Arctic, Antarctic, and Alpine Research, Vol. 32, No. 4, 2000, pp. 375–380

Winter Photosynthesis in Red Spruce (*Picea rubens* Sarg.): Limitations, Potential Benefits, and Risks*

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Abstract

Numerous cold-induced changes in physiology limit the capacity of northern conifers to photosynthesize during winter. Studies of red spruce (*Picea rubens* Sarg.) have shown that rates of field photosynthesis (P_{field}) and laboratory measurements of photosynthetic capacity (P_{max}) generally parallel seasonal ambient temperature trends; carbon exchange decreases in the fall, remains negative or close to zero for much of the winter, and increases in the spring. However, increases in P_{field}, P_{max}, and foliar carbohydrate concentrations can occur during winter thaws. Thaw-induced increases in photosynthesis are probably not the result of increased stomatal conductance, but may result from other changes in physiology associated with thaw-induced improvements in water relations. In addition to increased photosynthesis, red spruce also decrease in cold hardiness during thaws. The co-occurrence of thaw-induced changes in photosynthesis and cold hardiness raises questions regarding their adaptive significance, particularly in the context of potential climate change. Red spruce may face a tradeoff between potentially beneficial increases in carbon capture and potentially detrimental reductions in cold tolerance. The physiological consequence(s) of this tradeoff may depend on the number and duration of thaws, as well as ambient temperature trends following thaw. Pollution-induced reductions in cold tolerance, and the low genetic variability of red spruce, may also influence the net outcome of thaw-induced changes in physiology.

Introduction

Photosynthetic measurements of conifers growing in mild winter climates have shown that significant carbon fixation can occur year-round (Fry and Phillips, 1977; Harrington et al., 1994). However, field results for conifers in cold northern regions suggest that winter carbon fixation may be intermittent, and limited to periods with temperatures predominantly above 0°C (Jurik et al., 1988; Schaberg et al., 1995). Carbon gains during winter could benefit evergreen trees by compensating for respiratory carbon losses of overwintering foliage (Sprugel, 1989), contributing to the accumulation of cryoprotective sugars (Parker, 1963) and providing added energy for spring bud break and growth (Kimura, 1969). However, the true impact of carbon gains during winter on the annual carbon budgets of northern conifers remains uncertain (Schulze et al., 1977; Matyssek, 1986).

Although questions concerning the occurrence and physiological importance of carbon exchange during winter exist for all northern conifers, information regarding this topic is of particular scientific interest for red spruce. Since the mid-1960s, growth rates of montane red spruce have declined in many parts of the eastern United States (Cook and Zedaker, 1992). Pollution-induced disruptions in foliar carbon relations (McLaughlin and Kohut, 1992) and cold tolerance (DeHayes, 1992; DeHayes et al., 1999) have both been linked to this decline. In addition, winter thaws also alter the cold tolerance (Strimbeck et al., 1995) and carbon exchange (Schaberg et al., 1995) of red spruce. However, the adaptive consequence of thaw-induced changes in physiology is less certain than direct pollutant impacts. This paper examines the influence of winter thaws on red spruce carbon relations and cold tolerance, and explores the possible implications of warming winter climates on the physiology, health, and survival of this species. Because baseline rates of carbon exchange are close to zero for red spruce exposed to typical (subfreezing) winter climates, this paper begins with a brief summary of the physiological limitations to carbon capture at low temperatures.

PHYSIOLOGICAL LIMITATIONS TO WINTER PHOTOSYNTHESIS

Numerous cold-induced alterations to the photosynthetic machinery of northern conifers occur during winter which limit potentials for carbon capture. For example, cold-associated changes in the appearance and positioning of chloroplasts have been reported for several conifers (Perry and Baldwin, 1966; Senser et al., 1975) including red spruce (Fincher and Alscher, 1992). Fall and winter reductions in the amount and activity of chlorophyll have also been documented (McGregor and Kramer, 1963; Martin et al., 1978), as have low temperature-induced reductions in photosynthetic enzyme activity (Gezelius and Hallén, 1980; Öquist et al., 1980). Reductions in photosynthetic electron transport (especially photosystem II) also occur (Martin et al., 1978; Öquist, 1982).

* A version of this paper was presented at the Ecological Society of America symposium "Life at the cold limit: Plant processes near- and below-freezing temperatures," 10 August 1999, Spokane, Washington, U.S.A.

Winter photosynthesis is often considered to be limited by water stress. Cold-induced reductions in water uptake and/or transport can result in decreased water replacement following transpiration and, thus, contribute to the development of low foliar water potentials (Kincaid and Lyons, 1981; Teskey et al., 1984). Cold-associated foliar water deficits can also lead to decreases in guard cell turgor and result in stomatal closure (Kramer, 1983), which could independently restrict opportunities for carbon capture. Numerous studies have documented cold-associated reductions in stomatal conductance and photosynthesis for conifers (DeLucia, 1987; Jurik et al., 1988; Day et al., 1989, 1991; DeLucia et al., 1991). However, there are conflicting reports as to whether or not winter photosynthesis is primarily restricted by low stomatal aperture: some reports suggest that it is (Jurik et al., 1988), some suggest that it is not (DeLucia, 1987), while others contend that stomatal limitations vary among species (Day et al., 1989) or across temperatures gradients (Day et al., 1991; DeLucia et al., 1991).

Low water availability in the cold may also limit photosynthesis by directly altering cellular physiology. Numerous reports document nonstomatal limitations to photosynthesis associated with low water availability (Sharkey and Badger, 1982; Kaiser, 1987; Epron and Dreyer, 1992). These limitations may result from reductions in photophosphorylation (Sharkey and Badger, 1982), Calvin cycle and more generalized enzyme activity (Kaiser, 1987; Martin and Ruiz-Torres, 1992), electron transport (Epron and Dreyer, 1992), and/or mesophyll conductance (Grieu et al., 1988). Initial phases of the dehydration-induced inhibition of photosynthesis probably involve reactions catalyzed by water-soluble enzymes and enzymes at the membrane-water interface (Kaiser, 1987).

Another possible impediment to photosynthesis at low temperatures is the feedback inhibition of photosynthesis by high foliar sugar concentrations. Concentrations of soluble carbohydrates (especially sugars) within needles of northern conifers increase during fall, remain high in winter, and decrease in the spring (Hinesley et al., 1992; Schaberg et al., 2000), whereas photosynthetic rates follow an opposite pattern (Jurik et al., 1988; Gage and DeHayes, 1992; Schaberg et al., 1995, 1998). Inverse changes in photosynthetic and foliar carbohydrate levels associated with changing temperature have led several investigators to hypothesize that conifers may experience cold-induced feedback inhibition (Parker, 1963; Day et al., 1989, 1991). Studies using herbaceous plants (Foyer, 1988) and cell cultures (Krapp et al., 1993) have provided experimental evidence of the feedback inhibition of photosynthesis by sugars. However, the possibility that the photosynthetic capacity of northern conifers is down-regulated by high foliar sugar concentrations during winter has not been experimentally tested.

THAW-INDUCED INCREASES IN WINTER PHOTOSYNTHESIS FOR RED SPRUCE

As a result of physiological and environmental limitations, net carbon exchange of the current-year foliage of northern red spruce is negative or close to zero during typical (low temperature) winter periods (Schaberg et al., 1995, 1998). However, net photosynthetic rates can increase substantially for this foliar age class during prolonged thaws (two or more days with air temperatures >0°C) (Schaberg et al., 1995, 1998). For example, mean rates of field photosynthesis (P_{field}) can reach 37% of mean growing season levels, and the rates for individual trees can approximate those occurring during the growing season (Schaberg et al., 1995, 1998). Correlations between P_{field} and ambient air temperature means during winter support the hypothesis that significant carbon capture occurs primarily in association with prolonged periods of elevated ambient temperature (Schaberg et al., 1995, 1998).

In order to determine how much winter photosynthesis in red spruce is limited by immediate environmental constraints, the photosynthetic capacity (P_{max}) of red spruce shoots has been measured both prior to and during natural and simulated thaws (Schaberg et al., 1998). Photosynthetic capacity is the maximum photosynthetic rate (the asymptote of the net CO_2 exchange curve during a 40-min measurement period) of excised shoots that are rehydrated and provided near-optimum growing season temperature and light conditions (e.g., 16.9 ± 0.2°C and 582 ± 16 μmol m^{-2} s^{-1} PAR; Schaberg et al., 1998). Because environmental limitations to photosynthesis are reduced during these measurements, P_{max} provides a more specific measure of the physiological capacity of the photosynthetic apparatus to capture carbon. Measurements of P_{max} for red spruce current-year shoots during a sustained period of below freezing ambient air temperatures were negative or close to zero (Schaberg et al., 1998), indicating that, even when provided a favorable environment, foliage was incapable of net carbon capture. However, periodic measurements of freshly cut shoots showed that P_{max} increased significantly within 48 h of the onset of a natural thaw (Schaberg et al., 1998). This increase in P_{max} can occur within 3 h for rehydrated shoots continuously exposed to simulated thaw conditions (17°C) (Schaberg et al., 1998). Even though increases from prethaw levels are considerable, for both natural and simulated thaws, P_{max} plateaued at only 37% of the mean photosynthetic rate reported for red spruce during the growing season. Thus, even though shoots were provided with near-optimal environmental conditions, and despite any thaw-induced changes in physiology, significant limitations to photosynthesis remained.

MECHANISM(S) OF THAW-INDUCED INCREASES IN PHOTOSYNTHESIS

Although the physiological mechanisms responsible for thaw-associated increases in photosynthesis have received little study, the rapidity of thaw-induced increases in P_{max} tends to exclude certain mechanisms (Schaberg et al., 1998). For example, alterations in thylakoid membrane composition and associated electron transport (Öquist, 1982), and reductions in chlorophyll content (Martin et al., 1978) have all been causally linked to the low photosynthetic rates typical of winter. However, it does not seem likely that these types of anatomical and/or physiological limitations would be rapidly reversible. In contrast, thaw-associated improvements in water relations and ensuing changes in physiology may better account for rapid increases in P_{max} observed by Schaberg et al. (1998).

Some evidence suggests that improvements in water availability precede the thaw-induced increases in photosynthesis reported for red spruce. Schaberg et al. (1996) found that red spruce seedlings exposed to a simulated thaw experienced large increases in xylem pressure potential at least 2 d before average net photosynthetic rates were positive. For these seedlings, xylem pressure potential and net photosynthesis values were positively correlated for the transitional period from net respiration to net photosynthesis, but were not correlated thereafter. Differences in the speed of the photosynthetic response to thaw for intact shoots (Schaberg et al., 1995, 1996) compared to rehydrated shoots (Schaberg et al., 1998) may also indicate that improved water relations are an important prerequisite for thaw-induced increases in photosynthesis. Although improved water

relations may be important to the photosynthetic response to thaw, thaw-associated increases in photosynthesis are probably not mediated through changes in stomatal aperture (Schaberg et al., 1995, 1996, 1998). Increases in net photosynthesis for intact shoots during natural (Schaberg et al., 1995) and simulated thaws (Schaberg et al., 1996), and increases in P_{max} for cut/rehydrated shoots brought indoors (Schaberg et al., 1998) all occurred without corresponding increases in stomatal conductance. Instead, thaw-induced increases in photosynthesis could occur following improvements in water relations and the partial reversal of nonstomatal limitations to photosynthesis (e.g., reductions in electron transport, photophosphorylation, enzyme activity, and mesophyll conductance resulting from low water availability). In addition, disruptions in chloroplast structure documented during winter (Perry and Baldwin, 1966; Senser et al., 1975; Fincher and Alscher, 1992) could be eased as thaw-induced rehydration allowed for conformational changes and enhanced function of protein and membrane components of the photosynthetic system.

TABLE 1

Means (standard errors) of photosynthetic capacities (P_{max}) and carbohydrate concentrations for current-year and year-old foliage of red spruce prior to (0 h, P_{max} only) and following (48 h) simulated winter thaw (48 h at approximately 17°C)

			Carbohydrate concentration (mg/g)				
Foliar age class	P_{max} (μmol m^{-2} s^{-1}) 0 hours	48 hours	Total sugar	Sucrose	Glucose	Fructose	Starch
			48 hours				
Current-year	−0.09	4.82	114	40	30	44	15
	(0.13)	(0.91)	(6.33)	(2.87)	(2.28)	(2.89)	(1.56)
Year-old	0.10	3.15	90	32	23	35	13
	(0.11)	(0.40)	(8.01)	(3.34)	(2.69)	(2.47)	(2.09)
$P \leq$*	0.65	0.01	0.04	0.01	0.07	0.04	0.07

* Statistical differences were determined using *t*-tests.

INCREASES IN CARBON STORES DURING THAWS

Whatever the physiological mechanism for thaw-induced increases in winter photosynthesis, its occurrence is of potential ecological significance, especially if photosynthesis results in measurable carbon gain. An increase in sugar concentrations within older (= 1 yr old) needles of native red spruce seedlings following a winter thaw was recently documented (Schaberg et al., 2000). This increase occurred without any reduction in foliar starch concentrations and without significant reductions in sugar or starch levels in new needles, stems or roots. This increase in sugar concentrations could have occurred through conversions from a noncarbohydrate fraction. However, because it occurred in association with a protracted thaw (the very circumstance for which winter photosynthesis has been documented for red spruce), it seems more likely that this increase in foliar sugars resulted from photosynthetic carbon fixation.

Additional evidence that photosynthesis during thaws can increase red spruce carbon stores was recently collected in my laboratory. We measured midwinter P_{max}, foliar sugar and starch concentrations of current- and year-old shoots from eight red spruce trees growing in a Vermont plantation following a period of sustained cold (12 d with mean air temperatures below 0°C prior to sample collection in February 1997). Gas exchange was measured at two times: immediately as shoots came in from the field (0-h measurement) and again on the same shoots after 2 d of simulated thaw conditions (300 ± 25 μmol m^{-2} s^{-1} PAR and 17°C) (48-h measurement). These thaw conditions can significantly increase the P_{max} of red spruce current-year foliage within 3 h (Schaberg et al., 1998). However, the time required for measurable thaw-induced increases in foliar carbohydrates had not previously been determined. We chose 48 h as the duration of thaw treatment because preliminary calculations indicated that photosynthetic capture could result in measurable carbon gains during this time, and because natural winter thaws often exceed 2 d in length (Strimbeck et al., 1995; Schaberg et al., 1998). Thaw-associated increases in sugar concentrations can occur preferentially in older red spruce foliage, although it is unknown how differences in P_{max} among foliar age classes may contribute to this (Schaberg et al., 2000). We simultaneously measured P_{max} of current-year and year-old foliage using separate, isolated cuvettes to assess potential differences in carbon capture between these tissues during a thaw. The base of each shoot containing both current-year and year-old foliage was cut under water and attached to a supply of distilled water using latex tubing to maintain hydration. Schaberg et al. (1998) provides a complete description of P_{max} methodologies. Immediately following the 48 h P_{max} measurement, foliage was frozen in liquid nitrogen, freeze-dried, ground, and stored at −60°C prior to carbohydrate analysis. Cuticular waxes were extracted using hexane, and soluble sugars were extracted using 80% ethanol (Hinesley et al. 1992). The remaining pellet was further processed and enzymatically analyzed for starch using the methods of Hendrix (1993). Chlorophyll was removed from the ethanol supernatant via filtration, and subsamples of supernatant were enzymatically assessed for sucrose, glucose, and fructose (Hendrix, 1993). *T*-tests were used to test for differences in P_{max}, sugar, and starch means between foliar age classes.

Results of this study (Table 1) support previous findings that red spruce foliage shows little photosynthetic activity when acclimated to ambient subfreezing temperatures (P_{max} at 0 h), but can greatly increase in photosynthetic capacity under prolonged thaw conditions (P_{max} at 48 h). New to this study, however, is an indication that the response to thaw is not equal for all foliar age classes: the increase in P_{max} was greater ($P \leq 0.01$) for current-year than year-old foliage. In fact, mean P_{max} of current-year shoots at 48 h was approximately 72% of the mean photosynthetic rate reported for red spruce during summer (Gage and DeHayes, 1992). Red spruce current-year foliage typically have comparable starch, but lower sugar concentrations than older foliage during winter (Schaberg et al., 2000). However, we found that current-year foliage had higher sugar ($P \leq 0.05$) and starch ($P \leq 0.07$) concentrations than did year-old foliage following 48 h of simulated thaw (Table 1). The atypically high carbohydrate concentrations in current-year foliage following 48 h of thaw was likely a result of the higher photosynthetic rates achieved by this foliage relative to older needles. The buildup of carbohydrates within current-year foliage could depict an early stage of carbohydrate distribution during thaw, although this pattern could also result from limited carbohydrate transport from needles due to a lack of distal sinks for these cut-shoots. Whatever the cause for observed patterns, these data raise questions about whether additions to the already high carbohydrate concentrations measured further inhibit photosynthetic activity during winter; current-year foliage had higher photosynthetic rates and higher sugar concentrations than year old foliage.

Neither evidence from the field (Schaberg et al., 2000) nor the results of this study (Table 1) provides definitive proof that thaw-induced increases in photosynthesis result in meaningful

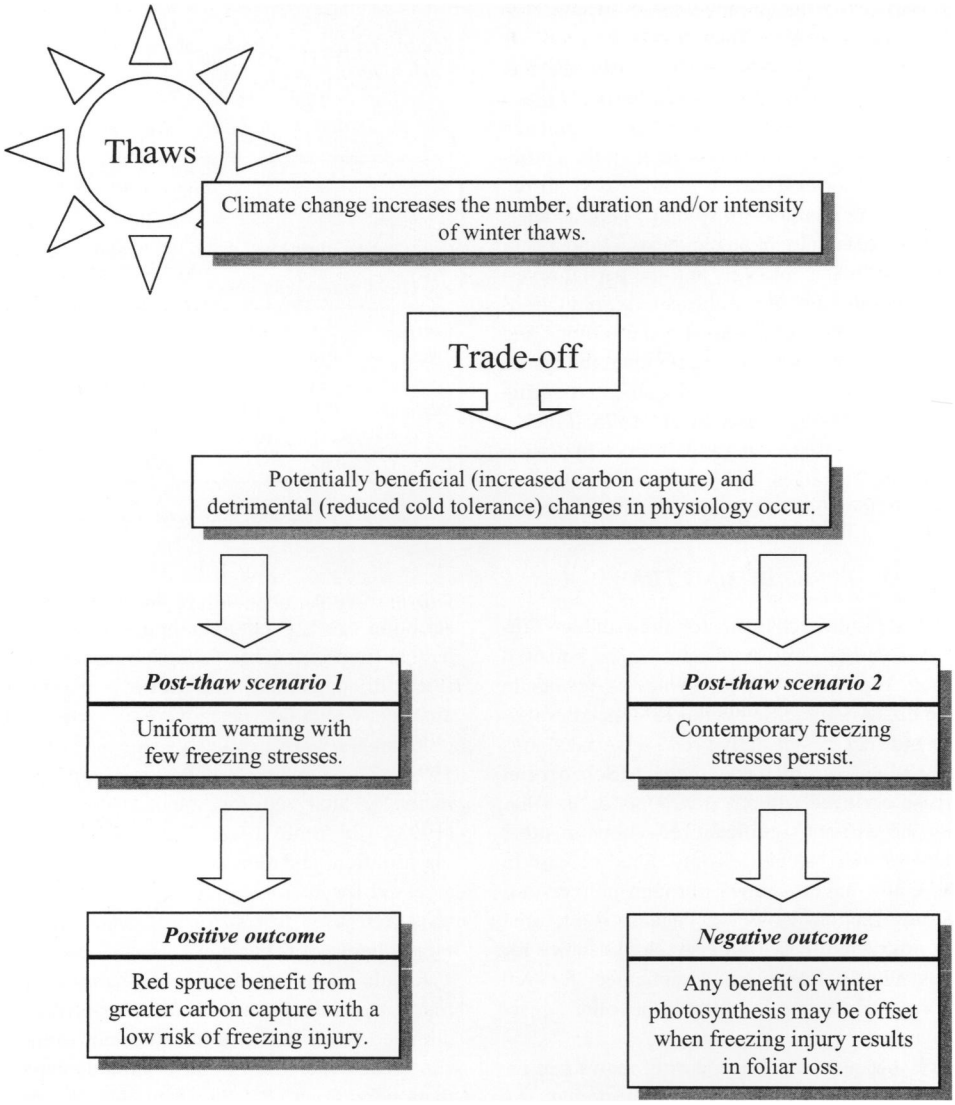

FIGURE 1. Potential response of red spruce to a warmer winter climate. The consequence of thaw-induced changes in physiology could depend on trends in ambient temperatures following thaws.

carbon gains for red spruce. However, together these data support the hypothesis that measurable carbon gains can occur during thaws.

THAW-INDUCED DEHARDENING AND POTENTIAL CLIMATE CHANGE

In addition to increased photosynthesis, red spruce also deharden during winter thaws. Several studies have documented reductions in cold tolerance up to 14°C for red spruce seedlings exposed to simulated thaws (5–10°C) for 4 to 5 d (DeHayes, 1992; Schaberg et al., 1996). Furthermore, Strimbeck et al. (1995) found mature montane red spruce dehardened an average of 9°C after only 3 d of exposure to ambient above-freezing (0–10°C) temperatures. Importantly, this dehardening persisted for up to 19 d after resumption of ambient air temperatures consistently below 0°C (Strimbeck et al., 1995). Red spruce attain a maximum cold hardiness that is barely sufficient to escape freezing injury at the lowest temperatures encountered during most winters (DeHayes, 1992; DeHayes et al., 1999). Thus, precocious dehardening could greatly increase the risk of freezing injury if a thaw is followed by low temperatures, or if foliage is subjected to other types of cold stress such as rapid freezing (Perkins and Adams, 1995) or repeated cycles of freezing and thawing (Lund and Livingston, 1998).

The co-occurrence of changes in photosynthesis and cold hardiness during thaws raises interesting questions regarding the ecology and survival of red spruce. Because both potentially beneficial (increased carbon capture) and potentially detrimental (reduced cold hardiness) changes in physiology can occur, the response of red spruce to thaw could be characterized as a tradeoff, with the adaptive consequences of thaw-induced changes in physiology dependent upon long-term probabilities of costs versus benefits.

This tradeoff could be particularly meaningful within the context of potential climate change. Anthropogenic climatic warming could have broad impacts on the health and distribution of forest trees (Krauchi, 1993), with montane trees, and species with limited genetic diversity, among the groups most likely to be affected (Krauchi, 1993). The distribution (Blum, 1990) and unusually low genetic variability (DeHayes and Hawley, 1992; Hawley and DeHayes, 1994) of red spruce may place this species at particular risk. However, the specific impacts of climate change would likely depend on the mix of environmental changes experienced (Fig. 1). For example, if the number or duration

of winter thaws increases, but winter low temperatures are also moderated, then red spruce might benefit from enhanced carbon capture without an increased risk of freezing injury. However, it is more commonly predicted that pollution-induced climate change could cause winter thaws to become increasingly common while existing low temperature extremes persist (MacCracken et al., 1991). Under this scenario, the increased risk of freezing injury following thaw could offset any physiological benefit of winter carbon capture. In fact, if thaw-induced reductions in cold tolerance resulted in greater foliar mortality, this loss would decrease potentials for carbon capture and remove access to carbon reserves within lost foliage. Thus, thaw-associated alterations in physiology could actually deplete plant carbon stores.

The consequences of climate change on the health and distribution of red spruce are particularly uncertain when considered in conjunction with the potential impacts of other pollution-induced stresses. For example, even if northern climates become uniformly warmer during winter, acid mist–induced reductions in hardiness (DeHayes et al., 1999) could cause red spruce to be vulnerable to freezing injury at temperatures currently thought to be safe. In addition, pollution-induced alterations in mineral nutrition may disrupt growing season energy relations (McLaughlin and Kohut, 1992) which, in turn, could alter the adaptive significance of cold season carbon capture. Likely exposures to heterogeneous mixes of pollution and temperature perturbations, along with red spruce's comparatively low genetic diversity (which may limit its ability to respond to changing environmental stresses), greatly complicate our ability to reliably predict the response of this species to warming winter climates. Nonetheless, experimental evidence of the combined influence of pollutant exposures and variable winter temperatures on red spruce cold tolerance and energy relations is needed so that adaptive outcomes of likely environmental change scenarios can be modeled and evaluated.

Acknowledgments

I thank Paula Murakami for her considerable research assistance and her help with this manuscript. I also thank Drs. M. T. Tyree (USDA Forest Service), D. H. DeHayes, J. R. Donnelly, B. Etherton, and T. D. Perkins (The University of Vermont) for reviewing an earlier draft of this manuscript. Special thanks are owed to J. B. Shane and G. J. Hawley (The University of Vermont) for their valuable inputs to the research described in this paper.

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