Oecologia (1994) 99:260–270

ORIGINAL PAPER

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Environmental and developmental controls over the seasonal pattern of isoprene emission from aspen leaves

Received: 17 December 1993 / Accepted: 31 May 1994

Abstract Isoprene emission from plants represents one of the principal biospheric controls over the oxidative capacity of the continental troposphere. In the study reported here, the seasonal pattern of isoprene emission, and its underlying determinants, were studied for aspen trees growing in the Rocky Mountains of Colorado. The springtime onset of isoprene emission was delayed for up to 4 weeks following leaf emergence, despite the presence of positive net photosynthesis rates. Maximum isoprene emission rates were reached approximately 6 weeks following leaf emergence. During this initial developmental phase, isoprene emission rates were negatively correlated with leaf nitrogen concentrations. During the autumnal decline in isoprene emission, rates were positively correlated with leaf nitrogen concentration. Given past studies that demonstrate a correlation between leaf nitrogen concentration and isoprene emission rate, we conclude that factors other than the amount of leaf nitrogen determine the early-season initiation of isoprene emission. The late-season decline in isoprene emission rate is interpreted as due to the autumnal breakdown of metabolic machinery and loss of leaf nitrogen. In potted aspen trees, leaves that emerged in February and developed under cool, springtime temperatures did not emit isoprene until 23 days after leaf emergence. Leaves that emerged in July and developed in hot, midsummer temperatures emitted isoprene within 6 days. Leaves that had emerged during the cool spring, and had grown for several weeks without emitting isoprene, could be induced to emit isoprene within 2 h of exposure to 32°C. Continued exposure to warm temperatures resulted in a progressive increase in the isoprene emission rate. Thus, temperature appears to be an important determinant of the early season induction of isoprene emission. The seasonal pattern of isoprene emission was examined in trees growing along an elevational gradient in the Colorado Front Range (1829–2896 m). Trees at different elevations exhibited staggered patterns of bud-break and initiation of photosynthesis and isoprene emission in concert with the staggered onset of warm, springtime temperatures. The springtime induction of isoprene emission could be predicted at each of the three sites as the time after bud break required for cumulative temperatures above 0°C to reach approximately 400 degree days. Seasonal temperature acclimation of isoprene emission rate and photosynthesis rate was not observed. The temperature dependence of isoprene emission rate between 20 and 35°C could be accurately predicted during spring and summer using a single algorithm that describes the Arrhenius relationship of enzyme activity. From these results, it is concluded that the early season pattern of isoprene emission is controlled by prevailing temperature and its interaction with developmental processes. The late-season pattern is determined by controls over leaf nitrogen concentration, especially the depletion of leaf nitrogen during senescence. Following early-season induction, isoprene emission rates correlate with photosynthesis rates. During the season there is little acclimation to temperature, so that seasonal modeling simplifies to a single temperature-response algorithm.

Key words Seasonal pattern · Isoprene emission
Nitrogen · Temperature induction · Biogenic emission inventory

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Introduction

Biospheric emissions of isoprene (2-methyl-1,3-butadiene) represent a principal control over the oxidative capacity of the continental troposphere (Brasseur and Chatfield 1991). Tropospheric oxidation, in turn, determines the lifetime of numerous atmospheric constituents, including methane and carbon monoxide. Biogenic isoprene emission also contributes to the generation of tropospheric ozone in urban atmospheres (Chameides et al. 1988). Given its prominent role in tropospheric oxidative chemistry, it is important to understand the ecological and physiological controls over isoprene emission as part of the emerging integration of biosphere/atmosphere processes.

The observed isoprene emission rate is controlled at two levels. The *basal emission rate* represents the inherent capacity of a leaf to emit isoprene, independent of prevailing environmental conditions. The basal emission rate is typically measured under a standard set of environmental conditions, and is controlled by the amount of biochemical machinery and substrate available to synthesize isoprene. The *instantaneous emission rate* is the rate observed for the prevailing set of environmental conditions. Mathematically, the instantaneous emission rate is the product of the basal emission rate and some factor that takes into account the influence of prevailing environment on basal emissions. Most past studies have focused on determinants of the instantaneous emission rate. The primary environmental controls over the instantaneous rate are light and temperature (Tingey et al. 1979, 1987; Monson and Fall 1989; Loreto and Sharkey 1990). Control by light may explain the often-reported linkage between isoprene emission rate and the instantaneous photosynthesis rate (Sanadze 1969; Rasmussen and Jones 1973; Tingey et al. 1987; Monson and Fall 1989; Loreto and Sharkey 1990, 1993a; Monson et al. 1991b). Temperature control is expressed through the temperature dependence of isoprene synthase, the enzyme that underlies isoprene biosynthesis (Silver and Fall 1991; Monson et al. 1992). Using empirical measurements of the basal emission rate, biochemical models of the light dependence of photosynthetic electron transport, and Arrhenius-based models of the temperature dependence of enzymes, Guenther and co-workers have successfully simulated the instantaneous isoprene emission rate in several species (Guenther et al. 1991, 1993a).

Recently, studies have also focused on determinants of the basal isoprene emission rate. Such determinants typically work through characteristics of plant development and growth regime. Developmental expression of the basal rate is delayed after leaf emergence despite the expression of a significant photosynthesis rate (Grinspoon et al. 1991; Kuzma and Fall 1993; Sharkey and Loreto 1993; Harley et al., in press). This developmental regulation appears to lie in controls over the level of isoprene synthase activity (Kuzma and Fall 1993). Growth temperature also affects the basal rate, as evidenced by the disappearance of isoprene emission from kudzu leaves after growth in a cool-temperature regime (Sharkey and Loreto 1993). Finally, leaf nitrogen concentration can influence the basal emission rate, with a higher emission rate observed when leaf nitrogen concentrations are higher (Harley et al., 1994). The influence of leaf nitrogen on isoprene emission rate may be due to its influence on the concentration of enzymes available for the synthesis of isoprene (Harley et al., 1994). When taken together, these recent results indicate possible controls over the early-season onset of isoprene emission – partly regulated by internal developmental traits and partly determined by seasonal increases in growth temperature. In this study, the interaction between these developmental and environmental controls was examined with regard to the seasonal pattern of isoprene emission in aspen trees (*Populus tremuloides* Michx.).

Materials and methods

Plant material

Most measurements were conducted on trees growing in their natural habitat in the Colorado Front Range of the Rocky Mountains. Three populations were studied. During the summer of 1992, measurements were made on ten different clones of one population 0.5 km east of the University of Colorado Mountain Research Station near Nederland, Colorado (elevation 2896 m, 40°05′N, 105°25′W). These clones had been the subject of past studies on the population biology of *P. tremuloides*, and thus had been mapped using electrophoretic markers and identified as different genets (Mitton and Grant 1980). During the summer of 1993, two additional populations were added to the study, one at an elevation of 2439 m and one at 1829 m.

Additional studies were conducted with potted, 3-year-old aspen saplings native to the Sangre de Cristo Mountains in southwestern Colorado. During May 1992 the saplings were planted in 20-l plastic pots, using a soilless potting mixture of 2 parts general purpose sorbent (diatomaceous earth, Siegel Co., Denver, Colo.): 2 parts vermiculite: 1 part perlite. All plants were grown outdoors, watered two to three times per week, and fertilized twice per week with 1 l full-strength Hoagland's solution. During the winter of 1992/1993 the pots were wrapped with plastic insulating material and placed in a sunny, protected site. This warm microenvironment caused the plants to break bud and initiate growth in late February. Following bud break, the plants were moved to a site where they would experience the natural cool temperatures of March and April, though they were placed beneath a plastic tent when temperatures were predicted to be below −5°C – approximately 10 days during the 2-month period of March and April. Temperatures in the tent were approximately 10°C warmer than outside. Maximum/minimum temperatures, midday light intensities, and representative midday leaf temperatures were measured daily during this spring-time growth period. By protecting the plants through this spring period, it was possible to study the developmental onset of isoprene emission in a cool temperature regime, in comparison to the warmer mid-summer regime that followed.

Isoprene emission measurements

During 1992, measurements of isoprene emission rate and photosynthesis rate were made in situ using a Li-Cor 6200 photosynthesis system (Li-Cor, Lincoln, Neb., USA) modified to allow samples of gas exiting the cuvette to be collected in locking, 10-cm^3 glass syringes. After collection, the syringes were stored on ice, in

the dark, to prevent photochemical reactions. The syringes were taken to a nearby laboratory and the contents were analyzed within 2 h of collection. Preliminary studies showed that when samples of known isoprene content were stored in the syringes there was no loss for at least 2 h. Samples were analyzed after injection into a self-constructed gas chromatograph (stainless steel column, packed with Unibeads 3S), and coupled to a reduction gas detector (model RGD-2, Trace Analytical, Palo Alto, Calif., USA). Peak integration was accomplished with an integrator (model 3390, Hewlett-Packard, Avondale, Pa., USA). The isoprene analysis system has been described in detail in a separate report (Greenberg et al. 1993), and has been shown to have a detection limit of less than 1 ppbv isoprene in a sample volume of 1 cm^3. The gas chromatograph was calibrated several times each day against an isoprene standard referenced to a National Institute of Standards propane standard using gas chromatography/flame ionization detection (GC/FID). An air sample entering the cuvette was obtained with each set of leaf measurements to assess background isoprene concentration. In all cases background concentrations were below the 1-ppbv detection limit.

The experimental measurement protocol consisted of inserting a leaf into the Li-Cor cuvette, measuring photosynthesis rate in the closed flow mode, toggling a switch (supplied by Li-Cor) to establish an open flow mode, waiting 20–30 s to allow for equilibration, then taking a syringe sample. Flow rate in the open mode was measured with a rotameter inserted into the air line going into the cuvette. At the time of syringe sampling, the flow rate, leaf temperature, and incident photon flux density were noted.

During 1993, measurements were made using branches collected in situ and transported to the laboratory for analysis. The branches were cut under water before dawn to avoid potential artifacts due to interruption of an established diel gas-exchange pattern. After cutting, the branches were stored with the cut end under water until analysis. Leaves on the cut branches were analyzed within 6 h of cutting. Laboratory measurements were made the 2nd year, rather than continued in situ measurements, to ensure repeatable conditions of light and temperature. Comparison of maximum photosynthesis rates and isoprene emission rates between cut branches and measurements made the previous year on the same trees in situ revealed no significant differences. (This relationship can be verified by comparison of the maximum isoprene emission rates reported for high-site leaves in situ and in the laboratory). Although Loreto and Sharkey (1993b) have shown that cutting plants affects isoprene emission rate in the exotic vine kudzu, measurements made in our laboratory have shown no influence of mechanical injury at one part of an aspen branch on the isoprene emission rate of leaves at a distant part of the branch (R. Fall, University of Colorado, unpublished).

Laboratory measurements of photosynthesis rate were made with the gas-exchange system described previously (Monson and Fall 1989; Hills et al. 1992). The GC portion of the system was modified, however, to include one of two instruments – a gas chromatograph with reduction gas detector (as described above) or a commercially available, portable gas chromatograph (Photovac 10S portable photoionization gas chromatograph, Deer Park, N.Y., USA). Approximately half of the measurements were made using the reduction gas detector, before reconfiguring the system to utilize the Photovac gas chromatograph. Comparative measurements using the same plants revealed no significant differences between the systems. The same calibration procedures as described above were used during 1993.

Unless stated otherwise, all gas exchange measurements were made with a photon flux density of 1000 μmol m^{-2} s^{-1} and an ambient CO_2 concentration of 345 μmol mol^{-1}. Leaf temperature was variable and is reported separately for each experiment.

Measurements of leaf nitrogen and miscellaneous micrometeorology measurements

All leaves for the nitrogen assays were dried at 60°C for 72 h. Leaf nitrogen concentrations were measured as total Kjehldahl nitrogen (TKN) according to the procedures described in Jaeger and Monson (1992). Maximum/minimum temperatures were measured with recording mercury thermometers (Weathermeasure, Sacramento, Calif., USA). Leaf temperatures, when not measured with the gas exchange systems, were measured with copper/constantan thermocouples appressed to the abaxial leaf surface and connected to a thermocouple thermometer (Wescor, Logan, Utah, USA). Incident photon flux density was measured with a Li-Cor solar monitor (model 1776, Li-Cor, Lincoln, Neb., USA).

In one analysis, the early-season induction of isoprene emission was analyzed as a function of heating degree days. This cumulative temperature index was calculated for the period between spring bud-break and the onset of isoprene emission from (1) trees at the three elevations for the 1993 season, (2) trees at the high-elevation for the 1992 season, and (3) potted trees for the 1993 season. This represented all available experiments in which trees were observed from the period of spring bud-break to the onset of isoprene emission. In this analysis we did not include leaves that emerged in mid-summer since they presumably emerged from meristematic cells that already possessed the capacity for isoprene emission. The cumulative heating analysis was only relevant to spring bud-break. Cumulative temperature was obtained from weather records collected at the various sites. For the high-elevation site, weather records were obtained from site C1, which is maintained by the Niwot Ridge Long Term Ecological Research Program (located approximately 1 km from the aspen clones that were used). For the low-elevation site, local weather records for Boulder were used. These records were collected approximately 10 km from the low-elevation site. For the potted plants, daily maximum temperatures were measured at the pots using maximum/minimum thermometers. For the middle-elevation site, the Boulder local weather data were used with corrections for the elevation difference (dry adiabatic lapse rate of 10°C per 1000 m). Cumulative temperatures were calculated using various bases ranging from 0 to 10°C. The cumulative deviation from each base temperature was summed for successive days between bud-break and the initiation of isoprene emission. In the results, data are presented only for base temperatures of 0 and 5°C, though the data from other base temperatures exhibited similar qualitative patterns.

Results

Field measurements of the seasonal pattern of isoprene emission

During 1992, leaf emergence at the high-elevation site occurred during the 2nd week of May. Beginning the week of 18 May, measurements were made on four clones (two male and two female) at approximately weekly intervals until leaf senescence in early- to mid-September. In a separate experiment, no systematic differences were observed between five male and five female clones, in either isoprene emission rate or photosynthesis rate (data not shown). Data for both sexes have therefore been combined in all further analyses. Because leaf temperatures were variable, all isoprene emission rates were normalized to 25°C using the algorithm of Guenther et al. (1991). Only measurements that were taken with incident photon flux density greater than 1000 μmol m^{-2} s^{-1} were recorded. Supplementary measurements made with an artificial light source showed that this was sufficient to ensure light saturation of isoprene emission and photosynthesis (data not shown). Thus, the data reported in Fig. 1A represent the developmental pattern of

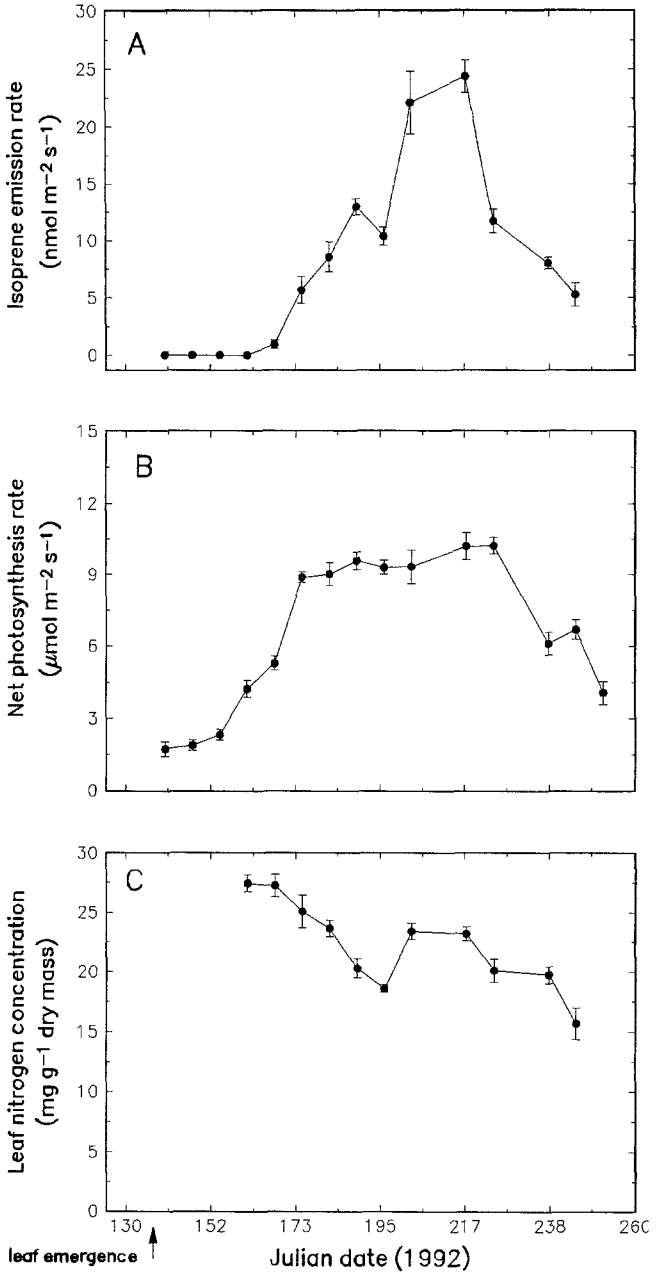

Fig. 1 **A** Seasonal pattern of isoprene emission for aspen leaves at the high-elevation site (2896 m) during the 1992 growing season. All rates have been normalized to 25°C, and only rates measured with a photon flux density greater than 1000 μmol m^{-2} s^{-1} were used. **B** Seasonal pattern of photosynthesis rate for the same leaves described in *panel A*. In this case rates have not been normalized to temperature, though they were determined at photon flux densities greater than 1000 μmol m^{-2} s^{-1}. **C** Seasonal patterns of leaf nitrogen concentrations for the same leaves described in *panel A*. In all panels points represent the mean ±SE of three to four leaves

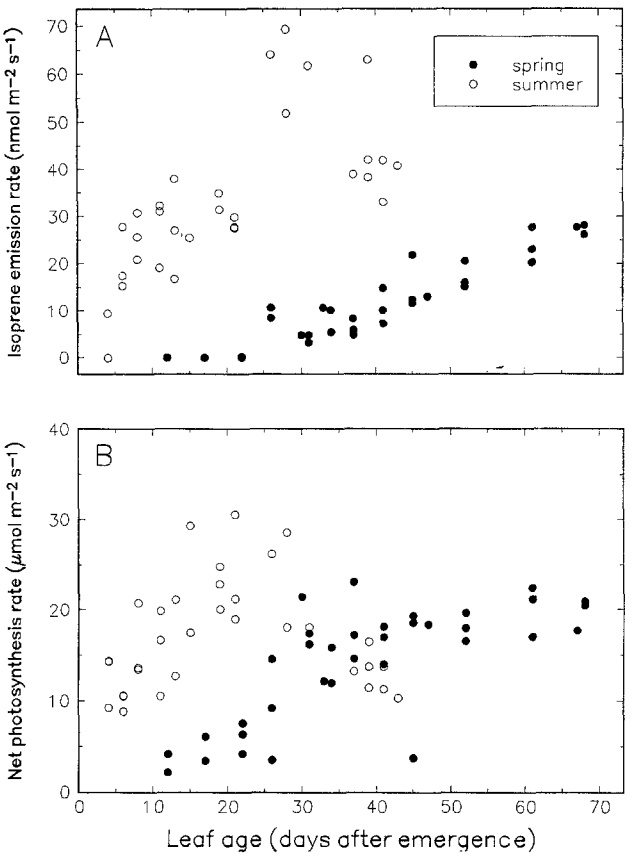

Fig. 2 **A** The relationship between isoprene emission rate and leaf age for leaves that emerged during the winter/spring (late February 1993; *closed circles*) or during the summer (late June 1993; *open circles*). All data were from potted trees maintained outdoors in Boulder, Colorado. Each *point* represents measurement of a separate leaf. **B** The relationship between net photosynthesis rate and leaf age for leaves that emerged during the winter/spring (late February 1993; *closed circles*) or during the summer (late June 1993; *open circles*). Isoprene emission rates and photosynthesis rates were measured at a leaf temperature of 30°C

seasonal isoprene emission rate, independent of environmental variation due to incident light or temperature (i.e., the basal emission rate). Isoprene emission was first detected in mid-June (Julian date 166) (Fig. 1A). Isoprene emission rates increased through mid-season, peaking in early August, then declining in concert with leaf senescence. Photosynthetic rates were low, but detectable, during the first measurement date and increased slowly until mid-June, when an increase in photosynthetic activity occurred in all clones (Fig. 1B). Photosynthetic rates had increased by early July to between 9 and 11 μmol m^{-2} s^{-1} and remained at about that level until late August, when rates declined as leaf senescence was initiated.

Leaf nitrogen concentration was at a maximum early in the season, exhibiting a general decline as the season progressed (Fig. 1C). The general pattern, therefore, showed a negative correlation between isoprene emission rate and leaf nitrogen early in the season, and a positive correlation late in the season.

Experiments with potted aspen trees grown across a seasonal temperature gradient

In the experiments reported in this section, 3-year-old aspen saplings were grown outdoors in pots and sampled

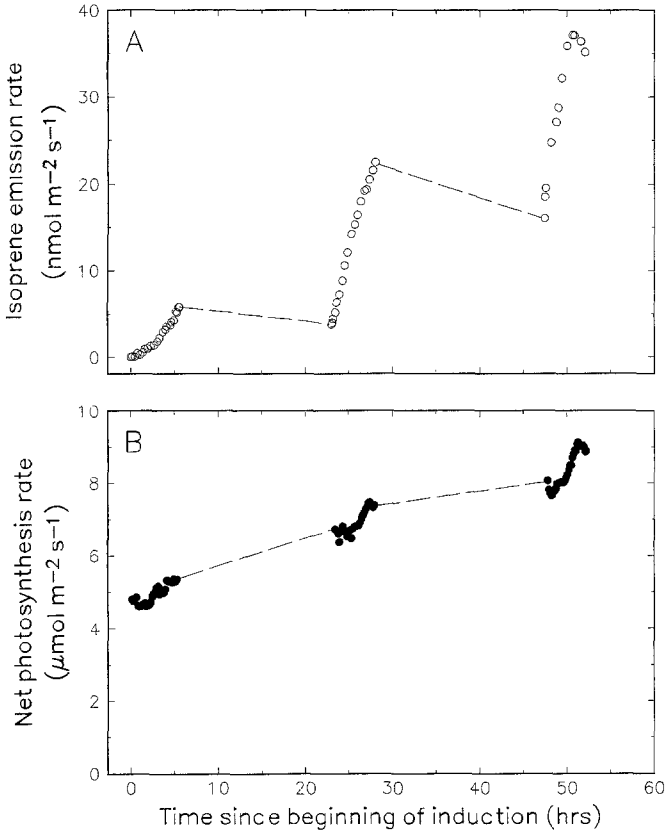

Fig. 3 **A** Isoprene emission rate measured during 3 successive days in which a non-induced leaf was exposed to 32°C for 6 h each day. The *broken lines* indicate the period during which plants were returned to their natural outdoor temperatures. Measurements were conducted on 20–23 March 1993. **B** Net photosynthesis rates measured for the same leaf described above. All points are for one leaf

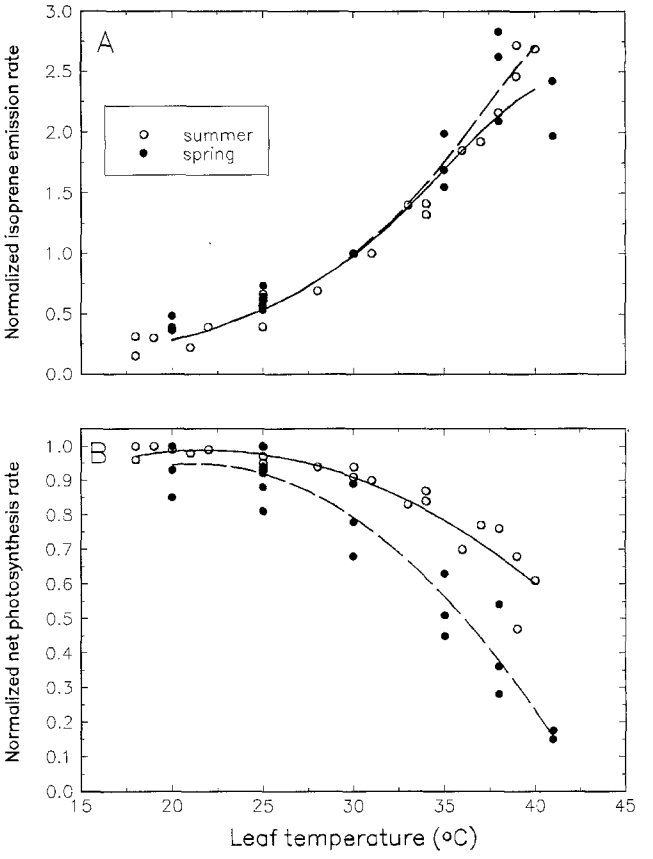

Fig. 4 **A** The temperature dependence of isoprene emission rate for leaves measured during the spring (late April 1993; *open circles*) or summer (mid-July 1993; *closed circles*). All isoprene emission rates have been normalized to a value of 1.0 at 30°C. The *broken line* represents the simulated temperature dependence as calculated by the algorithm of Guenther et al. (1991) using a high-temperature threshold (T_m) value of 45°C (the mean value determined from the three late-April replicates obtained by determining the best fit of each leaf's data to the Guenther algorithm). The *solid line* represents the simulated temperature dependence as calculated by the algorithm of Guenther et al. (1991) using a T_m value of 43.5°C (the mean value of best fit determined for the mid-July replicates). **B** The temperature dependence of photosynthesis rate for leaves measured during the spring (late April; *open circles*) or summer (mid-July; *closed circles*). Photosynthesis rates have been normalized to the maximum observed rate which is taken as 1.0

across the seasonal weather gradient of 1993. Leaves that emerged in late February and developed under cool spring temperatures did not emit isoprene until 23 days after emergence (Fig. 2A). Measurable photosynthesis rates occurred on the earliest sampling date, demonstrating that the development of photosynthetic competence occurred well before the development of isoprene emission (Fig. 2B). The mean maximum air temperature between the time of leaf emergence and the onset of isoprene emission was 12°C. After the initiation of isoprene emission, the rate increased slowly and continuously over the next 7 weeks, concomitant with a progressive increase in air temperature. Leaves that emerged in late June and developed under hot mid-summer temperatures initiated isoprene emission within 6 days, increasing rapidly to a maximum by 30 days after leaf emergence (Fig. 2A). The mean maximum air temperature between the time of leaf emergence and the onset of isoprene emission was 30°C. Once again, photosynthesis rates were well-developed by the first sampling date, 4 days after emergence (Fig. 2B).

To directly examine the role of temperature as an agent of the springtime onset of isoprene emission, a leaf from one of the potted aspen trees was placed in a cuvette maintained at 32°C for 6 h per day for 3 consecutive days. The experiment was performed in mid-March when ambient temperatures were still cool (daily maximum temperature during this period was 0–19°C) and before the tree had initiated seasonal isoprene emission. The plant was kept outdoors during the night in between the warm-temperature treatment. Within 2 h of being exposed to the warm-temperature treatment, the leaf began to emit detectable levels of isoprene (Fig. 3A). Upon continued exposure of the heated leaves, the isoprene emission rate continued to increase. During the intervening cool nights, the isoprene emission rate decreased slightly, but exhibited a striking recovery and further increases during each successive day's warm-temperature

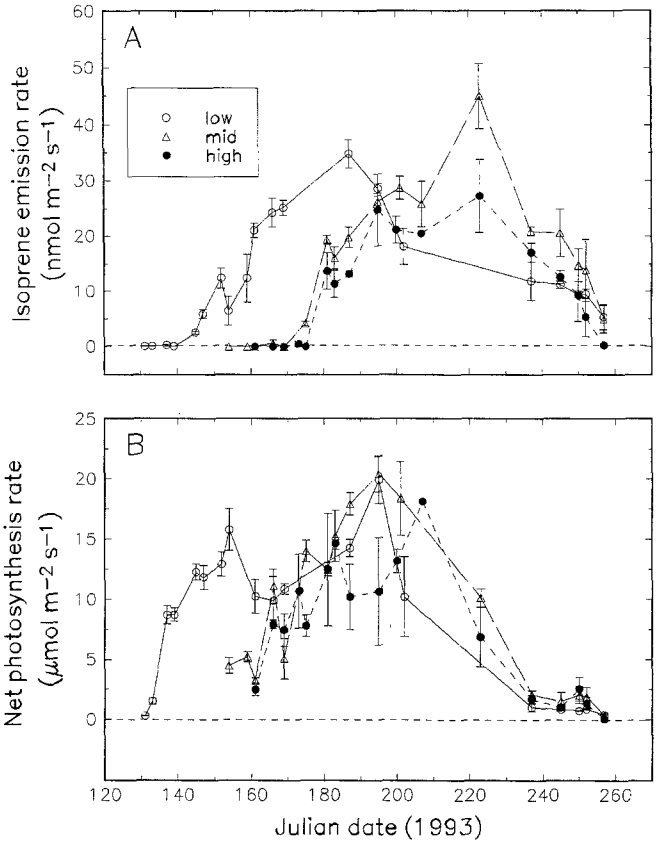

Fig. 5 **A** Seasonal pattern of isoprene emission for aspen leaves at the high-elevation site (2896 m), middle-elevation site (2439 m) and low-elevation site (1829 m) during the 1993 growing season. **B** Seasonal pattern of photosynthesis rate for the same leaves described in *panel A*. All rates were measured at 25°C. Points represent the mean±SE of three replicates

treatment. Photosynthesis rates showed a slow but continuous increase over the 3-day sampling period, and a progressive hourly increase during the daily warm-temperature treatments (Fig. 3B). Control trees that were not exposed to the elevated temperatures did not initiate seasonal isoprene emissions until 1–2 weeks after this experiment.

Seasonal temperature acclimation of the isoprene emission rate was examined by observing the temperature dependence during late April, when growth temperatures were still cool (range of daily maximum temperatures was 9–22°C), and during mid-July, when growth temperatures were relatively hot (range of daily maximum temperatures was 25–33°C). Photosynthesis rates exhibited some acclimation to warmer temperatures as the summer proceeded, but the overall shape of the temperature response curve did not change significantly (Fig. 4B). The data for isoprene emission were normalized to a value of 1.0 at 30°C so that the temperature algorithms developed by Guenther et al. (1991, 1993a) could be applied. Isoprene emission rates were similar, between 20 and 35°C at both times of the year (Fig. 4A). Isoprene emission rates above 35°C are notoriously unstable and dependent on the time of measurement (Guenther et al. 1993a). Thus, these measurements have not been included. Besides, the temperature range 20–35°C is the most relevant to the temperature regimes actually experienced by aspen trees in the Rocky Mountain Front Range. The temperature algorithm developed by Guenther et al. (1991) was used to simulate the temperature response of normalized isoprene emission rates during the spring and summer. This algorithm was accurate in predicting the temperature response of isoprene emission between 20 and 35°C with no adjustment required for seasonal acclimation (Fig. 4A).

Studies of isoprene emission over an elevational gradient

In the experiments reported in this section, observations were made of trees growing in their native habitat at three elevations along the Colorado Front Range. Trees at different elevations exhibited staggered patterns of bud-break and initiation of photosynthesis and isoprene emission (Fig. 5). This pattern was in concert with the progressive delay in spring thaw that characterizes elevational gradients in the Colorado Front Range. Once induced, isoprene emission rates increased rapidly. As was the case for measurements during 1992, leaf nitrogen concentrations declined during the period of leaf expansion (data not shown). This resulted in a negative correlation between isoprene emission rate and leaf nitrogen concentration during the period preceding expression of the maximum isoprene emission rate (Fig. 6A). During the late summer and autumn period, following the seasonal maximum emission rate, rates were positively correlated with leaf nitrogen concentration (Fig. 6B).

The seasonal onset of isoprene emission was approximately equal for all three elevations when expressed as a function of heating degree days above 0 or 5°C (Fig. 7). Accumulated temperature of approximately 400 degree days above 0°C was a fairly good predictor of the seasonal induction point. This predictor held true despite a phenological spread of 30 days between the lowest and highest elevation sites. It also held true for the potted trees that broke bud in late February and did not initiate isoprene emission until late March.

Discussion

On the mechanisms underlying the seasonal pattern

Although past studies have reported seasonal variation in isoprene emission rate (e.g., Ohta 1986), this is the first study to address the underlying causes. The springtime onset of isoprene emissions clearly interacts with prevailing temperature (Figs. 2, 3). In recent studies of isoprene emission from kudzu leaves, Sharkey and Loreto (1993) first reported the existence of temperature induction, observing that growth at <20°C completely suppressed isoprene emission. By treating leaves at 30°C,

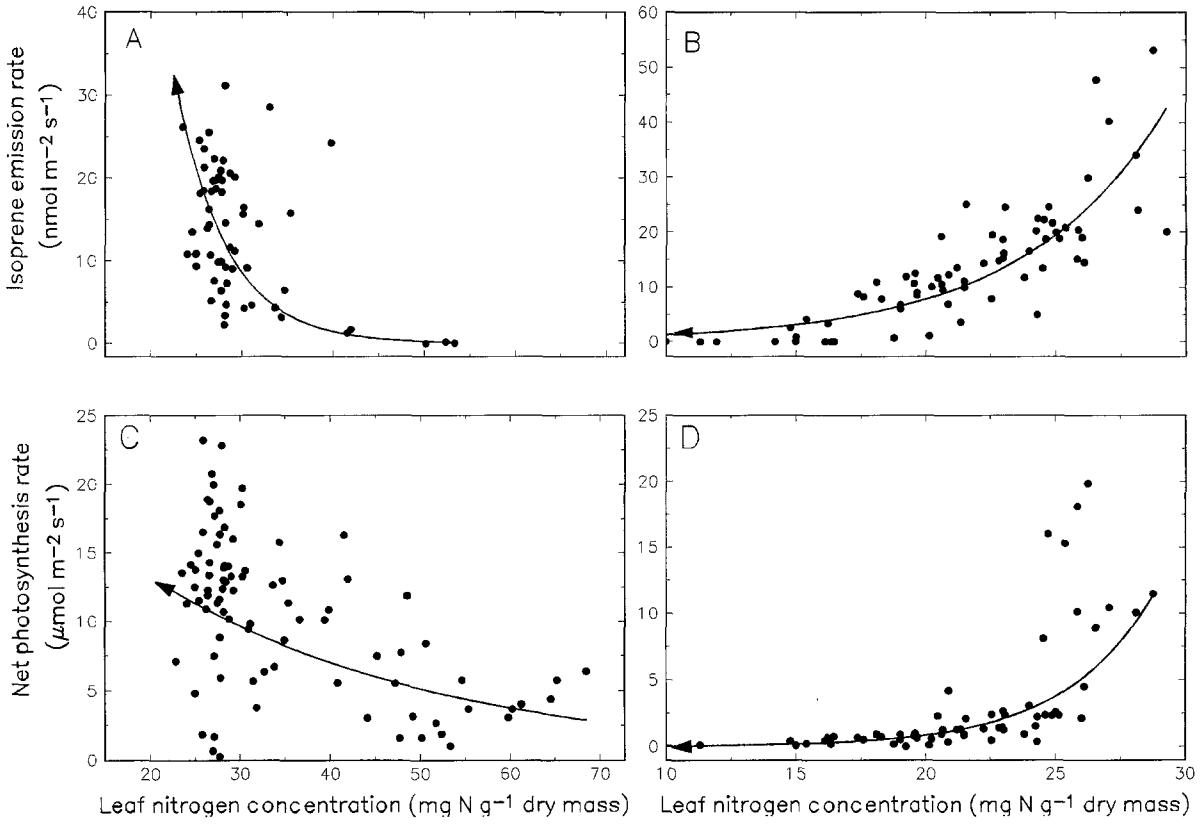

Fig. 6 **A** The relationship between isoprene emission rate and leaf nitrogen concentration for leaves measured before seasonal maximum isoprene emission rates at all three elevation sites. Data were pooled for all three elevations. Each *point* represents measurement of a separate leaf. **B** The relationship between isoprene emission rate and leaf nitrogen concentration for leaves measured after the seasonal maximum isoprene emission rates at all three elevation sites. **C** The relationship between net photosynthesis rate and leaf nitrogen concentration for leaves measured before the seasonal maximum isoprene emission rate at all three elevations. **D** The relationship between net photosynthesis rate and leaf nitrogen concentration for leaves measured after the seasonal maximum isoprene emission rate at all three elevation sites. The *arrows* represent the exponential best fit to the data, with the *direction* of the arrow indicating temporal pattern of data collection

these workers could induce isoprene emission in a matter of hours. The current study, and the observed early-season interaction of isoprene emissions with temperature, has provided an ecological context for these past results.

Temperature controls also appear to explain elevational variation in the seasonal pattern of isoprene emission. As temperatures progressively warmed along the elevational gradient, a staggered pattern of induction of isoprene emission was observed (Fig. 5). When expressed as a function of cumulative temperature (in this case as heating degree days), the lowest and highest sites exhibited similar requirements for springtime warming (Fig. 7). Numerous previous studies have reported a correlation between cumulative temperature and forest phenology patterns (e.g., Olson et al. 1959; Hellmers 1962; Boyer 1978; Thomson and Moncrief 1982). The phenological expression of isoprene emission rate is likely dependent on the production of isoprene synthase activity (see Kuzma and Fall 1993). At this time it is not clear whether the temperature effect is due to a slow accumulation of temperature (e.g., Fig. 7) or exposure to a critical high temperature threshold (e.g., Fig. 3). It may be that cumulative heat or some threshold temperature following bud-break is required for seasonal activation of the isoprene synthase gene. In this context, it would be worth investigating possible similarities between the temperature-induced activation of isoprene synthase and patterns of induction for heat-shock proteins (Vierling 1991). Alternatively, cumulative heat may regulate leaf traits that are only indirectly linked to isoprene emission (e.g., leaf area or leaf starch concentration), triggering the onset of emissions when the expression of such traits reaches a critical level. One limitation of the experiments in this study is that they are correlative. They do not differentiate between a direct inductive effect of temperature on some aspect of metabolism and an effect of temperature on the rate of leaf development, with concomitant developmental control over the onset of isoprene emission.

Previous studies have shown that isoprene emission rate is positively correlated with leaf nitrogen concentration when examined at the same phenological stage for velvet bean plants (Harley et al. 1994), and for aspen and oak trees (M. E. Litvak, T. D. Sharkey, F. Loreto, P. C. Harley, and R. K. Monson, unpublished results). In interpreting the results of the current study, we assume that positive correlations should also be observed for the aspen trees used here, unless phenological or other envi-

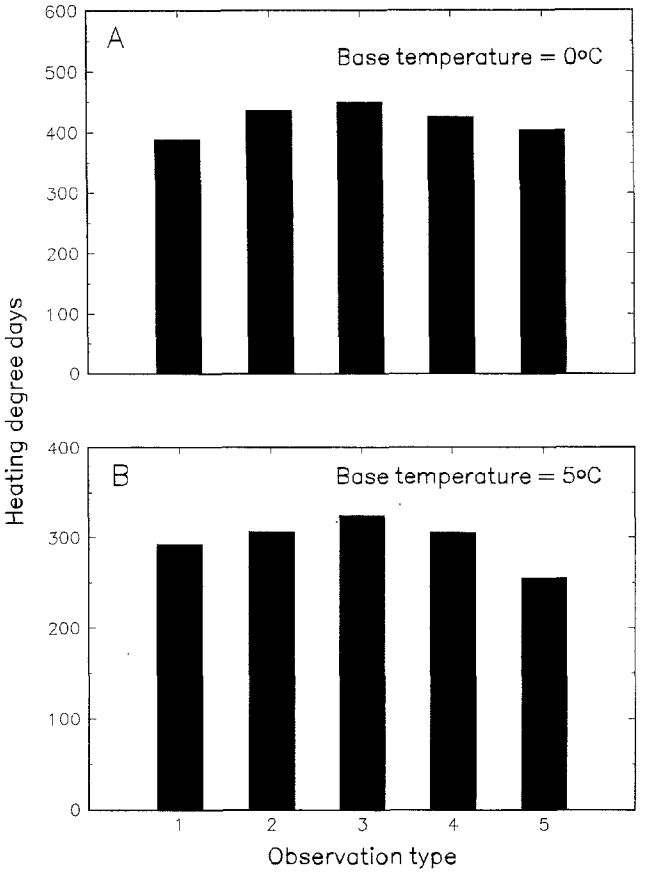

Fig. 7 **A** Heating degree days calculated for the period between spring bud-break and the onset of spring isoprene emission using a base temperature of **A** 0°C and **B** 5°C. *Observation type* refers to one of five different experimental observations (*1* trees observed at the lowest elevation site during the 1993 growing season, *2* trees observed at the middle elevation site during the 1993 growing season, *3* trees observed at the highest elevation site during the 1993 growing season, *4* potted trees grown outdoors and observed at Boulder from late February to early April 1993, *5* trees observed at the highest elevation site during the 1992 growing season)

ronmental controls intervene and exert dominance. The results of the current study make clear the dominance of phenology and temperature over leaf nitrogen concentration in controlling the springtime onset of emissions. In fact, a negative correlation was observed between isoprene emission rate and leaf nitrogen concentration during the spring (Fig. 6). During the late summer and early autumn, leaf nitrogen concentrations were positively correlated with isoprene emission (Fig. 6). Thus, although temperature controls the early season pattern of isoprene emission, the autumnal translocation of nitrogen out of leaves during senescence appears to control the late-season pattern. At present it is not clear whether autumnal decreases in leaf nitrogen concentration influence isoprene emission rate directly, or reflect a metabolic linkage between isoprene emission rate and decreases in photosynthesis rate. (Photosynthesis rate is known to be highly dependent on leaf nitrogen concentration; Field and Mooney 1986.)

Several past studies have provided evidence of a metabolic linkage between isoprene biosynthesis and photosynthesis (e.g., Sanadze 1969; Rasmussen and Jones 1973; Tingey et al. 1987; Monson and Fall 1989; Loreto and Sharkey 1990, 1993; Monson et al. 1991b; Delwiche and Sharkey 1993). There appears to be no role for this linkage in determining the early-season onset of isoprene emission. This is especially clear in the results shown in Figs. 1 and 5, which show a lag in isoprene emission, despite substantial photosynthesis rates. Once isoprene emission was induced, however, there was a general correlation with photosynthesis rate. This was observed both in seasonal patterns in which seasonal maxima of isoprene emission rate and photosynthesis generally coincided (e.g., Fig. 1), and in diurnal patterns in which hourly increases in daytime isoprene emission rate generally matched increases in photosynthesis rate (e.g., Fig. 3).

What could be the nature of the general seasonal correlation between isoprene emission rate and photosynthesis rate? Three possible explanations are as follows: (1) Following the seasonal onset of isoprene emission, and at least until the commencement of autumnal senescence, the emission rate might be controlled by chloroplast carbon supplies, specifically the diel accumulation of starch or other forms of stored photosynthate. (2) There could be a coordinated, direct developmental linkage of photosynthesis rate and post-induction isoprene synthase activity. There is currently no support for this. In fact, at present it is difficult to reconcile this possibility with the observations that onset of isoprene emission is independent of expression of early-season photosynthetic capacity. (3) It is possible that isoprene emission and photosynthesis rate are indirectly linked through their common dependence on leaf nitrogen concentration (Fig. 6). The difficulty with this explanation is that it does not readily account for the opposite directions of the correlations of these fluxes with nitrogen for early- and late season data (Fig. 6). It is possible that these explanations are interactive, with one underlying the early-season linkage between these processes and another underlying the late-season linkage.

Past studies have demonstrated a lag in the development of isoprene emission, compared to photosynthesis rate, when plants are grown in warm environments (Grinspoon et al. 1991; Sharkey and Loreto 1993). This is due to a developmental lag in the appearance of isoprene synthase activity (Kuzma and Fall 1993). The results of the current study show that such developmental control can be influenced by the prevailing temperature regime. In leaves that developed in warm temperatures isoprene emission appeared within 6 days, compared with 23 days for leaves that developed under cool conditions (Fig. 2). The results of these experiments are interpreted as indicating that isoprene emissions can be delayed for a considerable fraction of the growing season if temperatures are cool and concomitant leaf development is slow.

Seasonal acclimation to changes in temperature regime was not observed in the isoprene emission rate or photosynthesis rate (at least at temperatures between 20

and 35°C) (Fig. 4). These results are similar to those observed for velvet bean plants grown in controlled-environment chambers (Monson et al. 1992). The temperature-dependence algorithm developed previously for several forest trees (Guenther et al. 1991, 1993a) accurately described the temperature dependence of isoprene emissions over the seasonal temperature gradient. These results reflect the robust nature of this algorithm in describing the dependence of isoprene emission rate on temperature – one of the most important environmental factors controlling emissions.

On the implications for modeling regional and global isoprene emissions

Numerous efforts have been made in recent years to model the emission of isoprene from local to global scales (recent reviews by Monson et al. 1991a; Fehsenfeld et al. 1992; Hewitt and Street 1992). In an effort to assess the adequacy of this modeling approach, simulated isoprene emission rates were calculated and compared with the observed emission rates of this study. The simulated rates were obtained by taking an average isoprene emission rate and adjusting it to the daytime maximum temperature. The average emission rate was determined by averaging over that portion of the season when emissions were at least 30% of the seasonal maximum. Acknowledging the subjectivity of this procedure, it is nonetheless a fair representation of how basal emission rates have been determined in past inventory efforts (see Lamb et al. 1993 for a discussion and sensitivity analysis of different methods used in choosing mean isoprene emission rates). Temperature dependence of the basal emission rate was determined using the previously described algorithm of Guenther et al. (1991). As in past modeling studies, early-season induction requirements were ignored. These modeled data were then compared to the measured emission rates (corrected to the maximum daily temperature using the same temperature algorithm described above). Trees from the highest- and lowest-elevation sites were compared.

Measured isoprene emission rates were significantly lower for the high-elevation site than for the low-elevation site (Fig. 8). This was due to the cooler seasonal temperatures at the high-elevation site. Using the modeled and actual emission rates, the seasonal patterns were integrated to determine differences in total emissions. From this analysis, it was calculated that the traditional inventory procedure precisely matched measured seasonal emissions at the low-elevation site, and overestimated measured seasonal emissions by only 17% at the high-elevation site. However, when one examines details in the modeled versus actual emission patterns it is evident that any success of the integrated modeling approach is serendipitous (Fig. 8). The model calculations consistently overestimate emissions during the early and late parts of the growing season, and consistently underestimate the seasonal maximum.

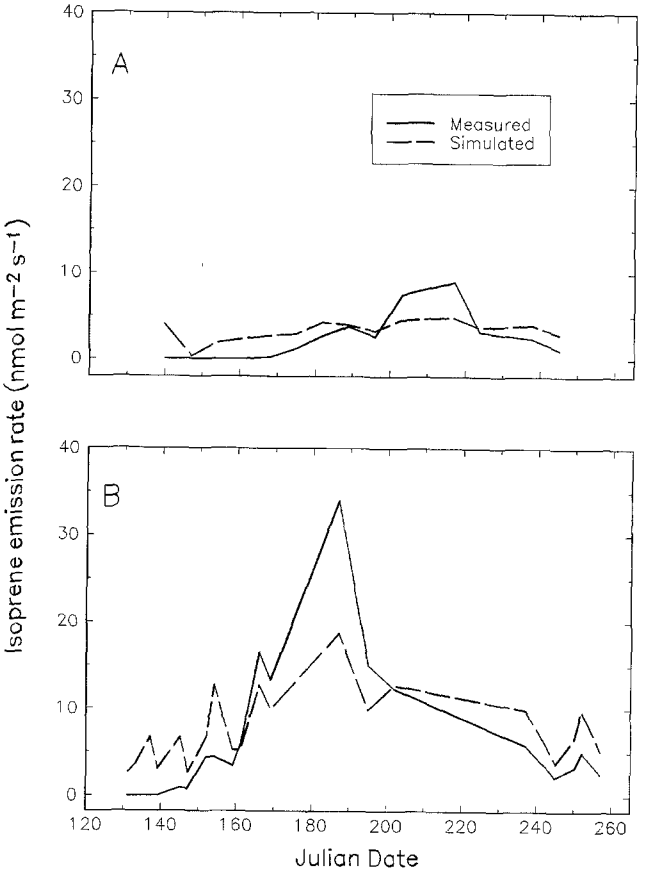

Fig. 8 The actual seasonal pattern of isoprene emission rate (*solid line*) compared to the simulated seasonal pattern of isoprene emission rate (*broken line*) using the procedures typically used for constructing isoprene emission inventories (see text). **A** Data and simulation for trees at the highest-elevation site. The integrated seasonal isoprene emission rate is 29.0 nmol m^{-2} for the observed data and 34.1 nmol m^{-2} for the simulated data. **B** Data and simulation for trees at the lowest elevation site. The integrated seasonal isoprene emission rate is 107 nmol m^{-2} for the observed data and 107 nmol m^{-2} for the simulated data

Recently, Guenther et al. (1993b) have recommended using a basal isoprene emission rate of 26 nmol m^{-2} s^{-1} (at 30°C and light saturation) for members of the genus *Populus*. Applying this rate to the model procedures described above, we calculate a 39% overestimate of the integrated seasonal emission rate at the low-elevation site and a 19% overestimate of the integrated seasonal emissions at the high-elevation site. These deviations reflect the fact that although an emission rate of 26 nmol m^{-2} s^{-1} accurately predicts the seasonal maximum isoprene emission rate from aspen (Fig. 1), it does not accommodate seasonal or developmental variation. The deviations would be even larger, but in the opposite direction, if one applied the basal emission rates used in the national emission inventories developed by Lamb et al. (1987, 1993). In these models an emission rate of 5–8.5 nmol m^{-2} s^{-1} (at 30°C and light saturation and assuming an aspen specific leaf mass of 80 g m^{-2} for conversion purposes) was used.

(Note that the latter rates are meant as an average of all isoprene-emitting species, and thus probably underestimate emissions from "high emitters" such as aspen.) Applying these emission rates to a model of seasonal aspen emissions results in a 55–73% underestimate of integrated seasonal emissions at the low-elevation site, and a 61–77% underestimate of integrated seasonal emissions at the high-elevation site.

In all these cases, the inadequacies of using one basal emission rate to represent an entire season are apparent. The results of this study show that such an approach has the danger of overestimating the early- and late-season isoprene emission rates, while underestimating the seasonal maximum (Fig. 8). Future models of seasonal isoprene emission will be improved by accommodating the tapered pattern by which the seasonal maximum is reached, as well as the potential for variation in early-season temperature regimes and its influence on developmental patterns.

Effective modeling of the early-season induction might be achieved through consideration of cumulative temperatures between the times of spring bud-break and initiation of isoprene emission (Fig. 7). This variable is likely to be species dependent, however, and thus dependent on empirical parameterization, at least until the mechanisms of seasonal induction are discovered. Modeling of the post-induction increase in isoprene emission might be best accomplished through exploitation of the correlation between isoprene emission rate and photosynthesis rate. Several ecosystem-level models currently include algorithms to estimate seasonal dynamics in photosynthesis rate (e.g., FOREST-BGC, Running and Coughlan 1988; Running and Gower 1991).

Most current models of isoprene emission recognize incident light intensity and temperature as dominant controls over the instantaneous emission rate (e.g., Lamb et al. 1987, 1993; Anastasi et al. 1991). Recent studies have demonstrated that a temperature algorithm based on enzyme activation energy accurately simulates the instantaneous temperature dependence of isoprene emission (Guenther et al. 1991, 1993a). One uncertainty in using this algorithm, however, has been the possibility of temperature acclimation over seasonal and spatial temperature gradients. The results of the current study demonstrate that temperature acclimation of isoprene emission rate does not occur in aspen when measured over fairly broad seasonal ranges. (At least this is true for the temperature dependence within the range 20–35°C.) This simplifies modeling of the temperature response to a single algorithm. There are still uncertainties about application of the algorithm for plants grown and measured at temperatures above 35°C. Additional studies within this range should be conducted with a high priority since many of the high ozone episodes reported for the southeastern United States occur during the hottest parts of the summer. Isoprene emission from urban forests in this region is known to play an important role in these events (Chameides et al. 1988).

Acknowledgements The authors gratefully acknowledge the talented technical assistance provided by Tracy Lynn, Erika Kelley, Delphina Margulis, and Taryn Mann. Additional thanks are offered to Dr. Beth Holland (National Center for Atmospheric Research, Boulder) for providing some of the nitrogen analyses and Dr. Manuel Lerdau (Stanford University) for comments on the manuscript. This research was supported by Environmental Protection Agency Grant no. R-819431–01–0 (to R.K.M. and R.F.), a National Science Foundation Equipment Grant (DIR-8908168) (to R.K.M.), an Environmental Protection Agency Interagency Agreement with the National Center for Atmospheric Research (DW-49934973–01–0) (to A.B.G. and P.R.Z.), and a Faculty Fellowship (to R.F.) from the Council on Research and Creative Work, University of Colorado. This research is part of the Southern Oxidants Study (SOS) – a collaborative university, government, and private industry study to improve understanding of the accumulation and effects of photochemical oxidants.

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