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Reduction of isoprene emissions from live oak (*Quercus fusiformis*) with oak wilt

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Summary Many plants emit isoprene, a hydrocarbon that has important influences on atmospheric chemistry. Pathogens may affect isoprene fluxes, both through damage to plant tissue and by changing the abundance of isoprene-emitting species. Live oaks (*Quercus fusiformis* (Small) Sarg. and *Q. virginiana* Mill) are major emitters of isoprene in the southern United States, and oak populations in Texas are being dramatically reduced by oak wilt, a widespread fungal vascular disease. We investigated the effects of oak wilt on isoprene emissions from live oak leaves (*Q. fusiformis*) in the field, as a first step in exploring the physiological effects of oak wilt on isoprene production and the implications of these effects for larger-scale isoprene fluxes. Isoprene emission rates per unit dry leaf mass were 44% lower for actively symptomatic leaves than for leaves on healthy trees ($P = 0.033$). Isoprene fluxes were significantly negatively correlated with rankings of disease activity in the host tree (fluxes in leaves on healthy trees > healthy leaves on survivor trees > healthy leaves on the same branch as symptomatic leaves > symptomatic leaves; isoprene per unit dry mass: Spearman's $\rho = -0.781$, $P = 0.001$; isoprene per unit leaf area: Spearman's $\rho = -0.652$, $P = 0.008$). Photosynthesis and stomatal conductance were reduced by 57 and 63%, respectively, in symptomatic relative to healthy leaves ($P < 0.05$); these reductions were proportionally greater than the reductions in isoprene emissions. Low isoprene emission rates in symptomatic leaves are most simply explained by physiological constraints on isoprene production, such as water stress as a result of xylem blockage, rather than direct effects of the oak wilt fungus on isoprene synthesis. The effects of oak wilt on leaf-level isoprene emission rates are probably less important for regional isoprene fluxes than the reduction in oak leaf area across landscapes.

Keywords: atmospheric chemistry, Ceratocystis fagacearum, fungal vascular disease, hydrocarbon emissions.

Introduction

Many plant species release the volatile hydrocarbon isoprene (2-methyl-1,3-butadiene) from their leaves. Global emissions of isoprene from vegetation have been estimated at 503×10^{12} g C year^{-1} (Guenther et al. 1995), greater than natural methane emissions ($300–480 \times 10^{12}$ g C year^{-1}, Cicerone and Oremland 1988). Isoprene plays a critical role in the atmospheric chemistry of several pollutants. When isoprene is oxidized by OH or O_3 in the presence of high concentrations of the pollutant NO, large amounts of O_3 and CO are produced (Trainer et al. 1987, Chameides et al. 1988, Brasseur and Chatfield 1991). Because isoprene consumes atmospheric OH, oxidation of methane is reduced, increasing the lifetime and greenhouse effects of methane in the troposphere (reviewed by Monson et al. 1991 and Fehsenfeld et al. 1992). These interactions make it vital to understand the factors that control the quantities and timing of isoprene emissions, such that isoprene can be appropriately incorporated into models of atmospheric dynamics and air quality.

Although plant pathogens may have strong influences on isoprene production, to our knowledge they have not been studied in this context. Pathogens may act at the leaf level by damaging tissue and changing leaf physiology, or at larger scales by changing the abundance of isoprene-emitting species (Lerdau et al. 1997, Harley et al. 1999). The fungal vascular disease oak wilt is dramatically reducing populations of live oak (*Quercus fusiformis* (Small) Sarg. and *Q. virginiana* Mill.) in Texas. Over 2300 oak wilt locations have been identified in the state (Texas Forest Service Oak Wilt Suppression Project, unpublished data), suggesting that the disease is widespread and could have a major impact on oaks in this region. Live oaks are among the most important isoprene-emitting species in the southern USA because of their abundance and high emission rates (Tingey et al. 1979, 1981). In this study, we investigated the effects of oak wilt on live oak leaf isoprene emissions in the field.

Oak wilt causes extensive leaf loss, reducing the tree's overall isoprene emissions. There may be additional important changes in emissions from leaves that remain in the canopy during the active phase of the disease, or in new leaves of trees that survive oak wilt, but to our knowledge this has not been studied. The epidemiology of oak wilt provides an excellent experimental opportunity to compare isoprene emissions in the field for leaves at various stages of infection. Sap-feeding beetles introduce spores of *Ceratocystis fagacearum* (Bretz) Hunt into a tree wound (Appel et al. 1986). The fungus colonizes the xylem and the tree responds by forming tyloses and secreting gums and resins that clog vascular elements and lead to leaf wilt (Beckman et al. 1953, Jacobi and MacDonald 1980). In live oak, symptoms include browning along leaf veins (veinal necrosis) and gradual canopy thinning (Appel 1994). Trees usually die within one year after symptoms appear, although some live for several years with a reduced canopy. These "survivors" generally represent 15–20% of infected trees at an oak wilt "center," a point on the landscape where oak wilt has been introduced and has spread to other trees through root grafts (Appel et al. 1989). Oak wilt centers in Texas average 3.6 ha and can be as large as 80 ha, and may include hundreds of trees (Appel and Maggio 1984).

Our specific goal was to determine how oak wilt influences leaf-level isoprene emissions in live oak leaves. Our approach was to measure isoprene emissions and leaf gas exchange characteristics in the field for leaves representing a range of symptomatic stages. These data provide a starting point for exploring mechanisms underlying the effects of plant pathogens on isoprene fluxes, and for estimating the influences of oak wilt on isoprene production at larger scales.

Methods

Study site description and tree selection for isoprene measurements

Measurements were taken at Camp Creek Resource Area, 80 km northwest of Austin, TX. The site is an open savanna containing mature live oak (*Q. fusiformis*, 10–15 m tall, 3.73 m mean canopy radius) interspersed with small (0.5–3 m tall) persimmon (*Diospyros texana* Scheele), mesquite (*Prosopis glandulosa* Torr.) and ashe juniper (*Juniperus ashei* Buchholz). The dominant grasses are King Ranch bluestem (*Bothriochloa ischaemum* (L.) Keng. var. *songarica* (Rupr.) Celerier & Harlan) and Texas wintergrass (*Nassella leucotricha* (Trin. & Rupr.) R.W. Pohl, formerly *Stipa leucotricha* Trin. & Rupr.). All oaks at the study site were surveyed visually for leaves with veinal necrosis in 1995 and 1996, and 24 trees had symptomatic leaves. The Plant Pathology Department at Texas A&M University successfully isolated *C. fagacearum* from branch samples from the site.

Twelve trees were chosen for studies of leaf-level isoprene emissions: four healthy trees that showed no evidence of oak wilt, four survivor trees with reduced canopies but no symptomatic leaves, and four symptomatic trees that had numerous leaves with veinal necrosis. Distances between trees in different categories ranged from approximately 10 to 50 m. Surveys of soil water and nutrients under and adjacent to trees across the site provided no evidence for resource gradients in the study area (L. J. Anderson, unpublished data). However, there was more soil water and less soil N under survivor trees than under healthy trees, presumably because of various environmental and resource use changes associated with reductions in the tree canopies (Anderson et al. 2000). Actively symptomatic trees that had been recently infected had only minor canopy losses. On three symptomatic trees, we measured both symptomatic and healthy leaves on the same branch. This resulted in four categories for comparison: leaves on healthy trees, healthy leaves on survivor trees, healthy leaves on symptomatic trees and symptomatic leaves.

Isoprene measurements

Isoprene fluxes, photosynthetic rates and stomatal conductances were measured on two to four leaves per tree between 0900 and 1730 h in late August and early September 1996. Leaves were chosen from the outer canopy on the south side of each tree, where the canopy was most accessible by ladder. Gas exchange was measured with an LI-6400 portable photosynthesis system (Li-Cor Inc., Lincoln, NE). An accessory LED light source (LI-6400-02) on the LI-6400 cuvette maintained irradiance at 1000 μmol m^{-2} s^{-1}. To measure isoprene fluxes, samples of cuvette air were withdrawn into the sample loop of a portable gas chromatograph through a T-junction in the airline leaving the cuvette. Isoprene was separated by means of a stainless steel column (1.3 m long × 2 mm i.d.) packed with Unibeads 3S, 60/80 mesh (Alltech Assoc., Deerfield, IL), and measured with a reduction gas detector (RGD2, Trace Analytical, Menlo Park, CA). A commercial integrator (Model 3390, Hewlett-Packard, Avondale, PA) was used for peak integration. The system was calibrated several times per day against a standard cylinder containing 51 ppb (v/v) isoprene, referenced to a National Institute of Standards and Technology neohexane standard.

We tried to maintain leaf temperatures at 30 °C, but hot, sunny conditions caused temperatures to vary from 27.3 to 35.4 °C. Isoprene emission rates were normalized to 30 °C with the temperature correction equations of Guenther et al. (1995). Leaf area was measured with an image analysis program (NIH Image, National Institutes of Health, Bethesda, MD) and leaves were oven-dried at 70 °C to constant weight. Isoprene emissions were expressed on both a dry mass (μg C g^{-1} h^{-1}) and leaf area (nmol m^{-2} s^{-1}) basis. Photosynthesis and stomatal conductance were expressed on a leaf area basis only (μmol CO_2 m^{-2} s^{-1} and mmol H_2O m^{-2} s^{-1}, respectively).

Statistical analyses

Multiple leaves in the same infection category from one tree were averaged to give an overall value for the tree. Mean isoprene emission rates, photosynthetic rates and stomatal conductances among infection categories were compared with non-parametric Kruskal-Wallis tests because of variance heterogeneity. Multiple pair-wise comparisons were made by the

methods of Dunn as described in Zar (1996). Spearman's rank correlation analyses were used to examine linear relationships between isoprene flux, gas exchange and disease activity in the host tree (the rank order of disease activity from greatest to least was symptomatic leaves > healthy leaves on symptomatic trees > healthy leaves on survivor trees > healthy leaves on healthy trees).

Results

Isoprene emission rates per unit dry leaf mass were 44% lower in symptomatic leaves than in leaves on healthy trees (Kruskal-Wallis, $P = 0.033$, Table 1). Isoprene emission rates expressed on a leaf area basis showed the same trend (Kruskal-Wallis, ns). Leaf mass per unit area (LMA) was somewhat lower in healthy leaves than in symptomatic leaves (Table 1, Kruskal-Wallis, ns), leading to reduced emissions in healthy leaves when values were calculated on a leaf area basis. Isoprene fluxes on both a dry mass and leaf area basis were significantly negatively correlated with rankings of disease activity in the host tree (fluxes in leaves on healthy trees > healthy leaves on survivor trees > healthy leaves on the same branch as symptomatic leaves > symptomatic leaves; dry mass: Spearman's $\rho = -0.781$, $P = 0.001$; leaf area: Spearman's $\rho = -0.652$, $P = 0.008$, Table 1). Photosynthesis and stomatal conductance were reduced by 57 and 63%, respectively, in symptomatic relative to healthy leaves (Kruskal-Wallis, $P = 0.042$ and 0.031, respectively, Table 1). There were no significant linear relationships between isoprene emission and photosynthetic rates within disease categories, although across categories higher photosynthetic rates were generally associated with higher isoprene fluxes (Figure 1).

Discussion

Leaf-level isoprene emissions were reduced in *Q. fusiformis* with oak wilt. The largest reductions were in symptomatic leaves, with small effects in healthy leaves on the same branch as symptomatic leaves, and healthy leaves on survivor trees. The simplest explanation for the low isoprene emissions in symptomatic leaves is that the reduction is caused by physiological constraints on isoprene production, rather than by any direct effect of *C. fagacearum*. For example, low isoprene emissions could result from including necrotic tissue in dry weight and leaf area calculations, because all symptomatic leaves had veinal necrosis. To identify the physiological mechanisms underlying oak wilt effects on isoprene fluxes, emission rates should be measured on the green portions of symptomatic leaves.

The influence of water stress on isoprene production in symptomatic leaves should also be explored, because there are similarities between the effects of oak wilt and drought on isoprene emissions. Isoprene emissions are known to decrease under severe (but not mild) water stress in several species (Tingey et al. 1981, Loreto and Sharkey 1993, Sharkey and Loreto 1993, Monson et al. 1995, Fang et al. 1996). Oak wilt blocks xylem conduits, inhibiting water flow (Beckman et al. 1953) and leading to stomatal closure, reduced photosynthesis and eventual leaf death (e.g., TeBeest et al. 1976). This mechanism suggests that reductions in isoprene emissions in symptomatic leaves may also be associated with water stress. In addition, isoprene production in droughted plants is often not affected by water stress as strongly as other leaf physiological processes (Tingey et al. 1981, Sharkey and Loreto 1993, Fang et al. 1996). In our study, photosynthesis and stomatal conductance in symptomatic leaves were reduced by 57 and 63%, respectively, compared with leaves on healthy trees. These decreases were proportionally greater than the decrease in

Table 1. Isoprene emission, photosynthetic rate, stomatal conductance and leaf mass per unit area (LMA) for *Quercus fusiformis* leaves in four disease categories (mean ± SE). Isoprene is expressed on both a dry mass and leaf area basis. Numbers in parenthesis refer to numbers of trees in each category; two to four leaves were measured and averaged to give a value for each tree. Isoprene emission rates were significantly different on a dry mass basis for leaves on healthy trees and symptomatic leaves (Kruskal-Wallis, $P = 0.033$). The same trend occurred for isoprene emission rates on a leaf area basis (Kruskal-Wallis, $P = 0.100$). Photosynthetic rates and stomatal conductances were also significantly different (Kruskal-Wallis, $P = 0.042$ and $P = 0.031$, respectively). The LMAs were not significantly different (Kruskal-Wallis, $P = 0.363$).

Variable	Healthy leaves on healthy trees ($n = 4$)	Healthy leaves on survivor trees ($n = 4$)	Healthy leaves on branches with symptomatic leaves ($n = 4$)	Symptomatic leaves ($n = 3$)
Isoprene emissions				
($\mu g\ C\ g^{-1}\ h^{-1}$)	60.9 ± 5.7	49.9 ± 2.3	46.6 ± 5.1	34.0 ± 5.7
($nmol\ m^{-2}\ s^{-1}$)	50.9 ± 5.0	42.8 ± 3.1	42.8 ± 5.3	33.2 ± 2.4
Photosynthetic rate				
($\mu mol\ CO_2\ m^{-2}\ s^{-1}$)	12.4 ± 0.7	13.9 ± 2.0	8.76 ± 1.6	5.35 ± 1.8
Stomatal conductance				
($mmol\ H_2O\ m^{-2}\ s^{-1}$)	154 ± 7	176 ± 40	100 ± 20	57 ± 16
Leaf mass per unit area				
($g\ m^{-2}$)	181.2 ± 11.1	184.7 ± 5.1	198.0 ± 10.5	219.7 ± 24.5

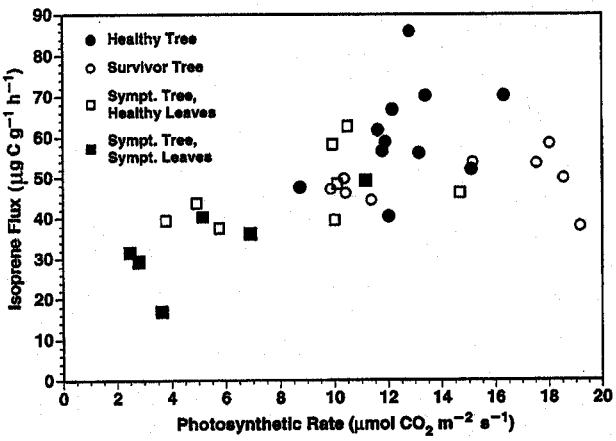

Figure 1. Relationship between isoprene flux and photosynthetic rate for individual leaves of *Q. fusiformis* in four disease categories. Each value is for a single leaf. Abbreviation: Sympt = symptomatic.

isoprene emission, particularly when emissions were expressed in terms of leaf area (33%). A similar trend was also evident for healthy leaves on the same branch as symptomatic leaves, where isoprene emissions were reduced by only 16% compared with a 29% reduction in photosynthesis relative to leaves on healthy trees. Although the apparently healthy leaves on the same branch as symptomatic leaves had no veinal necrosis, they were probably under water stress as a result of xylem blockage.

Leaves on survivor trees also showed a slight, non-significant reduction in isoprene emissions on a dry mass and leaf area basis (18 and 16%, respectively), but exhibited no reductions in photosynthetic and stomatal conductance rates. Given our sample sizes, these small differences must be interpreted cautiously; however, we note that the minor reductions in isoprene emissions in survivor trees are not easily explained by drought stress. Presumably, leaves on survivor trees are maintained by xylem that was either successfully isolated in the initial oak wilt attack or produced after the attack; therefore, survivor leaves have an adequate supply of water. Leaf water potentials do not differ significantly between healthy and survivor trees on the site, supporting this assumption (L. J. Anderson, unpublished data). It is possible that compounds produced by *C. fagacearum*, such as polysaccharides, indoleacetic acid and pectolytic enzymes (MacDonald and Hindal 1981), interfere with isoprene production in survivor and symptomatic trees. However, the roles of these compounds in disease progression are not understood, making it difficult to suggest mechanisms for their effects on isoprene production.

The importance of isoprene in atmospheric chemistry has led to efforts to quantify regional and global emissions, and to clarify the role of isoprene in atmospheric pollution and global change (Lamb et al. 1987, Monson et al. 1991, Hewitt and Street 1992, Guenther et al. 1996). One of our objectives was to explore whether models of isoprene budgets in areas with oak wilt should account for variation in emissions among leaves at different disease stages. Of particular interest were emission characteristics of leaves on survivor trees, because these may persist for long periods (in contrast to symptomatic leaves that die quickly once necrosis appears). Emissions were slightly reduced in leaves on survivor trees compared with leaves on healthy trees, but differences were not significant. Overall, leaf-level effects on isoprene emissions for leaves on survivor trees were minor compared with isoprene reductions caused by decreased canopy leaf area. We conclude, therefore, that the largest effects of oak wilt on regional isoprene emissions will occur through loss of healthy leaf area, rather than as a result of differences in emission rates per unit leaf area from leaves at different stages of infection.

Although our findings are preliminary, they highlight the potential importance of pathogen effects on isoprene emissions. Because leaves in a canopy are not uniform in their gas exchange and emission rates (Harley et al. 1996, Owens 1996, Lerdau and Throop 1999), future work should include detailed studies of the physiological mechanisms underlying emissions reductions in leaves with oak wilt, and research on variation in emissions within healthy oak canopies is needed. In particular, data on oak canopy leaf areas, isoprene fluxes from oaks in different sites and across seasons, numbers of oaks affected by oak wilt, and species replacing dead oaks are needed to estimate the influences of the disease on larger-scale isoprene fluxes. Factors such as local meteorology, hydrocarbon loading of the atmosphere from other biogenic and anthropogenic sources, and NO concentrations will determine whether reduced isoprene emissions from oaks will strongly affect local and regional atmospheric chemistry. Despite many uncertainties, we suggest that oak wilt may have a significant effect on isoprene fluxes at a range of scales. Further study of the interactions between oak wilt and isoprene could lend important insights into oak physiology and atmospheric chemistry in areas where oak wilt is prevalent.

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