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Metabolic and Ultrastructural Changes Associated with Flooding at Low Temperature in Winter Wheat and Barley[1]

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ABSTRACT

Cold-hardened winter wheat (*Triticum aestivum* L. cv. Fredrick) and winter barley (*Hordeum vulgare* L. cv. Dover) were exposed to total flooding at 2 C. Dover seedlings were damaged more quickly than Fredrick, and after 3 weeks of flooding the survival of Dover was reduced to 10% and Fredrick to about 50%. Tissue moisture was slightly greater in Dover than Fredrick throughout the 4-week flooding period. Carbon dioxide and ethanol accumulated throughout the 4-week flooding period in both cultivars. Lactic acid increased rapidly during the 1st week of flooding, and remained relatively constant during the remainder of the flooding period. Oxygen consumption of seedling shoot tissue after exposure to flooding declined abruptly after only 1 day of flooding, but recovered somewhat during the subsequent 2 weeks. The effect of flooding was more pronounced on the ultrastructure of Dover than Fredrick. Although proliferation of endoplasmic reticulum was observed in the early stages of flooding in both cultivars, the occurrence of distinct parallel arrays and concentric whorls of membranes was prevalent in the flooded barley. Severe ultrastructural damage to a large proportion of apical cells in both cultivars was observed after 2 to 3 weeks of flooding.

Exposure of plants to partial or total flooding results in major alterations in various physiological and metabolic processes (5, 8, 9, 21, 22). In flooding-intolerant species, normal respiration is blocked or severely inhibited resulting in enhanced rates of glycolysis, accumulation of one or more potentially toxic metabolites of anaerobic metabolism, and injury to the plant (5, 17). Respiratory processes of flooding-tolerant species are not as adversely affected by flooding (15, 16), and hence the accumulation of anaerobic metabolites and associated injury is generally less pronounced than in flooding-intolerant species (5, 8). Therefore, we concluded (8) that control of the level of glycolysis is essential for plants to withstand prolonged periods of partial or total anoxia that occur during flooding.

The metabolites that accumulate during flooding vary among plant species and are dependent on the conditions of flooding. Beletskaya (5) reported a 3- to 4-fold increase in the level of ethanol and lesser increases in lactic acid and acetaldehyde levels during warm-temperature flooding of winter wheat and rye. The greater degree of injury observed in rye than in wheat was related to a higher rate of glycolysis and a greater accumulation of glycolytic products in rye. Other reports (7–9) also indicated that ethanol is often the major end product of anoxia or flooding-induced glycolysis, but that other metabolites including lactic acid, malic acid, succinic acid, and acetaldehyde may accumulate to a lesser degree in some plant species. Ethylene was shown to increase sharply during flooding and was associated with injury in several plant species (12, 13). It also was observed that tolerance to anoxia in germinating seeds (7) and grasses (4) is greater when the metabolic rate is reduced at low temperature.

Flooding of winter cereals frequently occurs during autumn and spring in northeastern United States and eastern Canada. Low temperature flooding is currently being investigated in this laboratory as one of the factors influencing the winter survival of cereals. This paper reports changes in survival, cellular ultrastructure, and metabolic properties of a winter wheat and a winter barley during low temperature flooding in controlled environment conditions.

MATERIALS AND METHODS

Cold-acclimated seedlings of Fredrick winter wheat (*Triticum aestivum* L.) and Dover winter barley (*Hordeum vulgare* L.) were used in this investigation. Seedlings were grown in soil for 5 days at 20 C light, 15 C dark with a 16-h day at a light intensity of 525 μE m^{-2} s^{-1} (about 35,000 lux) and then were transferred to 2 C light, 0 C dark with a 16-h day at 145 μE m^{-2} s^{-1} (about 10,000 lux) for 6 weeks. Seedlings were prepared for flooding treatments by washing the roots free of soil under cold tap water and by removing endosperms. Groups of 15 seedlings with shoots and roots trimmed to 2 and 4 cm, respectively, were dipped in 3% sodium hypochlorite for approximately 5 s, washed under running tap water, and then immersed in 25 ml of glass-distilled H_2O in 40-ml glass tubes with culture caps. The vials and seedlings were transferred to 2 C day, 0 C night with a 16-h day at 145 μE m^{-2} s^{-1}. The level of microbial contamination in the system was monitored by determining levels of ethanol and CO_2 in water controls without plants, and in leachates from tubes from which sterilized seedlings were removed after 2 weeks at 2 C. After a further 2 weeks, there was no significant microbial contribution to ethanol levels, and only a slight contribution (about 10%) to final CO_2 levels.

After various intervals at low temperature, eight replicate vials were removed. From four vials, 10 plants were removed for survival determination and five were used for ethanol and lactate measurements. Seedlings from the other four treatment vials were used to determine respiratory rates and tissue moisture content. All experiments were carried out in duplicate. Moisture content of shoot tissue was determined by blotting surface water from the seedlings, weighing, drying at 80 C, and reweighing. Survival was determined by transplanting seedlings into moist Vermiculite and transferring to a growth room at 20 C day, 15 C night with a 16-h day at 525 μE m^{-2} s^{-1}. After 2 weeks, survival of seedlings was recorded.

Respiratory activity of shoot tissue after removal of seedlings from flooding conditions was determined on the basal 1-cm of shoots. The 1-cm portions were cut into 2-mm segments, weighed,

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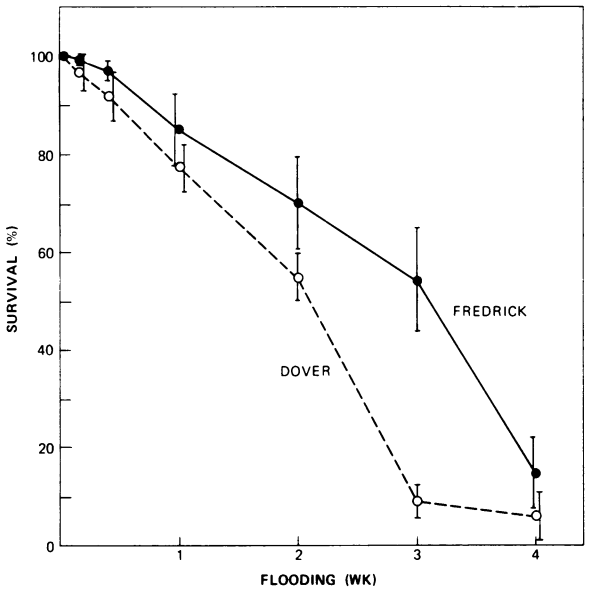


FIG. 1. Survival of cold-hardened Fredrick winter wheat (●——●) and Dover winter barley seedlings (○---○) after flooding in distilled H_2O at 2 C under controlled environment conditions. In Figures 1–5, each point represents an average (with SE) of two experiments, each with four replicates, and the absence of error bars indicates that the SE was smaller than the symbol used.

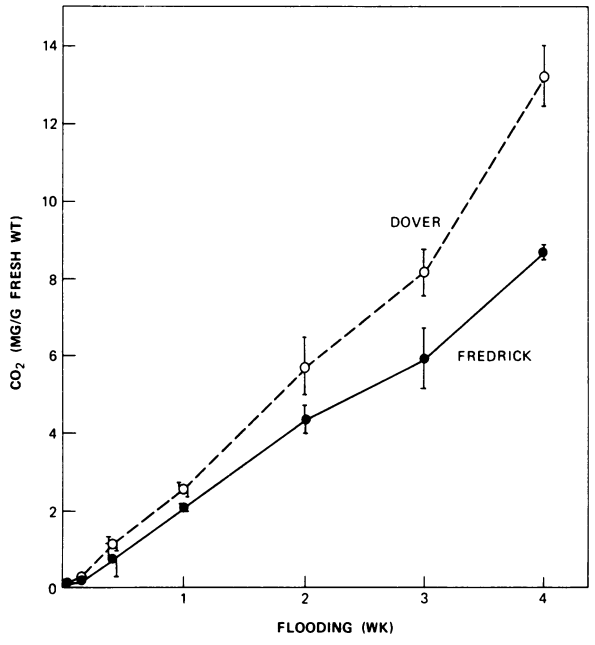


FIG. 2. Accumulation of CO_2 in the gas phase above the water during flooding of Fredrick (●——●) and Dover (○---○) in distilled H_2O at 2 C.

infiltrated with reaction media (18) under reduced pressure for 5 min, and O_2 uptake determined on 0.1-g lots using a standard Clark O_2 electrode. Ethanol and lactic acid were determined for groups of five seedlings and their leachates, as previously described (1, 19).

For CO_2 determinations, groups of 10 plants were immersed in 25 ml chilled H_2O in 35-ml serum bottles with five replicate bottles of each cultivar available for each flooding period. After flooding at 2 C, the bottles were agitated to ensure equilibrium between plant, water, and gas phase, and a 0.5-ml sample of the gas phase above the water surface was removed through the septum with a syringe. The samples were injected into a N_2 stream entering a

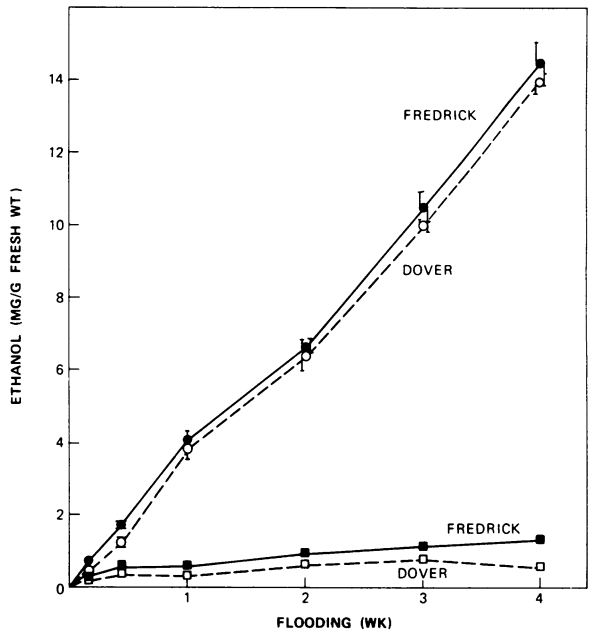


FIG. 3. Concentration of ethanol within plant tissues and of Fredrick (■——■) and Dover (□---□) and total ethanol production by Fredrick (●——●) and Dover (○---○) during flooding in distilled H_2O at 2 C.

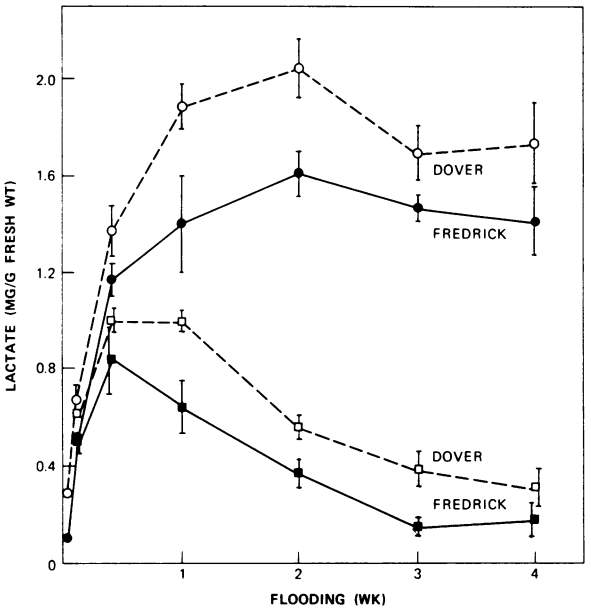


FIG. 4. Concentration of lactate within plant tissues of Fredrick (■——■) and Dover (□---□) and total lactate production by Fredrick (●——●) and Dover (○---○) during flooding in distilled H_2O at 2 C.

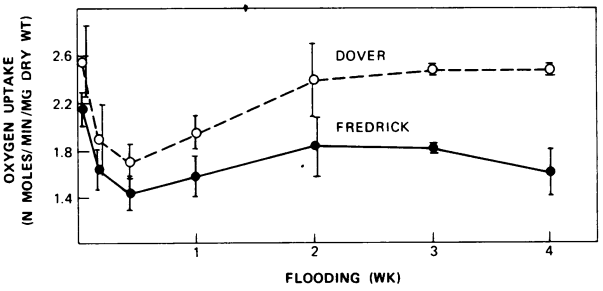


FIG. 5. O_2 consumption of tissue segments from shoots of Fredrick (●——●) and Dover (○---○) after flooding in distilled H_2O at 2 C. Seedlings were removed from flooding conditions, rinsed in distilled H_2O, and aerobic respiration measured using a Clark O_2 electrode.

Beckman 864 IR gas analyzer and responses compared with those of known quantities of CO_2. This is a procedure similar to that recently reported by Clegg *et al.* (6). The CO_2 values obtained in this way were adjusted to account for residual CO_2 dissolved in the water. This proportion was obtained by determining in a similar experimental arrangement the disappearance of CO_2 from a known amount of CO_2 injected into the gas phase over water. Under these conditions, CO_2 dissolved in water increased linearly at a ratio of 1.96:1 with increasing concentration in the gas phase. No attempt was made to determine residual CO_2 in the plants, due to uncertain interconversions among CO_2 molecules and carbonate and biocarbonate ions, and the high solubility of CO_2 in water at low temperature.

Shoot apices were dissected from seedlings and processed for electron microscopy (20) after various periods of flooding. The tissues were fixed in $KMnO_4$ for 4 h since earlier studies indicated better preservation of membranes with this fixative than with glutaraldehyde followed by postfixation in osmic acid (20).

RESULTS

The survival of cold-hardened Fredrick and Dover gradually declined during complete flooding at 2 C for 4 weeks (Fig. 1). Reduced survival and inhibition of growth after transplanting were observed within a few days in both cultivars. The reduction in survival was greater in Dover than in Fredrick throughout the flooding treatment, and after 4 weeks, survival of Dover and Fredrick was reduced to approximately 5 and 15%, respectively. The water content of seedling shoot tissue from both cultivars increased significantly throughout the flooding period, with the maximum rate of increase occurring during the first 3 days. Tissue moisture increased in Fredrick from 84 to 90% and in Dover from 87 to 93%.

The level of CO_2 produced by flooded plants increased throughout the flooding period (Fig. 2). The rate of CO_2 produced from Dover was considerably greater than from Fredrick, and after 4 weeks, the level was 50% greater in Dover than Fredrick, despite the fact that the mortality of plants was high for both cultivars. The amount of CO_2 produced by either cultivar was not significantly reduced by flooding with deoxygenated H_2O, or by N_2 atmosphere in the gas phase above the water (data not shown).

Total ethanol production (plant plus leachate) increased at a similar rate in the two cultivars throughout the flooding period (Fig. 3). The level of ethanol within the plant tissues increased slightly during the first few days of flooding, and then remained relatively constant thereafter (Fig. 3).

Lactic acid accumulated very rapidly but only to low levels, during the early stages of flooding in both Fredrick and Dover, reaching a maximum level within the plant after 3 days (Fig. 4). The lactate content of flooded seedlings then declined steadily, and after 4 weeks less than 50% of the maximum lactic acid levels were observed. Total lactic acid content increased for 2 weeks and then declined slightly during the final 2 weeks. The levels of total lactic acid and of those within the plants were consistently higher for Dover than for Fredrick.

The rate of O_2 consumption of shoot tissue after removal of the seedlings from flooding conditions was generally slightly higher for Dover than for Fredrick (Fig. 5). O_2 uptake declined to approximately 75% of nonflooded levels after 3 days of flooding and then gradually increased. After 2 weeks, O_2 uptake in Dover had returned to preflooding levels but that of Fredrick remained lower than initially observed. After 4 weeks when the seedlings had been severely damaged, O_2 uptake in Dover remained at this high level, and only a slight decline was observed in Fredrick tissue.

Shoot apex cells of cold-hardened, nonflooded wheat and barley seedlings were characterized by a large centrally located nucleus, copious mitochondria and plastids of various sizes and shapes, occasional dictyosomes, and ER in the cisternal form (Fig. 6). After flooding for only 3 days and prior to any significant reduction in seedling survival, minor alterations in ultrastructural features were detected in both cultivars. Concentric whorls of ER were observed frequently in cells of Dover (Fig. 7) but not in Fredrick. In both cultivars, plastids often were distended and irregular in shape, and the occasional appearance of large pores and formation of loops in the nuclear membrane were observed (Fig. 8).

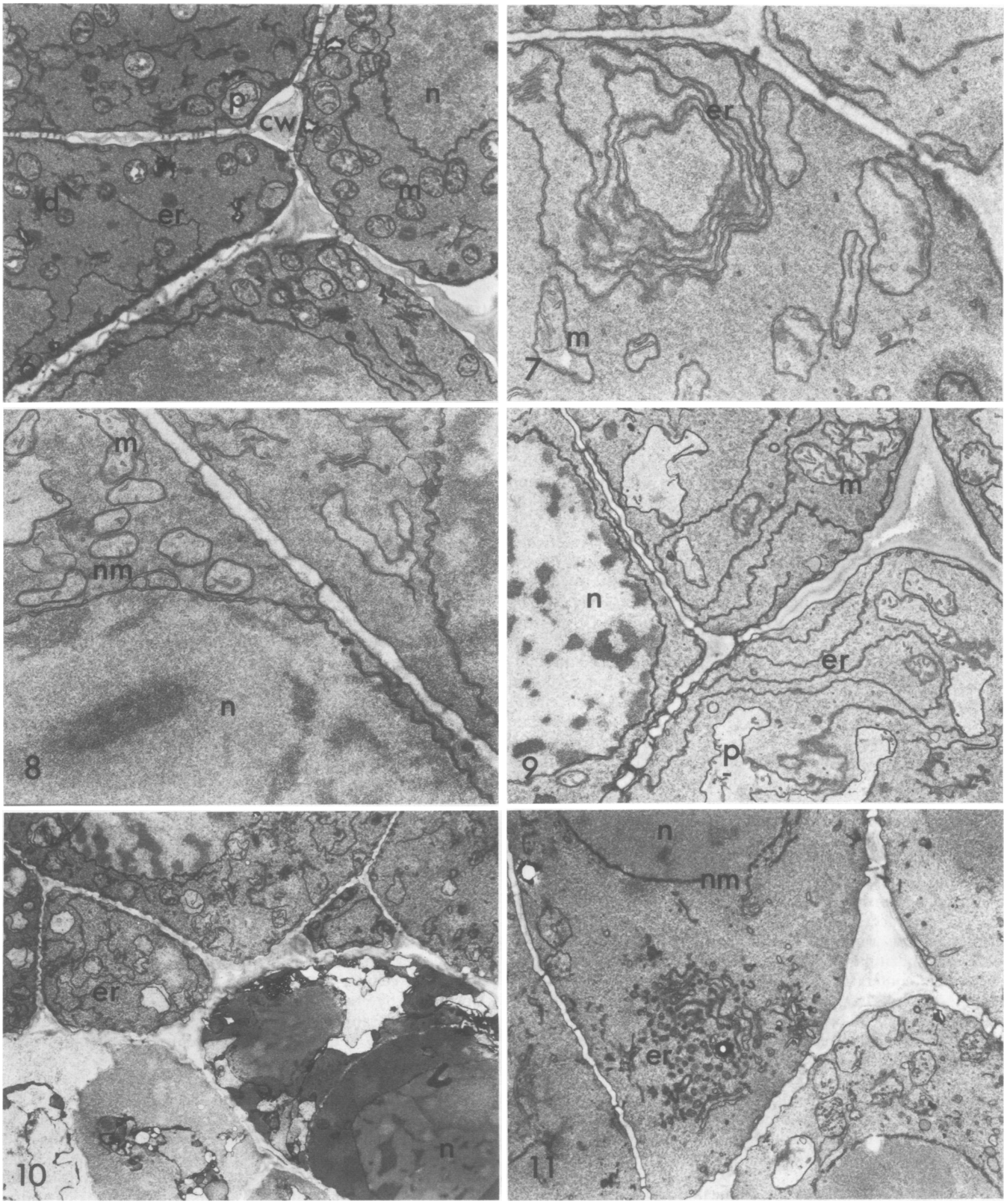
The quantity of cisternal ER increased appreciably in wheat and barley after flooding for 1 week (Fig. 9), but occasional whorls of concentric membranes were observed only in Dover. All apical cells of Fredrick retained their ultrastructural integrity, whereas a small proportion (10–20%) of Dover cells exhibited severe structural disorganization. After flooding for 2 weeks, approximately 50% of cells of Fredrick (Fig. 10) and nearly all cells of Dover (Fig. 11) were severely damaged. Many of the disintegrating Dover cells appeared to contain concentric whorls of membranes in various stages of breakdown. Frequently, severely damaged cells of Fredrick were found adjacent to cells which still retained much of their structural integrity. After 3 weeks flooding, continued structural disorganization in Dover made it difficult to identify cellular organelles (Fig. 12). In contrast, many cells of Fredrick were not severely disrupted, but occasional whorls of membranes were observed (Fig. 13).

DISCUSSION

The relatively slow rate of decline in viability of cereal seedlings observed in this study is a reflection of the generally lower rates of metabolism induced by reduced temperatures. This results in a decreased incidence and severity of damage, as compared to that observed during flooding at 25 C, where severe injury to both wheat and barley was observed after only a few days (unpublished results). The aerobic respiratory system of the plant is not totally incapacitated even after prolonged flooding, when survival of seedlings is markedly reduced. The continued consumption of O_2 in seedlings removed from flooding conditions may be due in part to the presence of uninjured or only partially damaged mitochondria (2) in the dying seedlings. However, the response is more marked in the more flooding-susceptible Dover, suggesting that O_2 is probably also being utilized by nonrespiratory processes in dead and injured tissues. Flooding at higher temperatures also inhibits the rate of aerobic metabolism and the effect is more pronounced in flooding-intolerant than flooding-tolerant species (5, 15).

The shift from aerobic to anaerobic metabolism due to flooding is accompanied by the accumulation of at least three metabolites: ethanol and CO_2 in generally similar quantities, and lactic acid to much lower levels. The level of this latter metabolite is limited by decreasing cellular pH (10). The greater production of CO_2 by Dover than by Fredrick during flooding may be directly associated with the more rapid decline in viability of this cultivar. In addition to ethanol, CO_2, and lactic acid, several other metabolites, including ethylene and various organic acids, have been reported to accumulate during flooding and other forms of anoxia (5, 7, 9, 12, 21). Ethanol frequently accumulates to higher levels than other metabolites during flooding (5, 7), but the mechanism of injury due to flooding has not been firmly established. Kawase (12, 13) reported that ethylene is responsible for cellular damage in flooded plants, but ethylene accumulates only to very low levels in cereals flooded at low temperature, and at this level is not toxic (Andrews and Pomeroy, unpublished results).

The exact relationship between metabolite accumulation and seedling damage during flooding has not been unequivocally established. Our earlier investigations (3) have shown that exogenously applied CO_2 and ethanol increase cell permeability and reduce survival of wheat seedlings at concentrations equivalent to or lower than those accumulating within the plant. Both ethanol and CO_2 also were shown elsewhere (11, 14) to alter cell permea-



FIGS. 6–13. Ultrastructure of shoot apex cells of Fredrick winter wheat and Dover winter barley after various periods of flooding in distilled H_2O at 2 C. Fixation for 4 h in aqueous 2% $KMnO_4$. cw: Cell wall; d dictyosome; er: endoplasmic reticulum; m: mitochondrion; n: nucleus; (nm: nuclear membrane; p: plastid.

FIG. 6. Fredrick, nonflooded (× 8,100).
FIG. 7. Dover, flooded 3 days (× 18,900).
FIG. 8. Fredrick, flooded 3 days (× 18,900).
FIG. 9. Fredrick, flooded 1 week (× 9,900).
FIG. 10. Fredrick, flooded 2 weeks (× 3,600).
FIG. 11. Dover flooded 2 weeks (× 9,000).

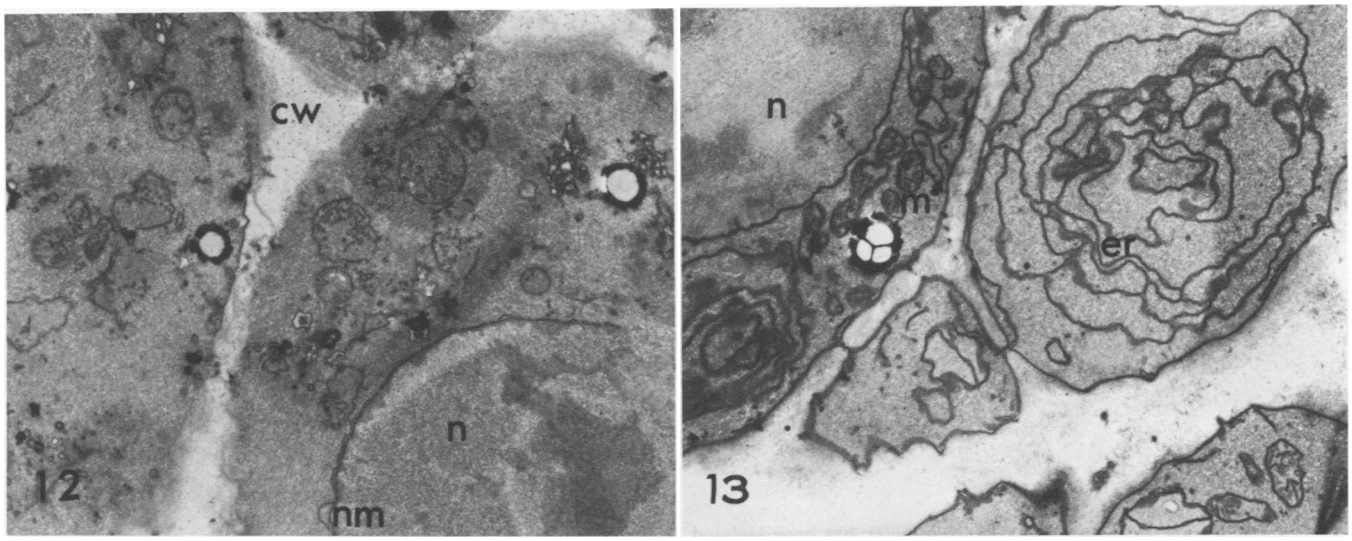


FIG. 12. Dover, flooded 3 weeks (× 8,100).
FIG. 13. Fredrick, flooded 2 weeks (× 9,000).

bility and damage cell membranes of other plant species after prolonged exposure. It is possible that endogenously produced ethanol and CO_2 results in similar damage during flooding.

Ultrastructural changes in flooded plants are more severe in Dover than in Fredrick and are undoubtedly associated with the greater susceptibility of Dover to flooding stress. However, the relationship of these ultrastructural changes to cellular metabolism and plant injury during flooding is uncertain. Other studies (19, and references therein) on many species of plants have shown that the formation of concentric whorls and parallel arrays of membranes can be induced by many types of nonlethal stress. The eventual breakdown of cell organelles and disruption of cellular integrity during flooding at low temperature appears to be associated with plant death.

The results obtained in this investigation have clearly shown that flooding, even at the low temperatures prevalent during autumn and spring, is potentially damaging to winter cereals. Under field conditions, metabolites would not accumulate to the high levels observed in the flood water of the experimental system employed in this study. However, this would not preclude damage to the plants, since the deleterious effects of the anaerobic metabolites result from their transient accumulation within the plant, prior to diffusion into the flood water. Autumn flooding could be expected to be particularly injurious in the colder winter wheat growing regions where damage incurred due to flooding could severely reduce the resistance of plants to subsequent injury from freezing and ice encasement.

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