

## SHORT COMMUNICATION

### Changes in Spectral Properties of Detached Birch Leaves\*

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A leaf begins to senesce as soon as it is removed from the plant and changes in metabolic processes and spectral properties are inevitable. The objectives of this study were to (1) determine the rate of changes in spectral properties of detached leaves and (2) evaluate the effectiveness of low temperature and cytokinin for delaying changes in spectral properties. Leaves from red birch (*Betula nigra* L.) were immersed for 5 min in water or 0.001M benzylaminopurine (BAP) and stored in plastic bags in the dark at either 5 or 25°C. Total directional-hemispherical reflectance and transmittance of the adaxial surface of leaves were measured over the 400-1100 nm wavelength region with a spectroradiometer and integrating sphere (LI-COR LI-1800). Spectral properties changed less than 5% of initial values during the first week when leaves were stored at 5°C. Storage at 25°C promoted rapid senescence and large changes in spectral properties. BAP delayed, but did not stop, senescence at 25°C. Low temperature was more effective than BAP in delaying senescence. It appears possible to store leaves at low temperatures in plastic bags in the dark for several days without significantly altering the spectral properties in the 400-1100 nm wavelength region.

#### Introduction

As models have been developed which describes the interactions of solar radiation with leaves, there has been increased need for accurate and reliable measurements of the spectral properties of leaves. Many factors affect the spectral properties of leaves, including chlorophyll content (Gausman et al., 1973; Hoffer and Johannsen, 1969; Knipling, 1970), water content (Sinclair et al., 1971; Thomas et al., 1971; Woolley, 1971), leaf age (Gausman et al., 1971), and internal structure (Sinclair et al., 1971; Gausman et al., 1969). To describe variability of spectral properties that occur in nature, one must not only select representative

samples but at the same time avoid introducing extraneous variation due to the measurement process.

When the spectroradiometer and its accessory equipment are located near the test site, spectral data of leaves can be acquired either in situ with leaves attached to the plant or immediately after removing the leaf from the plant. If precautions to minimize water loss are followed, then changes in spectral properties of the leaves are small during the first several hours after excision (Sinclair et al., 1971; Gausman et al., 1969). However, when the spectroradiometer cannot be located near the test site, several days may elapse from the time leaves are removed from the plant until the spectral properties can be measured. During this time, significant metabolic, and possibly spectral, changes can occur. The mag-

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nitude and rate of changes in spectral properties have not been documented for periods of time greater than 6 h after excision. The objectives of this study were to (1) determine the rate of changes in the spectral properties of detached leaves and (2) to evaluate the effectiveness of low temperature and cytokinin for delaying changes in spectral properties.

In oat leaves (*Avena sativa* L.), proteolysis may begin within 6 h and chlorophyll degradation may be evident within 24 hours after excision (Tetley and Thimann, 1974; Martin and Thimann, 1972). In wheat leaves (*Triticum aestivum* L.) detached for 3–4 days, the cytoplasm, tonoplast, and cellular organelles were in various stages of degradation (Hurkman, 1979). Starch deposits in chloroplasts disappeared and the ultrastructure of chloroplasts, mitochondria, and other cellular organelles were swollen and distended, indicating loss of energy (Hurkman, 1979; Thimann, 1980).

Senescence is an active metabolic process and can be affected by temperature, light, plant hormones, and various other compounds (Thimann, 1980). As temperature of excised oat leaves kept in the dark for 3 days increased from 5 to 30°C, chlorophyll concentration decreased rapidly (Tetley and Thimann, 1974). Senescence proceeded at its maximum rate at 30°C. At higher temperatures, the rate of chlorophyll degradation decreased until temperatures exceeded 40°C and thermal effects began to cause secondary yellowing (Tetley and Thimann, 1974; Thimann, 1980).

Light delays both proteolysis and chlorophyll degradation, but the effect of light is readily reversible in the dark (Tetley and Thimann, 1974). Rapid senescence in dark may be due to deprivation of energy

(Hurkman, 1979) or to accumulation of abscisic acid (Thimann, 1980). Photoperiod also influences the rate of senescence. For example, leaves of *Hibiscus rosa-sinensis* which senesce in 20 days in dark, survive to 35 days in 8-h photoperiods and 45 days in 16-h photoperiods (Misra and Biswal, 1973).

Cytokinins delay proteolysis and chlorophyll loss in leaves in the dark, perhaps by maintenance of a tight coupling between respiration and phosphorylation (Thimann, 1980). Although the relative activities of the cytokinins vary with plant species, benzylaminopurine (BAP) is generally several times more active than kinetin or zeatin (Tetley and Thimann, 1974). Treatment with BAP as preharvest spray or postharvest dip has been effective in prolonging storage life of some vegetables (Dedolph et al., 1962) and cut flowers (Heide and Oydvin, 1969).

In summary, when leaves are detached, they begin to senesce, and changes in metabolic processes and physiological and spectral properties are inevitable. However, the rate of senescence can be altered. If senescence can be delayed for several days without significant changes in spectral properties, then samples of leaves from plants at remote test sites can be prepared and shipped to laboratories which have the equipment to measure spectral properties.

## Materials and Methods

### Experimental conditions

Two experiments were conducted during 1983 using leaves from red birch (*Betula nigra* L.). In the first experiment, 0.5M apical segments of branches with leaves fully exposed to the sun on the south side of the trees were cut and the

bases of the branches were immediately placed in water. Within an hour, four whole, fully expanded leaves per branch were randomly selected, placed in resealable polyethylene bags, and stored in the dark at  $5 \pm 2^\circ\text{C}$ . In the second experiment, leaves were collected and prepared as in the first experiment, except that the leaves were immersed for 5 min in either distilled water or 0.001M BAP (Heide and Oydvin, 1969). Afterwards the leaves were blotted dry with paper towels, placed in resealable polyethylene bags and stored in the dark at either  $5 \pm 2^\circ\text{C}$  or  $25 \pm 3^\circ\text{C}$ . Within 2 h after excision and then on selected days after excision, the leaves were removed from storage, and their spectral properties were measured.

#### Spectral data

Total (diffuse and specular) directional-hemispherical reflectance and transmittance of the adaxial surface of the birch leaves were measured over the 400–1100 nm wavelength region with a spectroradiometer and integrating sphere (LI-COR LI-1800). The incident angle of illumination was  $5^\circ$  from normal. An area between the major veins of each birch leaf was identified and measured each time to minimize within-leaf variation in spectral properties. Spectral properties of the leaves were measured 0, 1, 3, 5, 8, 11, and 25 days after excision in the first experiment and 0, 3, 9, 17, and 23 days after excision in the second experiment. All data were corrected for reflectance of the barium sulfate reference surface in the integrating sphere and expressed in directional-hemispherical reflectance and transmittance factor units. Absorptance was calculated as 1.0 minus the sum of reflectance and transmittance. Changes in spectral properties were calculated as

differences and ratios and plotted as functions of wavelength and time after excision.

#### Results and Discussion

In the visible spectral region (400–700 nm), the low reflectance and transmittance and high absorptance (Fig. 1) of radiation by healthy, green leaves are due to pigments, primarily chlorophylls, carotenoids, xanthophylls, and anthocyanins (Hoffer and Johannsen, 1969; Knippling, 1970). Reflectance and transmittance of leaves in near infrared (700–1100 nm) are high and are related to internal cell structure of leaves. An important parameter in determining the level of near infrared reflectance is the number or total area of air-wall interfaces (i.e., discontinuities in refractive index) and not the volume of air space (Sinclair et al., 1973). Factors that change the number of interfaces also change the light-scattering properties of the leaves.

During initial stages of senescence, internal leaf volume may decrease but the number of interfaces may increase as cell walls dehydrate, split, and reorient themselves (Sinclair et al., 1971). These changes increase the radiation diffusing capacity and thus increase near infrared reflectance of leaves. In advanced stages of senescence, breakdown and deterioration of leaf tissue eventually decrease infrared reflectance (Colwell, 1956; Knippling, 1970).

In these birch leaves, no significant changes in reflectance, transmittance, or absorptance (Fig. 1) were evident within 3 days of excision, regardless of the treatment. Leaves stored longer than 3 days at  $25^\circ\text{C}$  showed marked changes in reflectance and transmittance, especially in the

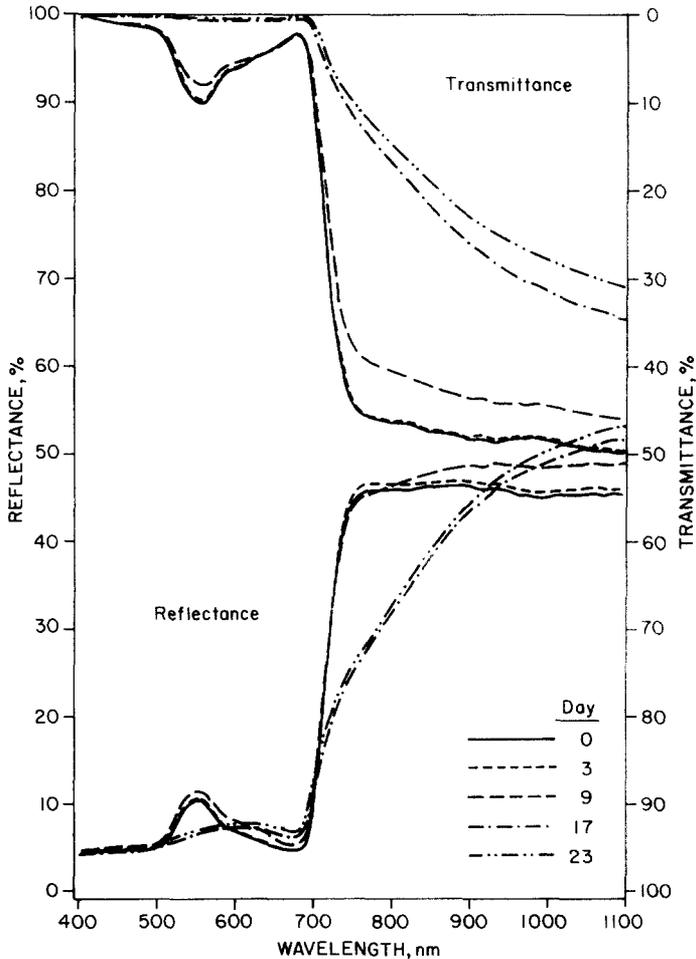


FIGURE 1. Reflectance, transmittance, and absorptance of birch leaves treated with water and stored at 25°C, as a function of wavelength and time after excision. Data are means of three leaves. Absorptance may be calculated as 100 minus the sum of reflectance and transmittance. Day: (—) 0; (---) 3; (- - -) 9; (- - -) 17; (- · - ·) 23.

green (550 nm), red (670 nm), and near infrared (700 and 1100 nm) wavelengths.

Changes in spectral properties of leaves are easily identified when the spectral property (reflectance, transmittance, or absorptance) on day  $n$  is divided by the spectral property on day 0. These ratios minimize differences in spectral properties among leaves and accentuate the changes in spectral properties within leaves. For birch leaves stored at 5°C,

reflectance (Fig. 2), transmittance (Fig. 3), and absorptance (not shown) at all wavelengths did not change more than 10% of the value on day 0 until 9 days after the leaves were excised from the trees. After 17 days at 5°C, changes in the visible reflectance (Fig. 2) exceeded 20% of initial values.

For leaves stored at 25°C, changes in reflectance (Fig. 2) and transmittance (Fig. 3) exceeded 10% of initial values

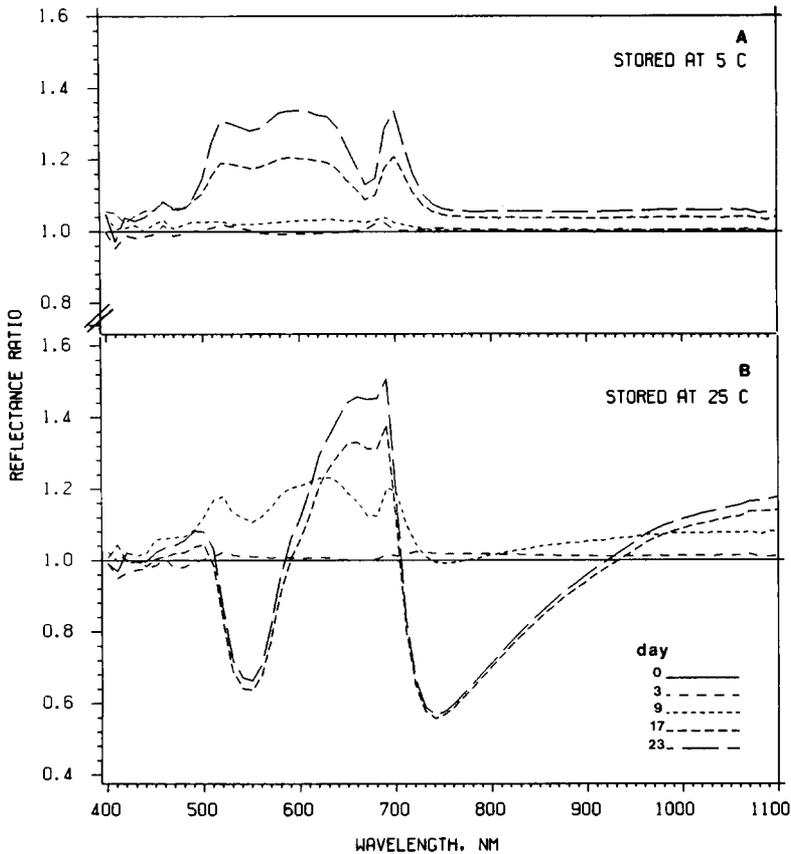


FIGURE 2. Changes in reflectance of birch leaves treated with water as a function of wavelength and time after excision. Data are ratios of reflectance on day  $n$  divided by reflectance on day 0. Day: (—) 0; (---) 3; (· · ·) 9; (- · - ·) 17; (- - -) 23.

after 3 days. The greatest changes in reflectance (Fig. 2) after 3 days occurred in green (550 nm), chlorophyll absorption (670 nm), and near infrared wavelengths. Leaves stored at 25°C for more than 9 days transmitted almost no visible (400–700 nm) radiation (Figs. 1 and 3). Transmittance of near infrared (700–1100 nm) radiation was significantly reduced also (Fig. 3) after 3 days at 25°C. Absorbance of radiation by leaves stored at 25°C increased in the green and near infrared wavelengths (Fig. 1) as the leaves senesced and changed from a bright green to a dull olive color. Changes in optical properties in the visible wavelengths indi-

cate significant chlorophyll degradation while the changes in near infrared (700–1100 nm) indicate alteration of cellular structure.

When the changes in reflectance, transmittance, and absorbance of these birch leaves were examined at two representative wavelengths (i.e., 670 and 750 nm), the effects of temperature on rate of senescence are evident (Figs. 4 and 5). Changes in the spectral properties were significantly smaller for leaves stored at 5°C than at 25°C. Tetley and Thimann (1974) reported that senescence, as measured by chlorophyll degradation or  $\alpha$ -amino nitrogen accumulation, progressed

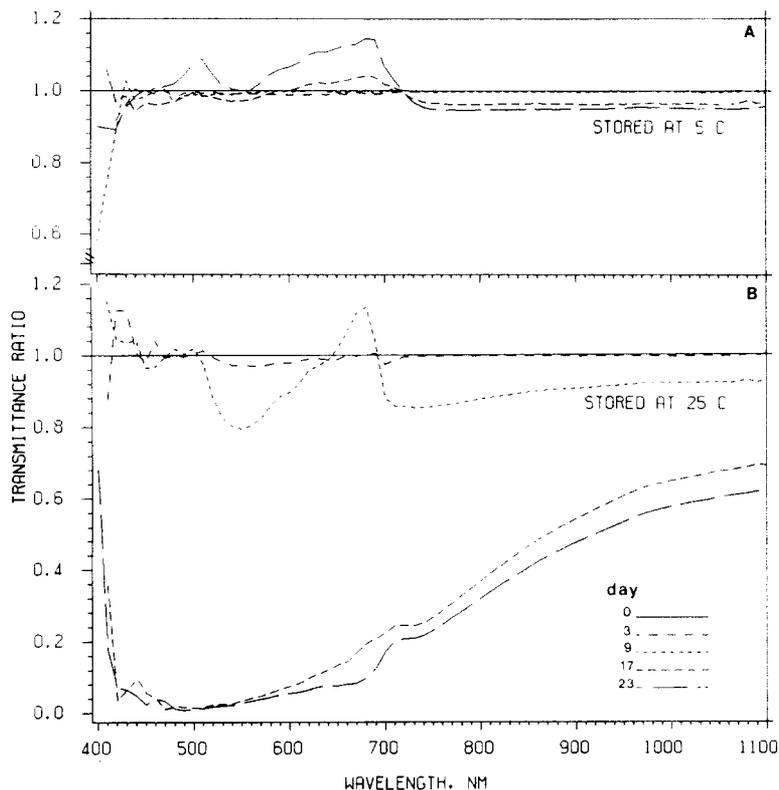


FIGURE 3. Changes in transmittance of birch leaves treated with water as a function of wavelength and time after excision. Data are ratios of reflectance on day  $n$  divided by reflectance on day 0. Day: (—) 0; (---) 3; (···) 9; (-·-) 17; (— · —) 23.

very slowly at 5°C but proceeded at its maximum rate at 26–30°C. Thus, for leaves stored at 5°C, the increases in reflectance at 670 nm (Fig. 5) indicate a gradual loss of chlorophyll. BAP tended to minimize changes in leaf spectral properties by delaying senescence (Figs. 5 and 6), presumably by slowing chlorophyll loss (Tetley and Thimann, 1974).

Leaves stored at 25°C senesced rapidly, and the increases in reflectance and transmittance at 670 nm (Fig. 5) at 9 days were greater than those of leaves stored at 5°C for 23 days. However, after 9 days, transmittance of leaves at 25°C was nil (Fig. 1).

Greater changes in near infrared reflectance, transmittance, and absorbance oc-

curred in birch leaves stored at 25°C than at 5°C (Fig. 6). Radiation scattering in the near infrared (700–1300 nm) wavelengths is strongly related to internal leaf structure, particularly the number of cell interfaces (Sinclair et al., 1971; 1973; Gausman et al., 1969). This pattern of changes in spectral properties is consistent with other metabolic and structural changes that occur in senescing leaves. Among the first evidences of cellular degradation is the onset of chloroplast breakdown (Hurkman, 1979). Ultrastructural changes in chloroplast during senescence include increases in size of osmiophilic globuli, changes in organization of internal membranes, swelling of chloroplasts, and loss of stroma (Hurkman,

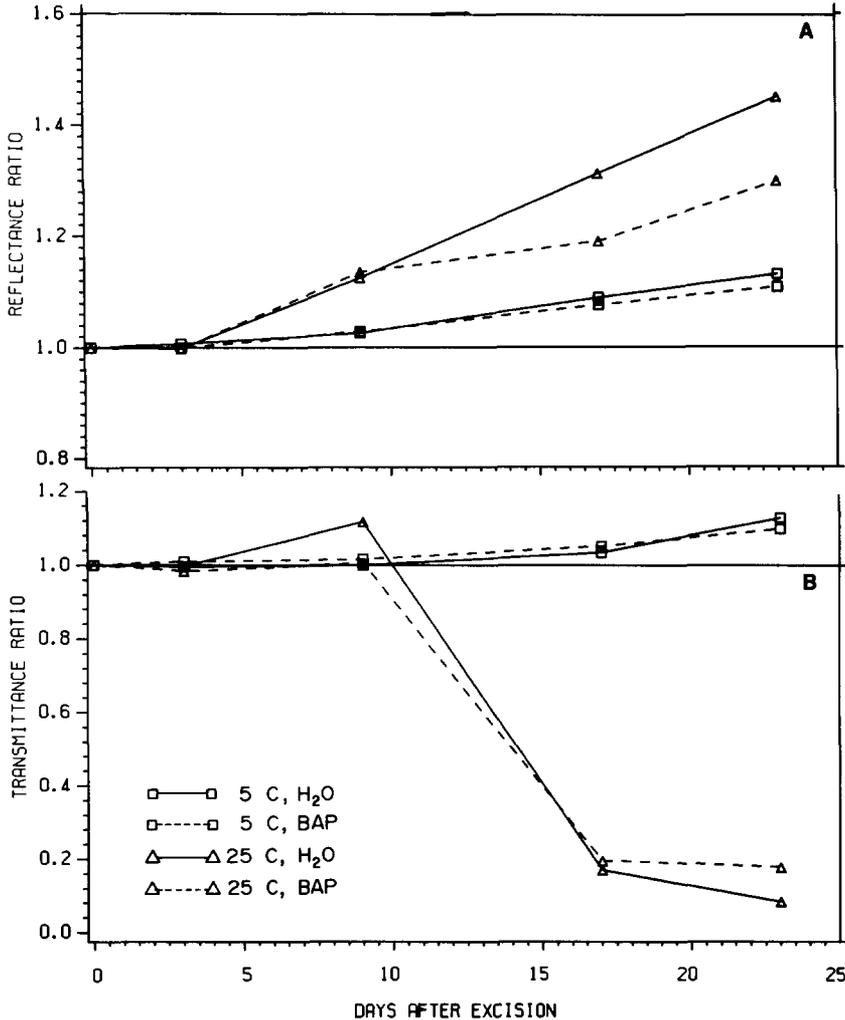


FIGURE 4. Changes in reflectance (A) and transmittance (B) of birch leaves at 670 nm as a function of time after excision. Data are ratios of reflectance on day  $n$  divided by reflectance on day 0: (□—□) 5°C, H<sub>2</sub>O; (□--□) 5°C, BAP; (△—△) 25°C, H<sub>2</sub>O; (△--△) 25°C, BAP.

1979). After the tonoplast disintegrates, other cell constituents degenerate rapidly, a process attributed to release of lytic material from the vacuole (Thimann, 1980). The rapid changes in spectral properties of leaves stored at 25°C probably occurred during the terminal stages of senescence as cell membranes disintegrated.

In summary, when leaves were stored at low temperatures (5°C) in the dark for

1 week, the changes in optical properties were less than 5% of the values on day 0. Storage at higher temperatures (i.e., 25°C) promoted rapid senescence and large changes in optical properties. A prestorage treatment of the leaves with BAP slowed but did not stop senescence at 25°C. Low temperature was more effective than BAP in delaying senescence. Thus it appears possible to store leaves at low temperatures in plastic bags for

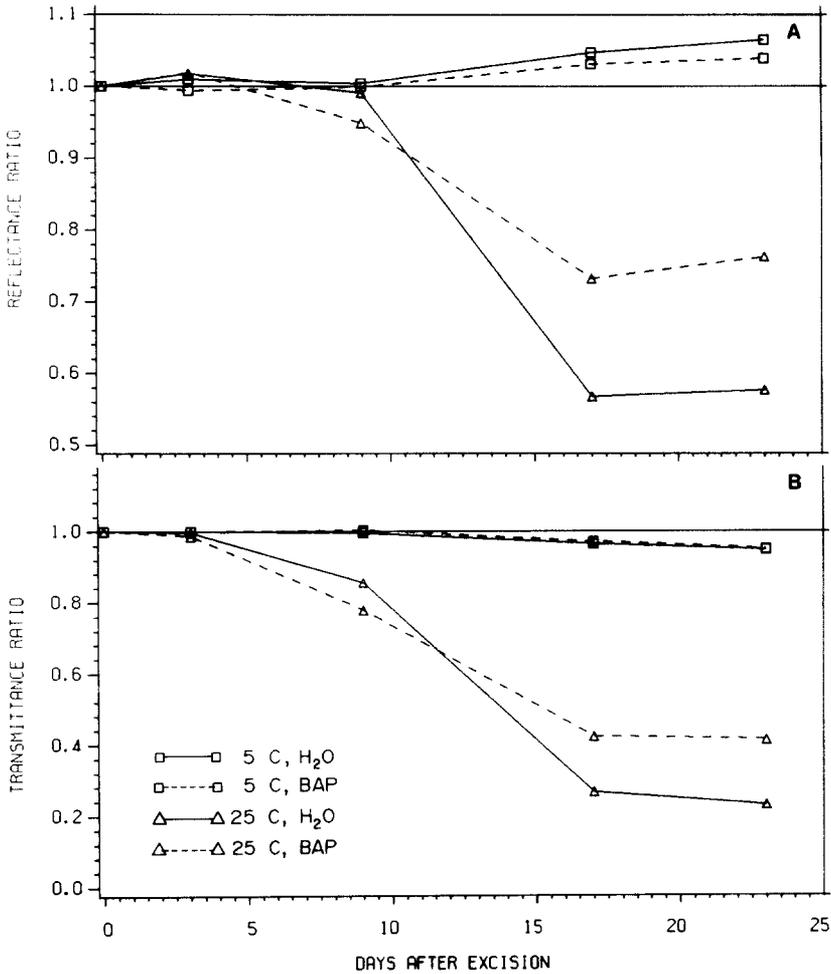


FIGURE 5. Changes in reflectance (A) and transmittance (B) of birch leaves at 750 nm as a function of time after excision. Data are ratios of reflectance on day  $n$  divided by reflectance on day 0: ( $\square$ — $\square$ ) 5°C, H<sub>2</sub>O; ( $\square$ - $\square$ ) 5°C, BAP; ( $\Delta$ — $\Delta$ ) 25°C, H<sub>2</sub>O; ( $\Delta$ - $\Delta$ ) 25°C, BAP.

several days without significantly altering the optical properties in the 400–1100 nm region. Because of limitations of the spectroradiometer, we could not examine the 1300–2400 nm region where optical properties of leaves are dominated by water absorption. However, spectral properties of turgid leaves may not be significantly affected until water content decreases to less than 80% (Sinclair et al., 1971; Gausman et al., 1971; Woolley, 1971; Knippling, 1970).

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