Seasonality of Potential Stem Photosynthesis / Respiration in Four Hardwoods

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Abstract

We observed an increasing reflectance of stems of four hardwoods in early spring. We hypothesized that it indicates an acclimation to spring conditions, likely associated with increased stem photosynthetic potential which is followed by re-acclimation in the fall to winter conditions. To test for changes in stem photosynthetic and respiratory capacity across seasons we contrasted, under laboratory conditions favorable to photosynthesis, CO_2 evolution rates of hardwood stems acclimated to field conditions of winter, spring, summer and fall. The four species studied included two that brighten strongly in spring [red-osier dogwood (*Cornus sericea*), brittle willow (*Salix fragilis*)] and two that brighten less [quaking aspen (*Populus tremuloides*), and black cottonwood (*P. trichocarpa*)]. Stem photosynthesis fixed, on average, 90 percent of carbon lost through respiration. We hypothesize that additional unmeasured photosynthesis is also occurring deeper within the stem. In all four species photosynthetic capacity increased in spring/summer and fell through fall to winter. In winter potential for photosynthesis fell markedly in both aspen and cottonwood and was eliminated in dogwood and willow. Responses of dogwood and willow provide the first known example of a complete down-regulation of stem photosynthesis in winter. Selective and selection-neutral hypotheses for this phenomenon are offered, i.e., that cessation of photosynthesis in winter has either been randomly fixed in shrubs (selection neutral) or created by selection of grazing animals against winter photosynthesis (palatability) in low shrubs greater than in trees with inaccessible twigs (natural selection). In contrast to photosynthesis, the potential for stem respiration appeared in all species and all seasons, and was usually lowest in winter and was highest in spring/summer.

Key words: acclimation, bark photosynthesis, browse, *Cornus sericea, Populus tremuloides, Populus trichocarpa, Salix fragilis,* stem photosynthesis, stem respiration

Introduction

Stems of some hardwoods, especially willow (*Salix*) and dogwood (*Cornus*) significantly brighten, or "glow," in early spring. We speculate that increased reflectance indicates acclimation to spring/summer conditions and a marked increase in stem photosynthesis, followed in the subsequent fall by re-acclimation to winter conditions. Thus, we tested the hypothesis that photosynthetic potential of these stems increases from winter to spring and declines again with oncoming winter. We considered two hardwood species which brighten strongly [red-osier dogwood (*Cornus sericea*) and brittle willow (*Salix fragilis*)] and two with less noticeable brightening [quaking aspen (*Populus tremuloides*), and black cottonwood (*P. trichocarpa*)].

Stem photosynthesis is important in other species. A capacity for stem photosynthesis has been shown in at least 36 plant families (Pfanz and Aschan 2001). In woody plants it is conducted by chloroplasts present in the phloem, xylem rays, pith and cork cambium (Aschan and Pfanz 2003, Tesky et al. 2008). Its importance is suggested by the fact that the bark of aspen

may contain 42 percent of the chlorophyll present (Kharouk et al. 1995). Seasonal variation in stem photosynthesis has been observed in many species including quaking aspen (Foote and Schaedle 1976), grape (*Vitis*) (Ortoidze et al. 1988), lilac (*Syringa*) (Pilarski, 1990), beech (*Fagus*) (Damesin 2003), and alder (*Alnus*), ginkgo (*Ginkgo*), spruce (*Picea*), pine (*Pinus*) and oak (*Quercus*) (Gerveiller et al. 2007). Stem photosynthesis changes with variation in stem chlorophyll concentration (Aschen and Pfanz 2003, Tesky et al. 2008), thus, paralleling our observation of changes in brightness. Photosynthesis in woody stems can contribute significantly to total plant carbon gain (reviewed in Aschan and Pfanz 2003, Tesky et al. 2008), even 50 percent of total photosynthesis of plants with short leafy seasons, e.g., those of deserts (Comstock et al. 1988) and high elevations (Kyte 1975).

To test our hypothesis, we contrasted potential cross-bark CO_2 flux (CO_2 evolution to physiologists) of stems of four field-acclimatized hardwood species among the four seasons. Measurements were made in the laboratory under carefully controlled conditions.

MATERIALS AND METHODS

Twig Samples

Twigs were collected along Mathew Byrd Creek, Bozeman, MT [latitude 45°40', longitude 111°3', elevation 1455 m. Red-osier dogwood, brittle willow, quaking aspen and black cottonwood collections were made from single mature individuals. Collections were made across the four seasons of 1991-1992. A single individual of each species was sampled to eliminate potentially confusing site effects. While the lack of replication, due to limited resources, of in-season measurements prevented evaluation of variation in stem photosynthesis/respiration within species, we gained generality among species by demonstrating parallel cross-species behavior. Thus, similarity of response among species demonstrated parallel behavior. Leaves and

buds were removed from the twigs to focus measurements on metabolism of current-year stems. To minimize the effects of wound respiration, i.e., to allow healing, all leaves and/or buds were removed from the most recent year's growth of the twigs one week before measurement.

Measurement of Photosynthesis and Respiration

We collected six twigs of each species for each run: five for measurement of CO_2 evolution, and one (a 'dummy') that was used only for monitoring temperature. The metabolism of current year's bark of each twig was measured in its individual chamber, a 200-ml glass test tube. Each test tube was closed with a three-hole stopper: one hole held the twig, and the two remaining holes allowed for air inflow and outflow. A fourth hole in the stopper of the dummy held a copper-constantan thermocouple that measured twig temperature. All six chambers were submerged in a water bath to provide temperature control. If any portion of the stem contained wood from previous year's growth it was masked with Parafilm® to minimize the influence of older internodes.

We measured CO_2 evolution first in the dark (respiration) and then in the light (simultaneous respiration and photosynthesis). Thus, before measuring respiration, we covered the water bath and allowed CO_2 evolution (respiration) to equilibrate for 10 min in the dark. When the respiration measurement was completed, we uncovered and illuminated the water bath. After stabilizing in the light (~20 min), twig CO_2 evolution was measured again with an Analytical Development Company (ADC®) infrared gas analyzer (IRGA). A pump delivered air (160 cm³ min⁻¹) to each chamber and the air return line passed through the IRGA.

To allow comparison of twig and season rates, we expressed CO_2 evolution as a rate, i.e., μmole CO_2 m⁻² sec⁻¹. The area of each stem was calculated by summing length x diameter x π across all exposed internodes. Larcher (1995) defined gross photosynthesis

as CO_2 evolution$_{dark}$ - CO_2 evolution$_{light}$. A two-tailed t-test for paired data (Box et al. 1978) was used to determine whether the treatment (light) increased or decreased CO_2 evolution significantly.

Conditions

In testing for this change in photosynthetic/respiratory 'potential,' we have measured plant performance under a set of lab conditions with no intention of determining actual photosynthesis/ respiration rates, or their integral, over the diversity of conditions appearing in the field during the year. To measure 'potential' photosynthesis and respiration, we needed to keep the twigs moist, warm (20 °C), and either dark or light saturated. Procedures for maintaining these conditions follow. First, to insure that the twigs were never dehydrated, they were excised under water on the day of measurement and the cut ends were kept immersed during transport to the laboratory. Approximately 1 hr after cutting, twigs were re-cut underwater to 20- to 25-cm lengths and kept underwater. Thus, cut ends were immersed during the entire experiment. Second, stem surface temperature was held at 20 °C by adding hot or cold water to the water bath surrounding the chambers to adjust twig temperatures. Twig temperature was read with a thermocouple inserted just under the bark, and near the midpoint of the dummy twig in each series. Third, the twigs, and their chambers, were held at an angle of 30° to the light by a test tube rack fastened inside the water bath. They were illuminated with a 6500W xenon lamp (Atlas Electric) which delivered 3200 umol m^{-2} sec^{-1} (~1300 ft-c) measured at an angle of 30° to the light. The spectrum of this lamp is very similar to daylight. Light saturation for aspen twig bark is between 800 ft-c (winter) and 1400 ft-c (summer, Foote and Schaedle 1976). Thus, we measured gross photosynthesis at or near natural light saturation in all cases.

Data Analysis

Differences between dark and light CO_2 evolution (gross photosynthesis) were determined using a paired t-test (Sigma Stat 3.0 for Windows 2003). Because two of the gross photosynthesis data sets were not normally distributed, we used a Kruskal-Wallis analysis of variance (ANOVA) on ranks to determine differences in gross photosynthesis between species within seasons (Sigma Stat 3.0 for Windows 2003). Differences were considered significant at α = 0.05. Regression of gross photosynthesis vs. dark respiration were made using Sigma Plot 8.0 for Windows (2003).

RESULTS

We observed three patterns in potential stem photosynthesis. First, potential photosynthesis increased from winter to spring/summer and fell through fall to winter (Table 1, Fig. 1). The trend is statistically significant for three species. Though the trend is weaker in aspen, where summer and fall photosynthesis were equal, aspen's photosynthesis also declined from summer/fall to winter. During spring, summer and fall the stems of all species had relatively high and constant capacities for photosynthesis under ideal conditions (high light, water available and 25 °C). Second, photosynthesis, on average, compensated for 90 percent of respiration across all species and seasons. Full compensation was rare. Among the six replicates in the four seasons studied, i.e., 24 cases/ species, four individual stems demonstrated net photosynthesis by compensating for respiration (negative light CO_2 evolution). We observed full compensation in aspen, cottonwood, and willow in summer and dogwood in spring (data not shown). Third, the potential for net photosynthesis for aspen and cottonwood was significantly reduced in winter and that of dogwood and willow was eliminated.

Respiratory potential, in contrast to photosynthetic, occurred at 20 °C in all seasons. Like photosynthesis, it was lowest in winter, except for willow (Table 1). The observed rates showed no seasonal pattern consistent across species. The respiratory potential of aspen, cottonwood and dogwood was greatest in spring/summer and least in winter. Willow respired most in summer and least in fall.

Table 1. Cross-season photosynthesis and respiration of four hardwood species.

Species	Season	Dark CO_2 evolution (μmol m^{-2} s^{-1})	Light CO_2 evolution (μmol m^{-2} s^{-1})	Gross photosynthesis (μmol m^{-2} s^{-1})[1]	p-value for paired t-test (dark vs. light)[2]
Red-osier Dogwood	Winter	0.48 ± 0.01	0.52 ± 0.02	**-0.04 ± 0.02**[3]	0.09[4]
	Spring	0.76 ± 0.03	0.14 ± 0.06	0.63 ± 0.05	<0.001
	Summer	1.10 ± 0.06	0.15 ± 0.04	0.96 ± 0.08	<0.001
	Fall	0.53 ± 0.03	0.20 ± 0.05	0.33 ± 0.04	<0.001
Quaking Aspen	Winter	0.81 ± 0.18	0.29 ± 0.15	0.52 ± 0.04	<0.001
	Spring	1.38 ± 0.07	0.23 ± 0.07	1.15 ± 0.07	<0.001
	Summer	0.86 ± 0.03	0.14 ± 0.07	0.72 ± 0.07	<0.001
	Fall	1.14 ± 0.08	0.23 ± 0.03	0.92 ± 0.08	<0.001
Black Cottonwood	Winter	0.81 ± 0.06	0.30 ± 0.04	0.50 ± 0.03	<0.001
	Spring	1.47 ± 0.09	0.39 ± 0.12	1.07 ± 0.18	<0.001
	Summer	1.38 ± 0.10	0.22 ± 0.10	1.16 ± 0.18	<0.001
	Fall	1.21 ± 0.04	0.24 ± 0.12	0.97 ± 0.06	<0.001
Brittle Willow	Winter	1.59 ± 0.05	1.71 ± 0.01	**-0.11 ± 0.04**[3]	0.06[4]
	Spring	1.17 ± 0.07	0.69 ± 0.05	0.48 ± 0.09	<0.001
	Summer	1.96 ± 0.18	0.24 ± 0.22	1.73 ± 0.13	<0.001
	Fall	1.28 ± 0.05	0.75 ± 0.15	0.53 ± 0.09	<0.004

[1]Gross photosynthesis = CO_2 evolution$_{dark}$ - CO_2 evolution$_{light}$. Data are means ± 1 SEM ($n = 6$).
[2]A two-tailed t-test for paired data was used to determine if CO_2 evolution was significantly different between light and dark.
[3]**Bolded** entries in dogwood and willow indicate no net photosynthesis in winter.
[4]Differences in CO_2 evolution$_{dark}$ - CO_2 evolution$_{light}$ are not significantly different from zero at $\alpha = 0.05$.

Aspen and cottonwood had higher gross photosynthetic potential than dogwood and willow (the shrubs) during all seasons except summer. In summer gross photosynthesis was significantly higher in willow than the other species. In fall, winter, and spring, gross photosynthesis was significantly higher for aspen and cottonwood than for dogwood and willow (Fig. 1). In winter both aspen and cottonwood maintained the ability to photosynthesize, although gross photosynthesis for dogwood and willow did not significantly differ from zero, which indicated a complete down-regulation of photosynthesis in these two species.

Regression of potential gross photosynthesis vs. dark respiration across all data points yielded a slope of 0.71 ($r^2 = 0.35$; Fig. 2). When the two data points representing the lack of winter photosynthesis in dogwood and willow were removed, the regression slope increased to 0.90 ($r^2 = 0.73$). This indicates that, on average, cross-bark photosynthesis compensates for ~ 90 percent of respiratory CO_2 escaping.

DISCUSSION

We have repeatedly observed that twigs of dogwood and willow brighten in spring. We hypothesized that the "glow" indicated acclimation to spring/summer conditions and the re-initiation of photosynthesis. To test for this change in photosynthetic/ respiratory 'potential,' we measured plant performance under a set of laboratory conditions (full sun, adequate water and 20 °C) and without any intention of determining actual photosynthesis/respiration rates or their integral over the diversity of conditions appearing in the field during the year. Our data supported this hypothesis for two mostly shrubby genera (dogwood and willow) and simultaneously rejects it for aspen and cottonwood trees. The brightening in dogwood and willow, which are visibly red and yellow, respectively, probably resulted from increased levels of
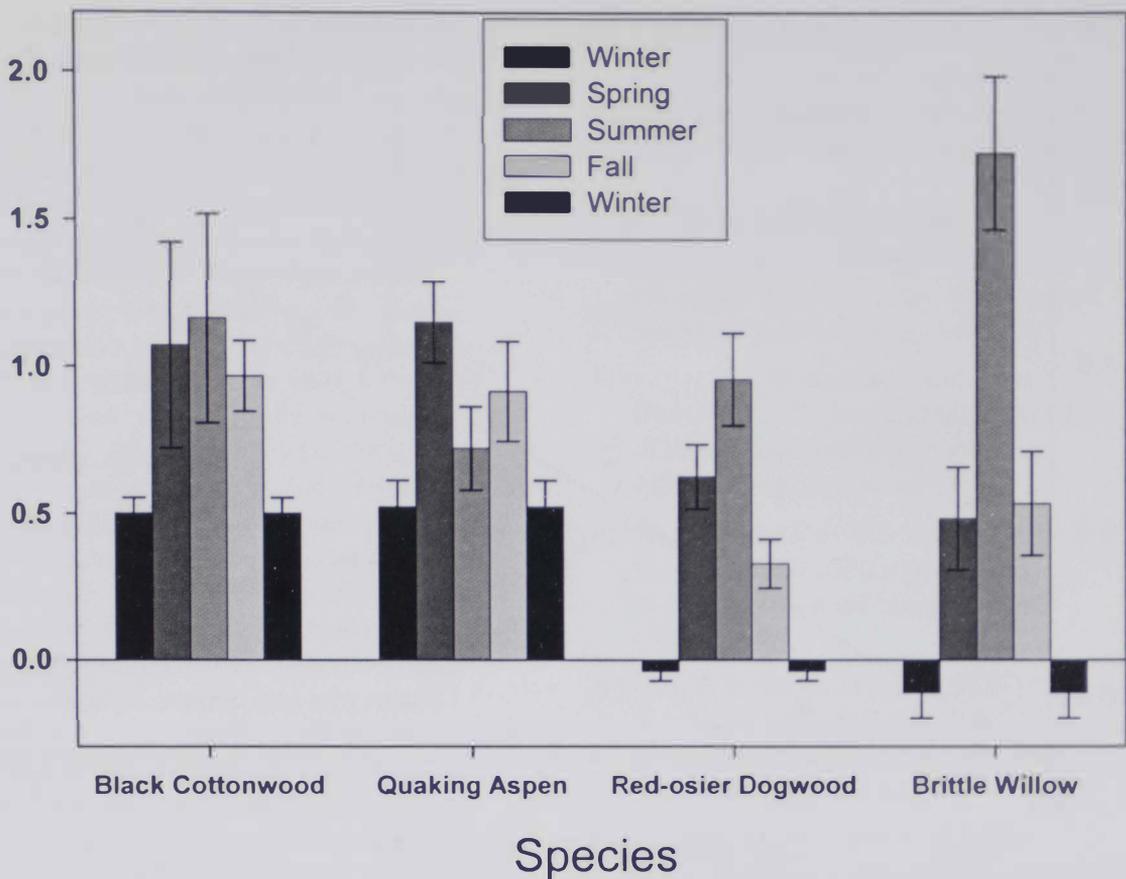
Figure 1. Gross photosynthesis (CO_2 evolution$_{dark}$ - CO_2 evolution$_{light}$) for each species during the four seasons. The second winter bar in each series is a repeat of the first winter bar made to emphasize the annual cycle of photosynthetic activity. Means with the same letter are not significantly different at $\alpha = 0.05$ within each species. Data are means $\pm$ 1 SEM ($n = 6$).

photo-protective compounds anthocyanins (red) and carotenoids (yellow, Steyn et al. 2002). Increasing chlorophyll concentrations commonly observed in many woody tree species might explain simultaneous, but lesser, brightening of aspen and cottonwood stems in spring (Berveiller et al. 2007), including aspen (Foote and Schaedle 1976).

Production

In our experiments, cross-bark photosynthetic potential is generally proportional to respiration potential, suggesting a possible parallel response to environmental conditions, e.g., temperature or drought. Our 90-percent cross-bark fixation rates are within the range of 31 to 126 percent fixation observed by others (reviewed in Teskey et al. 2008). With the exception of our winter observations of dogwood and willow, the mean fixation rate observed in our work is very near

that observed in the studies of Foote and Schaedle 1976, Han and Suzaki 1981, Wittmann et al. 2001, Wittmann et al. 2006, and Berveiller et al. 2007.

We hypothesize that the benefits of stem photosynthesis exceed those measured at the twig surface due to metabolic activity occurring in tissues too deep to readily exchange CO_2/O_2 with the external atmosphere. At this depth, photosynthesis fuels two processes. First, in a short (isolated) segment of the stem, photosynthesis and respiration continually cycle CO_2/O_2 as a source of energy (ATP). In this role the photosynthate cannot be withdrawn as a substrate for synthesis without interrupting (or breaking) the cycle. As an energy source this process is more efficient than leaf photosynthesis because the high internal CO_2 concentration of stems increases production both by mass
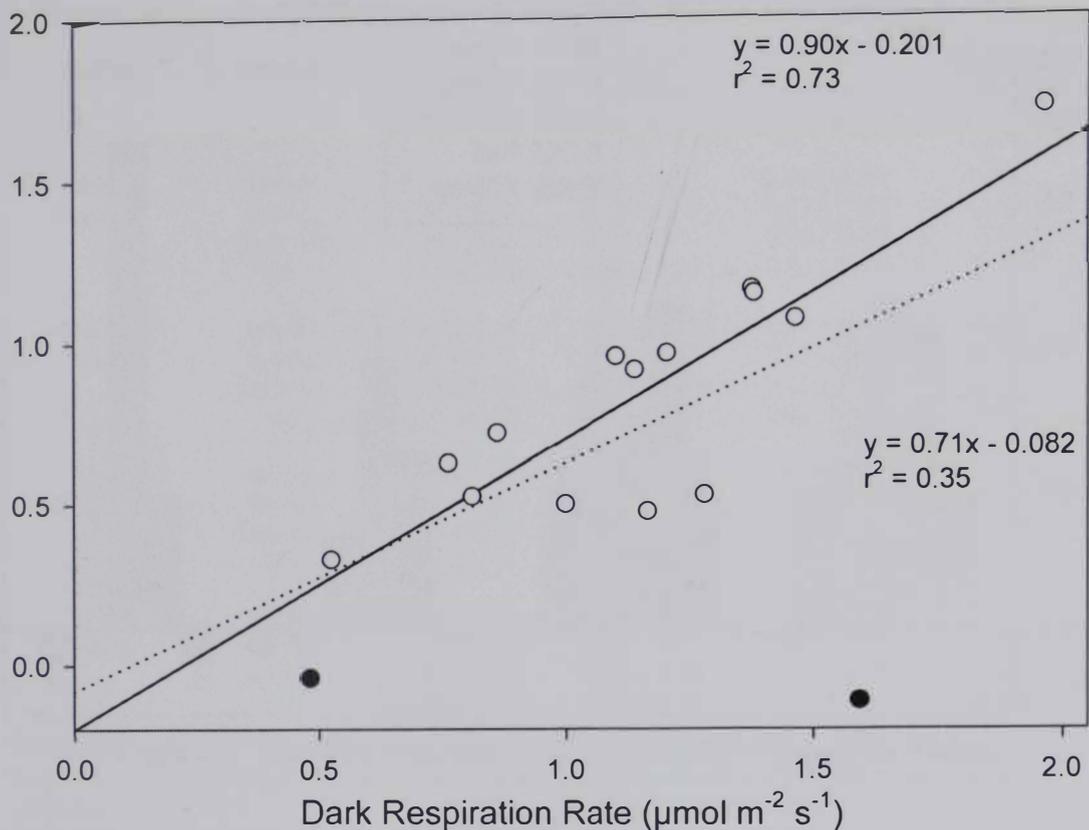
Figure 2. The relationship of mean gross photosynthesis (CO_2 evolution$_{dark}$ - CO_2 evolution$_{light}$) to dark respiration rate for all species during all seasons (r^2 = 0.37, dashed line). And for all species and seasons when anomalous winter data for dogwood and willow (closed circles) were removed (r^2 = 0.73, solid line) n = 6 for each data point.

action and reducing photorespiration (Aschan and Pfanz 2003, Tesky et al. 2008). Simultaneously, photosynthesis occurring without exchange with the outer atmosphere was more water-use efficient than surficial photosynthesis because it does not involve water losses associated with leaf photosynthesis. Second, internal photosynthesis incorporated for growth concentrated CO_2 rising from respiration in the stem, roots and soil (Billings and Godfrey 1967, Teskey et al. 2008). Because the source is continual, resultant sugars can be used either as a substrate for wood production or to fuel production or phloem activity. Because metabolic processes are generally reduced by cooling, we expect the effects of seasonal warming and cooling on internal photosynthesis to parallel those of external photosynthesis and respiration.

Seasonality

The winter observations of red-osier dogwood and brittle willow provide the first examples of complete winter cessation (or 'down-regulation') of stem photosynthesis. In contrast, our tree species maintained positive winter photosynthetic rates (aspen 50% and cottonwood 63%). Similarly, only partial winter down-regulation of photosynthetic capacity (34-90%) has been observed in six tree species (Damesin 2003, Berveiller et al. 2007).

Why has winter-time down-regulation, apparently absent in trees, evolved in typically shrubby genera (dogwood and willow). We offer two hypotheses. First, the difference could be due to random (non-selective) fixation of a winter down-regulation of photosynthesis in the two shrubby genera. If so, it seems somewhat odd that both of the shrub genera tested

shut down while this phenomenon has not been observed in any tree species studied. The significance of this difference could be tested by comparing established tree behavior with a larger sample of shrubs. Alternatively, the winter-time down-regulation of photosynthesis in shrubby genera may have been under greater selective pressure than in trees. That is, because low shrubs are more subject to browsing than are taller trees, one might expect the twigs of shrubs to be more strongly selected for low palatability, i.e., low sugar and/or high toxin contents. Under this scenario, twig photosynthesis of shrubs might be down-regulated in winter to reduce the contents of palatable sugars in the browsing season, and up-regulated again in the spring when stem photosynthesis is needed (to support bud growth and recover carbon lost through increasing stem respiration) and browsing is reduced (due to the appearance of alternative forage). Circumstantial evidence for this hypothesis might be found by comparing the winter and summer contents of twigs with respect to presumed attractants, e.g., sugars, and repellents, e.g., phenolics.

CONCLUSIONS

The potential photosynthetic and respiratory capacities of four hardwoods, red-osier dogwood, brittle willow, quaking aspen and black cottonwood, were measured across the four seasons. Respiration in all four species occurred in all seasons, usually rising from winter to summer and falling again to winter. On average, measured carbon fixation compensated for 90 percent of stem respiration. Unmeasured internal photosynthesis is hypothesized to add to cross-bark photosynthesis, probably resulting in positive total photosynthesis. All species photosynthesized during spring, summer and fall, but in winter potential photosynthesis was reduced in aspen and cottonwood and completely down-regulated in dogwood and willow. To explain the unusual cessation of photosynthesis during winter in dogwood and willow, we offer alternate hypotheses regarding random non-selected vs. browsing selected evolution.

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