for subsequent comparisons.

Fatty Acid Analysis. Wheat roots were harvested, frozen with Dry Ice, lypholized, and stored at −20 C until analyzed. Freeze-dried roots were ground with a mortar and pestle and extracted with chloroform:methanol (2:1, v/v) (11). The extracted lipids were purified, and analyzed by GC as previously described (22).

Mitochondria were isolated for fatty acid analysis by grinding wheat roots in isolation buffer using a mortar and pestle. All isolation procedures were performed between 0 and 5 C. The isolation buffer contained 0.5 M mannitol, 10 mM Tes buffer, 1 mM EDTA, 0.5% cysteine-HCl (w/v), and 0.05% BSA (w/v) (pH 7.5). After grinding, the sample was filtered through nylon cloth and the filtrate centrifuged at 2500*g* for 15 min. Subsequently, the pellet was discarded and the supernate was centrifuged at 12,000*g* for 15 min. The supernate was then discarded and the pellet resuspended in 10 mM Tes buffer containing 0.5 M mannitol (pH 7.5) and centrifuged again at 12,000*g* for 15 min. The supernate was discarded and the pellet was extracted with chloroform:methanol (2:1, v/v) (11). Subsequent lipid purification and chromatography were performed as described previously.

Phospholipid Extraction and Purification. Five g of wheat seedling roots were harvested for phospholipid extraction. The roots were enclosed in an aluminum foil envelope and placed at 100 C for 15 min to inactivate phospholipases (20). The roots were then frozen in Dry Ice and ground using a mortar and pestle. A Folch

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extraction (11) was performed on the Dry Ice powder. Subsequently, the sample was filtered through a 0.4 μm pore size polycarbonate membrane filter (Bio-Rad Laboratories[2], Richmond, CA), the solvent was evaporated with a stream of N_2, and the sample was dissolved in 0.5 ml chloroform.

Liquid chromatographic analyses were performed with a Waters Associates liquid chromatograph (Waters Associates, Milford, MA) as described previously (1). A Porasil column packed with 10 μm diameter silica gel particles was used. Detection of eluting components was by *A* at 206 nm.

The solvent system was a mixture of hexane, 2-propanol and water (12). Hexane and propanol were commercially distilled in glass (Burdick and Jackson Laboratory, Inc., Muskegon, MI). The water was distilled, deionized, and filtered through a 0.45 μm Millipore filter prior to use. The ratio of hexane to propanol was maintained at 3:4 (v/v), and the solvent polarity was controlled by varying the water content. Solvents were sonicated in an ultrasonic bath prior to use to minimize dissolved gases. Solvent flow was maintained at 1.5 ml/min. Solvent composition was initially hexane:propanol:water (6:8:0.5, v/v/v). A linear increase in the water content during a 25-min period resulted in a final solvent composition of 6:8:1.5.

The phosphorus content of collected fractions was determined using Bartlett's modification (2) of the Fiske-SubbaRow method (10).

Respiration Studies. The rate of O_2 consumption by wheat root tips was measured using a Gilson differential respirometer. Forty root tips, 2 cm long, were harvested and transferred to standard respirometer flasks containing 5 ml of 25 mM phosphate buffer (pH 7.0). Ten per cent KOH and a filter paper wick were placed in the center well of each flask to trap evolved CO_2. Four replicate flasks were used for each treatment. The respiration rate at each temperature was monitored for three successive 20-min intervals, and corrected for standard conditions of temperature and pressure. O_2 consumption was expressed on the basis of root dry weight. Root tips were collected from each flask, dried in a forced draft oven at 95 C overnight and dry weight was determined. The respiration rates at two different temperatures were determined using a single sample of root tips. Comparable samples were used to determine root tip respiration over the entire range of temperatures tested.

RESULTS AND DISCUSSION

The phospholipids of wheat roots were separated into five fractions using high performance liquid chromatography. PC[3] and PE were the predominant phospholipids isolated and accounted for one-half and one-third of the total lipid phosphorus, respectively (Table I). Smaller quantities of PG, PI, PA, and PS were isolated. The phospholipid composition of wheat roots did not change in response to temperature. The total quantities and the distribution of the phospholipid classes were the same in seedlings grown at a range of temperatures from 10 to 25 C (Table I). Earlier work by de la Roche and co-workers (5) supports these observations. They determined that the phospholipid compositions of wheat seedlings during germination at warm (24 C) and cold (2 C) temperatures were nearly equivalent. However, a slight increase in PA and a slight decline in PC during germination at 2 C was observed. Willemot (25) measured the incorporation of radioactive phosphorus into phospholipids. PC, PE, PI, and PG were the predominant phospholipids labeled and the relative proportions of radioactivity incorporated in each lipid changed only slightly during cold hardening of wheat. In contrast to the results of de la Roche and co-workers, Willemot observed a slight increase in PC at low temperatures. There were limitations in both the previous studies. de la Roche and co-workers (5) examined the phospholipid composition of entire seedlings (root, shoot, seed). The degradative processes of the seed may have led to the high levels of lysophosphatides observed. Also, changes in the root and shoot lipids during temperature acclimation may have been masked by the phospholipids of the endosperm (24). In the labeling study (25) the biosynthetic rate was determined; no information regarding lipid turnover and the actual levels of the phospholipids was provided. However, from two earlier studies and the present study, we can conclude that the phospholipid composition of wheat roots is unaffected by temperature.

Table I. *Phospholipid Composition of Wheat Seedling Roots*

Phospholipids were separated into five fractions using high performance liquid chromatography. The fractions were identified as PE, PG, PI, PA-PS, and PC. Fractions were collected and the phosphorus content determined by Bartlett's modification of the Fiske-SubbaRow method.

Temperature	Growth Temperature	Phospholipid Fractions				
		PE	PG	PI	PA-PS	PC
	C	*% lipid phosphorus*				
Control	25	34	5	7	4	51
	20	37	5	4	6	48
	15	31	8	7	3	52
	10	38	6	7	4	45
BASF 13 338 (100 μM)	25	35	6	5	4	50
	20	33	6	5	2	54
	15	34	5	7	4	50
	10	37	5	4	3	51

The phospholipid composition of some plant species is reported to be affected by temperature. When rye (*Secale cereale* L.) seedlings were exposed to cold temperatures (4 C), the relative levels of PC and PI decreased and the proportion of PA increased (24). Kuiper (13) observed that the levels of PG, PI, and sulfolipid in alfalfa (*Medicago sativa* L.) leaves decreased and the levels of monogalactosyl diglyceride, digalactosyl diglyceride, PC, and PE increased when plants were exposed to low temperatures. Dogras and co-workers (8) compared the phospholipid biosynthetic rates of three species at 25 and 10 C. The relative proportions of the phospholipid classes in broad bean (*Vicia faba* L.) and pea (*Pisum sativum* L.) changed little in response to temperature. In lima beans (*Phaseolus lunatus* L.) the synthesis of PC decreased and that of PE and PG increased.

No consistent effects of temperature on the distribution of phospholipid classes have been observed. Biophysical studies have demonstrated that the polar group of a phospholipid can alter membrane properties and influence the temperature at which membrane physical changes occur (14). Changes in the distribution of phospholipid classes may be an adaptive feature which utilizes the different physical properties of the phospholipid classes in order to maintain proper membrane ordering over a range of temperatures. If this were the case, uniform trends among plant species as to which phospholipids predominant at low temperatures might be expected. The differences observed may result from differences among species, types of tissues studied, or the method of extraction.

PC, PE, and the total polar lipid fraction of wheat roots had the same fatty acid composition. At low temperatures the level of

[2] Mention of a trademark, proprietary product, or vendor does not constitute a warranty of the product by the United States Department of Agriculture and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

[3] Abbreviations: PC, phosphatidyl choline; PE, phosphatidyl ethanolamine; PG, phosphatidyl glycerol; PI, phosphatidyl inositol; PA, phosphatidic acid; PS, phosphatidyl serine.

linolenic acid increased and the level of linoleic acid decreased to the same degree in all three fractions (Tables II, III, IV). The levels of palmitic, stearic, and oleic acids were not affected by temperature and remained at 23, 1, and 4%, respectively. The increase in the linolenic acid content of wheat roots was neither the result of changes in the level of polar lipid classes differing in the degree of unsaturation, nor the result of changes in the degree of unsaturation of a particular class of phospholipids. Instead, a logical explanation is that the enzyme catalyzing the desaturation of linoleic acid to form linolenic acid is responsive to temperature. When alfalfa was grown at low temperatures (15 C) the levels of lipid classes high in unsaturated fatty acids (monogalactosyl diglyceride, digalactosyl diglyceride, and PC) increased and the levels of lipid classes with low levels of unsaturated fatty acids (PG and PI) decreased resulting in an increased unsaturation of the leaf lipids (13). In eastern white pine (*Pinus strobus* L.) the increase in linolenic acid levels in the chloroplast lamellae during winter hardening appeared to result from the preferential loss of glycerolipids containing predominantly saturated and monounsaturated fatty acids (7).

Table II. *The Effect of Temperature and BASF 13 338 on the Fatty Acid Composition of Wheat Seedling Roots*

Seedlings were grown for either 5 days at 25 and 20 C, 8 days at 15 C, or 12 days at 10 C.

Treatment	Growth Temperature	Fatty Acid				
		16	18	18:1	18:2	18:3
	C	% by weight of fatty acids				
Control	25	23	1	4	50	21
	20	24	1	3	44	28
	15	22	1	4	36	38
	10	22	2	5	32	39
BASF 13 338 (100 μM)	25	23	1	4	63	9
	20	22	0	3	62	12
	15	20	1	4	64	11
	10	20	0	3	61	16

Table III. *Fatty Acid Composition of Phosphatidyl Choline from Roots of Wheat Seedlings*

Phospholipids were separated using high performance liquid chromatography. The phosphatidyl choline fraction was collected and the fatty acid composition determined.

Treatment	Growth Temperature	Fatty Acid				
		16	18	18:1	18:2	18:3
	C	% by weight				
Control	25	27	2	5	49	16
	20	22	3	10	44	21
	15	26	5	9	34	26
	10	24	2	6	32	36
BASF 133 338 (100 μM)	25	25	2	5	63	5
	20	23	3	7	61	5
	15	21	4	9	58	7
	10	19	2	5	63	11

Table IV. *Fatty Acid Composition of Phosphatidyl Ethanolamine from Roots of Wheat Seedlings*

Phospholipids were separated using high performance liquid chromatography. The phosphatidyl ethanolamine fraction was collected and the fatty acid composition determined.

Treatment	Growth Temperature	Fatty Acid				
		16	18	18:1	18:2	18:3
	C	% by weight				
Control	25	32	3	4	47	14
	20	25	3	6	45	21
	15	32	4	7	32	24
	10	27	6	7	25	34
BASF 133 338 (100 μM)	25	27	5	7	56	4
	20	27	5	7	56	4
	15	26	4	8	55	7
	10	24	3	5	56	11

Treatment of wheat seedlings with the substituted pyridazinone, BASF 13 338, reduced the level of linolenic acid and increased the level of linoleic acid (Table II). BASF 13 338 treatment did not affect the levels of palmitic, stearic, and oleic acids (Table II). This effect was first observed by St. John (21) and subsequently confirmed by others (3, 22, 23, 26). BASF 13 338 reduced the level of linolenic acid in mono- and digalactosyl diglycerides (21), in the polar lipid fraction (3, 22, 23) and in the total lipid fraction (3, 26). In our study BASF 13 338 was equally effective in reducing the linolenic acid levels in PC, PE, and the total polar lipid fraction (Table II, III, IV), but did not alter the distribution of phospholipid classes (Table I). Possibly, the fatty acids of these lipids originate in the same pool and BASF 13 338 inhibits the desaturation of linoleic acid.

The observation that numerous plant species increase the degree of fatty acid unsaturation in response to reduced temperatures has led to speculation that this is an adaptive mechanism which facilitates functioning at cold temperatures. However, we found that an increase in the linolenic acid content of wheat roots in response to low temperature was not required for the continued normal growth and development of wheat at the temperatures tested. Treatment with BASF 13 338 inhibited the accumulation of linolenic acid which accompanies exposure to low growth temperatures. However, BASF 13 338 did not affect the germination and growth rate of wheat seedlings at 10, 15, 20, and 25 C (Fig. 1). de la Roche (3) also has reported that BASF 13 338 had no effect on the germination and growth of wheat seedlings at 2 C. Respiration studies demonstrated that the relative proportions of linoleic and linolenic acids did not influence the ability of wheat roots to respire over a range of temperatures. Roots from plants grown at 25 and 10 C had markedly different levels of linolenic acid (Table II) yet respired at similar rates from 4 to 30 C (Table V). Likewise, treatment with 100 μM BASF 13 338 inhibited the accumulation of linolenic acids in roots (Table II) and root mitochondria but did not affect the rate of O_2 consumption over the range of temperatures tested (Table V). It seems that the accumulation of linolenic acid when wheat is exposed to cold temperatures is not a prerequisite for continued normal respiration at the temperatures tested.

When the respiration rate of wheat root tips at various temperatures was presented as an Arrhenius plot, the data did not yield a straight line. Instead, an increase in the activation energy below 19 C was observed (Fig. 2). Changes in activation energy have been correlated with membrane phase transitions (15, 16), and it has been suggested that the temperature at which these transitions occur is influenced by the degree of membrane lipid unsaturation. In our studies, both seedling growth temperature and BASF 13 338 treatment altered the level of linolenic acid, yet neither treatment affected the activation energies of respiration nor the temperature at which the change in activation energy occurred. Similar observations were made by Pomeroy and Andrews (18). They measured the effect of temperature on the respiration of mitochondria and tissue segments from wheat and rye grown at 2

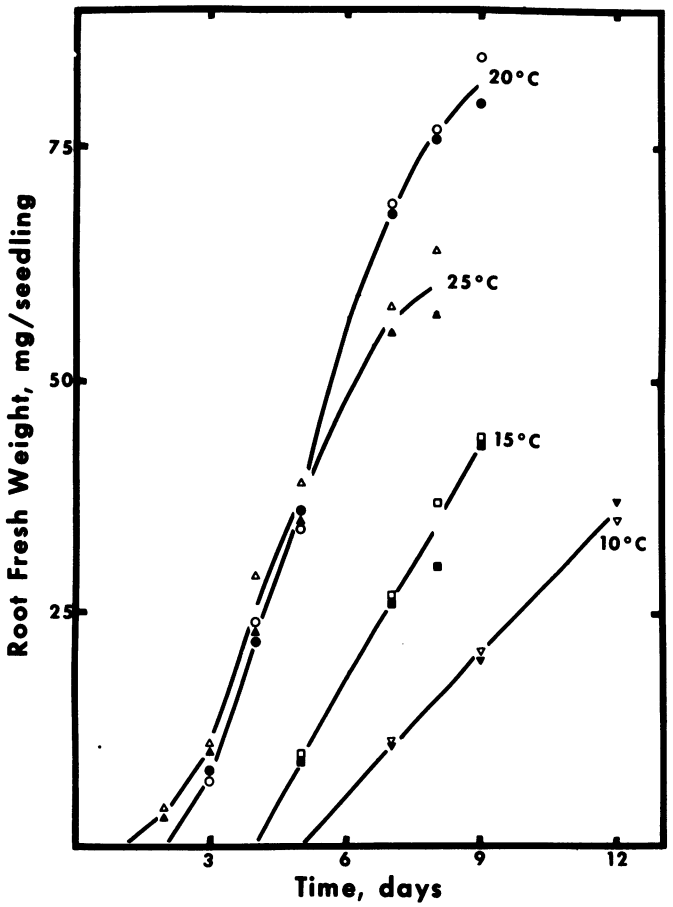

FIG. 1. Effect of temperature on the root growth of wheat seedlings. Seedlings were grown at 25 C (○, ●); 20 C (△, ▲); 15 C (□, ■); and 10 C (▽, ▼) in the absence (○, △, □, ▽) or presence of 100 μM BASF 13 338 (●, ▲, ■, ▼).

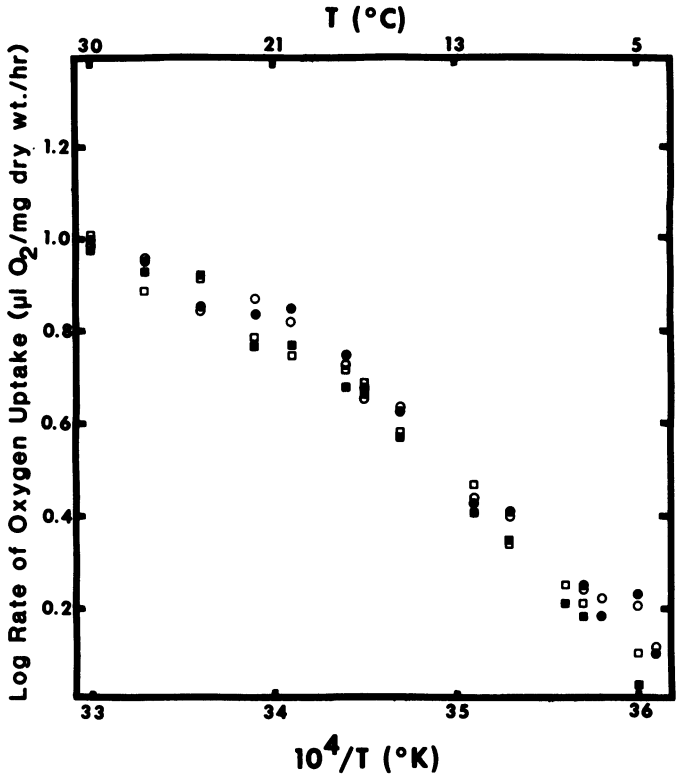

FIG. 2. Arrhenius plot of wheat respiration. Roots were harvested from seedlings grown at either 25 C (■, □) or 10 C (●, ○) and in the absence (■, ●) or presence of 100 μM BASF 13 338 (□, ○).

Table V. *Respiration Rate of Wheat Root Tips at Different Temperatures*

Wheat seedlings were grown at either 25 or 10 C to an equivalent size. Plants were grown in distilled water (control) or in 100 μM BASF 13 338. Values are the mean of three readings on four replicates.

Temperature	25 C Wheat		10 C Wheat	
	Control	BASF 13 338	Control	BASF 13 338
C	*μl O_2 consumed/mg dry wt·h*			
30	9.5	10.3	10.0	9.6
27	8.5	7.8	9.0	9.1
25	8.3	8.3	7.1	7.1
22	5.8	6.1	7.0	7.4
20	5.9	5.6	7.1	6.6
18	4.8	5.3	5.6	5.3
17	4.6	4.9	4.8	4.6
15	3.7	3.8	4.2	4.2
12	2.6	3.0	2.7	2.9
10	2.2	2.2	2.6	2.6
8	1.6	1.8	2.5	2.6
7	1.5	1.6	1.8	1.7
6			1.5	1.7
5	1.1	1.3	1.7	1.6
4			1.2	1.3

and 24 C. Changes in the activation energy of respiration were observed at 6 to 10 C in shoot segments and 10 to 14 C for isolated mitochondria. Neither the temperature at which the plants were grown nor the hardiness of the cultivars examined affected the temperature at which the transition occurred. They concluded that fatty acid unsaturation did not influence the temperature at which membrane phase transitions occur. Miller and co-workers (17) used electron spin resonance spectroscopy to measure the physical properties of wheat mitochondrial membranes. They detected three temperatures (6–10 C, 20–24 C, and 38–40 C) at which changes in membrane lipid ordering occurred and found that the transition temperatures for mitochondrial membranes isolated from cold-hardened wheat were 1 to 4 C lower than those observed in controls. In contrast, Raison and co-workers (19) measured the oxidation of succinate and α-ketoglutarate by wheat mitochondria and observed no change in activation energies from 2 to 28 C. They also used electron spin resonance spectroscopy and observed changes in membrane lipid ordering at 0 and 30 C. They did not compare warm and cold acclimated tissues, so the influence of lipid unsaturation could not be addressed. No explanation for the range of transition temperatures observed in these studies is available.

Based on the results of the present study and on those of Pomeroy and Andrews (18), we concluded that the increase in linolenic acid and decrease in linoleic acid in wheat in response to low temperature did not affect the rate of respiration from 4 to 30 C, the activation energies of respiration, or the temperature at which the change in activation energy occurs. This did not eliminate the possibility that the increase in linolenic acid in response to cold temperature was an adaptive mechanism which facilitated survival. Changes in fatty acid composition may influence other membrane-associated processes such as active transport, water and ion permeability, and membrane resilience.

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