Biochemistry, Molecular Biology

The Future of Isoprene Research

Thomas D. Sharkey

Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, Michigan, 48824 USA

(Presented by Academy Member T. Beridze)

ABSTRACT. This is the year of celebration of the 80th birthday of Academician Guivi Sanadze, the discoverer of isoprene emission from plants. Since the initial discovery, work in his laboratory has laid the foundation of our current understanding of many important aspects of isoprene emission. Among the most important are: the very large temperature sensitivity of isoprene emission from plants, its dependence on light, and its close association with photosynthesis. In this opinion paper I shall describe major understandings that have come about in the forty years of research since the discovery of isoprene emission, several current controversies and my opinions about them, and most importantly, the future for isoprene research. *© 2009 Bull. Georg. Natl. Acad. Sci.*

Key words: isoprene, dissipating energy, thermoprotection.

EARLY MAJOR DISCOVERIES

The first reports of isoprene emission from plants were received with significant skepticism. The mass spectrometry and NMR required to prove that the gas coming from plants was indeed isoprene is described by Sanadze [1]. Reinhold Rasmussen is given credit for independently discovering isoprene emission several years later. Rasmussen was confident that isoprene was being emitted and in Rasmussen and Went [2] stated that "the oak produces large amounts of a volatile material with the same absorption characteristics as isoprene". (The paper was read in 1964 and published in 1965.) He proved that isoprene was the compound emitted using mass spectrometry in 1970 [3]. The early period of isoprene research has been described in detail by Sanadze [1] and he puts the initial discovery in the 1950s. This work was clearly the first to describe isoprene emission. In a second period of isoprene research (as described by Sanadze), which I take to extend from 1970 (Rasmussen's confirmation) to 1989 (the beginning of a proliferation of papers), many details were added to the major observations originally made in the Institute of Botany (Academy of Sciences of Georgian SSR). This period saw confirmation that isoprene emission was light dependent, highly temperature dependent, and that the trait is found in a number of plant species, mostly trees but by no means in all plants. The middle period of research was primarily carried out by Sanadze, Rasmussen, and Tingey and their colleagues. It was during this period that I became fascinated with this topic as a graduate student in the mid-1970s, though it would be 15 years between my first isoprene experiments and my first publication on isoprene emission from plants. The publication in 1989 by Monson and Fall [4] and one year later by Sharkey and Loreto [5] began an intensive period of research on isoprene emission from plants, which continues to this day.

CURRENT MAJOR QUESTIONS

Two major questions characterize the current research on isoprene emission from plants; simply put they are "how" and "why".

How

This question can be addressed in two parts: 1. What is the pathway of carbon for isoprene synthesis; and 2. How is the rate of synthesis controlled? Initially it was assumed that all terpenoids were made by the mevalonic acid (MVA) pathway [6]. However, it had been known for some time that, for example, carotenoids were not easily labeled when labeled acetate (the precursor for the MVA pathway) was fed to chloroplasts. Carotenoids could be labeled when labeled CO_2 was fed so the chloroplasts were competent to make carotenoids. Sanadze postulated a second carboxylation reaction to account for this and other anomalies (reviewed in [1]). One of the anomalies was the labeling of carbons in the isoprene molecule when $^{13}CO_2$ is fed to leaves. The papers reporting on this work were very innovative and there was a significant analysis of mass spectrometry data [7,8]. But I was unable to confirm that some carbons are labeled more readily than others [9] and I now suspect the difference is related to whether the air continues to flow during the experiment so that the leaf does not run out of CO_2. However, these anomalies were also explained by the discovery of the methyl erythritol phosphate (MEP) pathway [10,11]. For me, it was compelling that feeding the inhibitor of one of the enzymes in the pathway, fosmidomycin, could almost eliminate isoprene emission without a significant effect on the rate of photosynthesis. I take this to mean that other carbon sources are insignificant relative to the MEP pathway but an alternate view is discussed by Sanadze [1].

1. *The Pathway* The prevailing view at present is that glyceraldehyde 3-phosphate from the Benson-Calvin cycle and pyruvate imported into the chloroplast as phosphoenolpyruvate are combined in the first step of the MEP pathway and dimethyl allyl diphosphate, the immediate precursor of isoprene, is formed in the last step. However, the second carboxylase theory is still put forward [1] and has been covered recently in this journal [12].

The activity of an isoprene synthase was reported by Silver and Fall [13,14]. The enzyme was first cloned from poplar by Miller et al. [15] followed by cloning of the genomic version from kudzu, aspen, and additional poplars [16-18]. The enzyme is closely related to monoterpene synthases but shows significant diversity among plants, and it has been suggested that the trait of isoprene emission may have evolved many times, perhaps once in *Quercus*, once in *Populus*, once in the legumes and another time in eucalypts. The gene structure of monoterpene synthases and isoprene synthase in angiosperms (six introns and reasonable conservation of exon size and phasing) is not found in other organisms known to emit isoprene such as gymnosperms or ferns and so it is likely that isoprene emission in these plants also evolved independently [17].

2. *Control of the rate* The rate of isoprene emission is highly controlled. Questions that need answers are why is isoprene emission light-dependent, why does it respond so strongly to temperature, why does it decline at high CO_2, why does it vary through the day, why does it vary through a season, and why is it developmentally delayed, with little or no isoprene emission until leaves are fully expanded? For the last question, it is clear now that expression of the isoprene synthase gene is delayed until full leaf expansion, although the effect is temperature-dependent [19,20]. For most of the other questions, evidence is accumulating that the provision of substrate (DMADP) is the most important determinant of the rate of isoprene emission [21,22]. There is some debate about the mechanism by which elevated CO_2 causes the rate of isoprene emission to decline [12,22] and further research will be required to determine the relative importance of the various mechanisms proposed. The capacity for isoprene emission varies through the season and appears to depend on the weather, mostly temperature, of the previous few days [23-26]. Attempts to find explanations in gene expression of either isoprene synthase or enzymes catalyzing regulatory steps in the MEP pathway were disappointing [27]. Variation through a day was investigated and several possible regulatory genes show significant circadian oscillations in expression but the gene products do not [28].

The biochemical mechanism for the two most characteristic emission rate responses, temperature and light, are still unexplained. There are several possible reasons for lack of isoprene emission in darkness. Since one of the substrates comes from the Benson-Calvin cycle, a lack of Benson-Calvin cycle activity could limit isoprene emission. The MEP pathway uses a lot of ATP and so low ATP at night could limit isoprene emission. However, in both cases, it is expected that there should be homeostasis so that some GAP and ATP would be present at night (and this is generally found experimentally). However, the supply of reducing power could become limiting very rapidly. There are three steps in the MEP pathway that require reducing power. One of the steps occurs very near the end of the MEP pathway and uses ferredoxin (hydroxymethyl butenol reductase). Rasulov

et al. [21] assume that this step accounts for the light dependence of isoprene emission. If so, then isoprene made after immediately turning off the light could only come from preexisting DMADP, making post-illumination isoprene emission a measure of the DMADP pool available for isoprene synthesis [21].

The temperature dependence of the rate of isoprene emission is complex. When measurements are made slowly, isoprene emission can peak at 37.5°C but if they are made quickly isoprene emission continues to increase up to 40 or more °C [29]. Above 35°C the rate of isoprene emission can overshoot and show other complex kinetics [30] but the metabolic basis for these effects is not known. Significant work on the metabolism of isoprene synthesis is still required.

Why

To ask "why" in biology is to ask whether plants that emit isoprene are more fit in some way, and fitness normally means reproductive success. Photosynthesis is often taken to be a proxy for fitness, higher photosynthetic rate should allow greater reproductive success. On the other hand, a process may be needed to correct some other metabolic defect, for example the photorespiration pathway is required because of the oxygenase activity of Rubisco. It is also possible that a particular trait is simply an evolutionary relic or metabolic mistake. Four hypotheses can be identified in current literature to explain why plants emit isoprene.

1. Metabolic relief valve for releasing phosphate inadvertently stuck in DMADP [31].
2. Mechanism for dissipating energy [1].
3. Protection against ozone, singlet oxygen and other reactive oxygen species [32-34].
4. Thermoprotection [35,36].

Each of these hypotheses has been described but I favor thermoprotection because it is consistent with the observations of physiology such as the large temperature dependence at several levels and the light dependence. I list here my opinions about why thermoprotection is the best explanation of "why" plants emit isoprene and suggest interested readers consult the cited papers for arguments for each of the alternatives.

The metabolic relief valve hypothesis is that plants are unable to control the production of DMADP and so need to make and release isoprene to recover the phosphate in DMADP. Phosphate imbalance in chloroplasts

has been demonstrated. At high CO_2 and low temperature too little phosphate is released by starch and sucrose synthesis [37,38]. Isoprene emission is low under these conditions so it is ill suited as a phosphate release mechanism. In addition, the MEP pathway has been shown to be under very strict feedback control, limiting carbon entry into the pathway when the DMADP concentration is sufficient for the plant's needs [39]. The relief valve hypothesis is that DMADP production is part of a futile cycle in which energy is consumed with no net usefulness to the plant. Generally, plants avoid futile cycles and we should resort to postulating futile cycles only in the absence of other compelling possibilities.

The dissipation of energy can be considered at two levels. First, in very broad terms, living organisms operate by using energy gradients in nature. Use of energy gradients can allow the low entropy situation of living beings and still allow for generally increasing entropy of the universe. This argument is laid out by Sanadze [1]. However, while it is true that life in general requires dissipative mechanisms, individuals are favored by energy efficiency, since a more energy-efficient individual is likely to have more resources for reproduction than inefficient individuals. Another aspect of the energy dissipation argument is that leaves can be subjected to sunlight intensity greater than can be used in photosynthetic reactions. If isoprene were a mechanism for coping with this kind of stress, then it would be expected that isoprene emission would be suppressed at low light and increase only once photosynthesis was saturated with light. Instead, the rate of isoprene emission follows photosynthetic rate and generally saturates at about the same level as photosynthesis. In addition, the amount of energy that can be dissipated by isoprene synthesis is small relative to the amount that needs to be dissipated and the amount that is routinely dissipated by energy dependent quenching mechanisms in photosystem II. For example, consider a plant that does not emit isoprene in which photosynthesis saturates with light at 500 μmol m^{-2} s^{-1} of light. In full sunlight (=2000 μmol m^{-2} s^{-1}), 1500 μmol m^{-2} s^{-1} of light energy will be dissipated, primarily by the zeaxanthin- and energy-dependent quenching mechanisms. As long as some other stress is not present, plants generally can dissipate this much energy with no damage. Isoprene emission, at a high rate of 100 nmol m^{-2} s^{-1} and assuming as much as 20 μmol photons m^{-2} s^{-1} per carbon emitted (allowing for the extra reduction of isoprene relative to sugar and some cycling in photorespiration) would

dissipate 2 μmol m^{-2} s^{-1} compared to the 1500 μmol m^{-2} s^{-1} dissipated by energy dependent quenching. Therefore, while it is true that isoprene emission will dissipate energy, the amount of energy dissipated this way is so low as to be meaningless. Finally, photoinhibition caused by excess light is generally a problem at low temperature while isoprene emission is greatest at high temperature. So, once again, like the phosphate release story, the characteristics of isoprene emission are not consistent with the dissipation hypothesis.

Isoprene protects against reactive oxygen species. Ozone causes cell death and collapse and isoprene can prevent this. It is not clear why some plants would use this antioxidant mechanism while others do not, especially in light of the universal and substantial antioxidant systems in chloroplasts and mitochondria. Surprisingly, isoprene emission is reduced in plants exposed to ozone for extended periods [40,41]. The antioxidant properties of isoprene have been suggested as a universal mechanism of action of isoprene to explain both antioxidant properties and thermoprotection [42]. I shall suggest below that membrane stability is a better candidate for a universal mechanism of action, if such exists.

Thermotolerance, especially tolerance of heat spikes that can occur in leaves at the tops of trees exposed to sunlight, remains the most attractive hypothesis to me for "why" plants emit isoprene. It is clearly consistent with the strong temperature response, the light response and other responses such as the four-fold difference in isoprene emission capacity between the top and bottom of a tree [43]. It has been shown using exogenous isoprene with species that do not emit isoprene and it has been shown by suppressing isoprene emission using fosmidomycin [36]. Some have worried about the use of a poison like fosmidomycin. For this reason the proper control experiment was done in which isoprene emission was suppressed by fosmidomycin, then isoprene was added in the airstream and thermotolerance was restored. With this control, the fosmidomycin data are very strong support for the thermotolerance hypothesis. Thermotolerance has been found by many other groups now, often using transgenic plants either knocking down isoprene emission in poplars or adding isoprene emission to plants that normally do not make it [44,45]. Another kind of thermotolerance has been reported in which extended high temperature that leads to cellular collapse can be counteracted in transgenic Arabidopsis plants transformed with an isoprene synthase gene [46].

The protection against short high temperature episodes could be explained by increased membrane stability. Model calculations indicate that membranes can be stabilized at high temperature [47] by intercalation of isoprene and recent data indicate that membrane leakiness can be a problem in short high temperature experiments [48]. Can the cell death and collapse of extended heat stress and oxidative stress also be explained by membrane stability? I think it can. Programmed cell death occurs in animals, the best-known pathway being apoptosis. The critical event in apoptosis is the permeabilization of the mitochondrial membrane. This converts the mitochondrion from an oxidant sink to an oxidant source. There is a mutual reinforcement of membrane permeability and ROS leakage from the mitochondria and it is possible that *either* reducing the permeability or quenching the ROS will stop programmed cell death. Much less is known about programmed cell death in plants than in animals but this is an important area for future research.

THE FUTURE OF ISOPRENE RESEARCH

The future of isoprene research should see significant advances in understanding the mechanism of action of isoprene within plants. There are likely to be major improvements of our understanding of the regulation of DMADP synthesis. This will have implications for other areas of biology to the degree that new regulatory mechanisms are discovered in the MEP pathway. Because the MEP pathway is so recently discovered there is significant opportunity for discovery.

The enzyme responsible for isoprene synthesis in species other than among the flowering plants will significantly advance our understanding of the evolution of isoprene emission among land plants. The relationship between terpene synthases and isoprene synthase in these other lineages will help in understanding how isoprene emission can evolve independently so often. It is almost certain that very different isoprene synthases will be found, especially considering that isoprene is emitted from humans and bacteria, for which the entire gene sequence is known and which do not have any gene resembling the flowering-plant isoprene synthase.

In addition to the controversies above that will be solved by future research, there is another major direction of isoprene research. Isoprene has potential as a biofuel. This has significantly accelerated interest in isoprene synthase research as metabolic engineers race

to improve the enzyme for use in fermentative organisms. Ethanol has been used as a biofuel for many years, with the country of Brazil being a notable success story. However, ethanol has a lower energy density and must be distilled from the fermentation broth, requiring significant energy. Isoprene could be made instead and collected from the gas phase of the fermentor, eliminating the need for distillation. Isoprene has a higher energy density than ethanol and does not absorb water and so should be less corrosive when used in automobiles. Isoprene could be made from the same inputs currently used to make ethanol. Starch from *Zea mays* grain is currently a major starting point in the ethanol industry in the US and this is generally considered unsustainable. As alternate inputs are developed there is no reason that those inputs could not be converted to isoprene instead of ethanol. On the other hand, isoprene emission is closely associated with photosynthesis so photosynthetic organisms engineered to make high levels of isoprene can also be envisioned for the future.

It may be some time before isoprene production is economically competitive, but in the meantime, there is a high value market for isoprene for making rubber and other products. There is a very large market for isoprene for rubber manufacture and at present this isoprene comes from petroleum. Biologically derived isoprene could substitute for petroleum-derived isoprene. The company Genencor delivered the first biologically derived isoprene to Goodyear Rubber Company in 2009. Genecor used several isoprene synthase genes that have been cloned and donated to public databases during the course of isoprene physiology research [49] including those of kudzu [17], *Populus alba* [16] and hybrid poplar [18].

(www.biofuelsdigest.com/blog2/2009/10/26/genencor-50-hottest-companies-in-bioenergy-candidate-profile) (accessed October 26, 2009).

Another promising compound very closely related to isoprene is 2-methyl-3-buten-2-ol (MBO)(isoprene with water across one of the double bonds). This compound is made by a limited number of pine species from the western United States [50]. This compound has almost identical light and temperature responses as isoprene and is made from the same precursor [51-53]. Despite its very limited distribution, it may prove a very useful compound in biobased products and biofuels.

In summary, it has been 50 years since Sanadze's discovery of isoprene emission from plants. The first 30 years of research on isoprene involved a very limited number of investigators but laid the groundwork for our current understanding of isoprene emission from plants. The past 20 years has seen much more intensive research but a number of questions remain open. The next phase of isoprene research is likely to be even more intense as the commercial prospects for isoprene (and MBO) biological production are explored. There remain many very interesting unanswered questions for the future of isoprene research. This year we can look back and celebrate the tremendous achievement of the Academician Sanadze's discovery of isoprene emission from plants and look forward to the future of isoprene research.

ბიოქიმია, მოლეკულური ბიოლოგია

იზოპრენის კვლევების მომავალი

თომას დ. შარკი

ბიოქიმიის და მოლეკულური ბიოლოგიის დეპარტამენტი, მიჩიგანის სახელმწიფო უნივერსიტეტი, ისთ ლანსინგი, მიჩიგანი, 48824 აშშ

წელს აღინიშნება აკადემიკოს გივი სანაძის დაბადებიდან 80 წელი, რომელიც არის მცენარეებიდან იზოპრენის გამოყოფის აღმოჩენის ავტორი. ამ აღმოჩენის შემდეგ მის ლაბორატორიაში შესრულებულმა კვლევებმა საფუძველი დაუდო იზოპრენის გამოყოფის მრავალ საჭურადღებო ასპექტს. ყველაზე მნიშვნელოვანი არის: მცენარეთა მიერ იზოპრენის გამოყოფის მაღალი ტემპერატურული მგრძნობელობა, მისი დამოკიდებულება სინათლეზე და მჭიდრო კავშირი ფოტოსინთეზთან. სტატიაში მე ავდვწერ მთავარ შედეგებს, რომლებიც ჩვენ მივიღეთ ამ მოვლენის აღმოჩენიდან ორმოცი წლის განმავლობაში, თანამედროვე დისკუსიების შინაარსს, ჩემს შეხედულებებს მათ შესახებ და, რაც ყველაზე მნიშვნელოვანია, იზოპრენის კვლევის პერსპექტივებს.

REFERENCES

1. *Sanadze G.A.* Biogenic isoprene (A review) // Russian Journal of Plant Physiology, 51, 729-741, 2004.
2. *Rasmussen R.A., Went F.W.* Volatile organic material of plant origin in the atmosphere // Proceedings of the National Academy of Sciences of the United States of America, 53, 220, 1965.
3. *Rasmussen R.A.* Isoprene: identified as a forest-type emission to the atmosphere // Environmental Science and Technology, 4, 667-671, 1970.
4. *Monson R.K., Fall R.* Isoprene emission from Aspen leaves. The influence of environment and relation to photosynthesis and photorespiration // Plant Physiology, 90, 267-274, 1989.
5. *Loreto F., Sharkey T.D.* A gas-exchange study of photosynthesis and isoprene emission in *Quercus rubra* L // Planta, 182, 523-531, 1990.
6. *Sharkey T.D., Loreto F., Delwiche C.F.* The biochemistry of isoprene emission from leaves during photosynthesis. In: Trace Gas Emissions by Plants (Sharkey T.D., Holland E.A., Mooney H.A., editors. Academic Press, San Diego. pp. 1991.
7. *Sanadze G.A., Dzhaiani G.I., Tevzadze I.M.* Incorporation into the isoprene molecule of carbon from 13CO2 assimilated during photosynthesis // Soviet Plant Physiology, 19, 17-20, 1972.
8. *Sanadze G.A., Dzhaiani G.I., Baazov D.I., Khakhubiya G.T., Ebralidze S.S., Gvantseladze L.G.* Probability-statistical model of distribution of the carbon of 13CO2 in the isoprene molecule during photosynthesis // Soviet Plant Physiology, 23, 580-586, 1976.
9. *Delwiche C.F., Sharkey T.D.* Rapid appearance of 13 C in biogenic isoprene when 13 CO 2 is fed to intact leaves // Plant, Cell and Environment, 16, 587-591, 1993.
10. *Rodríguez-Concepción M., Boronat A.* Elucidation of the methylerythritol phosphate pathway for isoprenoid biosynthesis in bacteria and plastids. A metabolic milestone achieved through genomics // Plant Physiology, 130, 1079-1089, 2002.
11. *Zeidler J.G., Lichtenthaler H.K., May H.U., Lichtenthaler F.W.* Is isoprene emitted by plants synthesized via the novel isopentenyl pyrophosphate pathway? // Zeitschrift für Naturforschung C, 52, 15-23, 1997.
12. *Monson R.K., Wilkinson M.J., Monson N.D., Trahan N., Lee S., Rosenstiel T.R., Fall R.* Biochemical controls on the CO_2 response of leaf isoprene emission: An alternative view of Sanadze's double carboxylation scheme // Annals of Agarian Science, 7, 21-29, 2009.

13. *Silver G.M., Fall R.* Enzymatic synthesis of isoprene from dimethylallyl diphosphate in aspen leaf extracts // Plant Physiology, 97, 1588-1591, 1991.

14. *Silver G.M., Fall R.* Characterization of aspen isoprene synthase, an enzyme responsible for leaf isoprene emission to the atmosphere // Journal of Biological Chemistry, 270, 13010-13016, 1995.

15. *Miller B., Oschinski C., Zimmer W.* First isolation of an isoprene synthase gene from poplar and successful expression of the gene in Escherichia coli // Planta, 213, 483-487, 2001.

16. *Sasaki K., Ohara K., Yazaki K.* Gene expression and characterization of isoprene synthase from Populus alba // FEBS Letters, 579, 2514-2518, 2005.

17. *Sharkey T.D., Yeh S., Wiberley A.E., Falbel T.G, Gong D., Fernandez D.E.* Evolution of the isoprene biosynthetic pathway in kudzu // Plant Physiology, 137, 700-712, 2005.

18. *Schnitzler J.P., Zimmer I., Bachl A., Arend M., Fromm J., Fischbach R.* Biochemical properties of isoprene synthase in poplar (Populus   canescens) // Planta, 222, 777-786, 2005.

19. *Wiberley A.E., Linskey A.R., Falbel T.G, Sharkey T.D.* Development of the capacity for isoprene emission in kudzu // Plant, Cell and Environment, 28, 898-905, 2005.

20. *Monson R.K., Harley P.C., Litvak M.E., Wildermuth M., Guenther A.B., Zimmerman P.R., Fall R.* Environmental and developmental controls over the seasonal pattern of isoprene emission from aspen leaves // Oecologia, 99, 260-270, 1994.

21. *Rasulov B., Copolovici L., Laisk A., Niinemets Ü.* Postillumination isoprene emission: in vivo measurements of dimethylallyldiphosphate pool size and isoprene synthase kinetics in aspenl eaves // Plant Physiology, 149, 1609-1618, 2009.

22. *Rasulov B., Huve K., Valbe M., Laisk A., Niinemets U.* Evidence that light, carbon dioxide, and oxygen dependencies of leaf isoprene emission are driven by energy status in hybrid aspen // Plant Physiology, 151, 448-460, 2009.

23. *Pétron G., Harley P., Greenberg J., Guenther A.* Seasonal temperature variations influence isoprene emission // Atmospheric Environment, 28, 1707-1710, 2001.

24. *Sharkey T.D., Singsaas E.L., Lerdau M.T., Geron C.* Weather effects on isoprene emission capacity and applications in emissions algorithms // Ecological Applications, 9, 1132-1137, 1999.

25. *Fuentes J.D., Wang D.* On the seasonality of isoprene emission from a mixed temperate forest // Ecological Applications, 9, 1118-1131, 1999.

26. *Fuentes J.D., Wang D., Gu L.* Seasonal variations in isoprene emissions from a boreal aspen forest // Journal of Applied Meteorology, 38, 855-869, 1999.

27. *Wiberley A.E., Donohue A.R., Meier M.E., Sharkey T.D.* Regulation of isoprene emission in *Populus trichocarpa* leaves subjected to changing growth temperature // Plant, Cell & Environment, 31, 258-267, 2008.

28. *Wiberley A.E., Donohue A.R., Westphal M.M., Sharkey T.D.* Regulation of isoprene emission from poplar leaves throughout a day // Plant, Cell & Environment, 32, 939-947, 2009.

29. *Singsaas E.L., Sharkey T.D.* The effects of high temperature on isoprene synthesis in oak leaves // Plant, Cell and Environment, 23, 751-757, 2000.

30. *Singsaas E.L., Sharkey T.D.* The regulation of isoprene emission responses to rapid leaf temperature fluctuations // Plant, Cell and Environment, 21, 1181-1188, 1998.

31. *Rosenstiel T.N., Ebbets A.L., Khatri W.C., Fall R., Monson R.K.* Induction of poplar leaf nitrate reductase: a test of extrachloroplastic control of isoprene emission rate // Plant Biology, 6, 12-21, 2004.

32. *Loreto F., Mannozzi M., Maris C., Nascetti P., Ferranti F., Pasqualini S.* Ozone quenching properties of isoprene and its antioxidant role in leaves // Plant Physiology, 126, 993-1000, 2001.

33. *Loreto F., Velikova V.* Isoprene produced by leaves protects the photosynthetic apparatus against ozone damage, quenches ozone products, and reduces lipid peroxidation of cellular membranes // Plant Physiology, 127, 1781-1787, 2001.

34. *Affek H.P., Yakir D.* Protection by isoprene against singlet oxygen in leaves // Plant Physiology, 129, 269-277, 2002.

35. *Sharkey T.D., Singsaas E.L.* Why plants emit isoprene // Nature, 374, 769-769, 1995.

36. *Sharkey T.D., Chen X.Y., Yeh S.* Isoprene increases thermotolerance of fosmidomycin-fed leaves // Plant Physiology, 125, 2001-2006, 2001.

37. *Sharkey T.D.* Photosynthesis in intact leaves of C 3 plants: Physics, physiology and rate limitations // The Botanical Review, 51, 53-105, 1985.

38. *Sharkey T.D., Vanderveer P.J.* Stromal phosphate concentration is low during feedback limited photosynthesis // Plant Physiology, 91, 679-684, 1989.

39. *Wolfertz M., Sharkey T.D., Boland W., Kühnemann F.* Rapid regulation of the methylerythritol 4-phosphate pathway during isoprene synthesis // Plant Physiology, 135, 1939-1945, 2004.

40. *Calfapietra C., Wiberley A.E., Falbel T.G, Linskey A.R., Mugnozza G.S., Karnosky D.F., Loreto F., Sharkey T.D.* Isoprene synthase expression and protein levels are reduced under elevated O_3 but not under elevated CO_2 (FACE) in field-grown aspen trees // Plant, Cell & Environment, 30, 654-661, 2007.

41. *Calfapietra C., Mugnozza G.S., Karnosky D.F., Loreto F., Sharkey T.D.* Isoprene emission rates under elevated CO_2 and O_2 in two field-grown aspen clones differing in their sensitivity to O_3 // New Phytologist, 179, 55-61, 2008.

42. *Vickers C.E., Gershenzon J., Lerdau M.T., Loreto F.* A unified mechanism of action for volatile isoprenoids in plant abiotic stress // Nature Chemical Biology, 5, 283-291, 2009.

43. *Sharkey T.D., Singsaas E.L., Vanderveer P.J., Geron C.D.* Field measurements of isoprene emission from trees in response to temperature and light // Tree Physiology, 16, 649-654, 1996.

44. *Vickers C.E., Possell M., Cojocariu C.I., Velikova V.B., Laothawornkitkul J., Ryan A., Mullineaux P.M., Hewitt C.N.* Isoprene synthesis protects transgenic tobacco plants from oxidative stress // Plant, Cell & Environment, 32, 520-531, 2009.

45. *Behnke K., Ehlting B., Teuber M., Bauerfeind M., Louis S., Hänsch R., Polle A., Bohlmann J., Schnitzler J.P.* Transgenic, non-isoprene emitting poplars don't like it hot // The Plant Journal, 51, 485-499, 2007.

46. *Sasaki K., Saito T., Lamsa M., Oksman-Caldentey K.M., Suzuki M., Ohyama K., Muranaka T., Ohara K., Yazaki K.* Plants utilize isoprene emission as a thermotolerance mechanism // Plant and Cell Physiology, 48, 1254-1262, 2007.

47. *Siwko M.E., Marrink S.J., de Vries A.H., Kozubek A., Schoot Uiterkamp A.J.M., Mark A.E.* Does isoprene protect plant membranes from thermal shock? A molecular dynamics study // Biochimica et Biophysica Acta - Biomembranes, 1768, 198-206, 2007.

48. *Zhang R., Cruz J.A., Kramer D.M., Magallanes-Lundback M.E., DellaPenna D., Sharkey T.D.* Moderate heat stress reduces the pH component of the transthylakoid proton motive force in light-adapted intact tobacco leaves // Plant, Cell & Environment, 32, 1538-1547, 2009.

49. *Cervin M.A., Whited G.M., Chotani G.K., Valle F., Fioresi C., Sanford K.J., Mcauliffe J.C., Feher F.J., Puhala A.S., et al.* Conditions and methods for producing isoprene. US2009/0203102 A1. United States, 2009.

50. *Schade G.W., Goldstein A.H., Gray D.W., Lerdau M.T.* Canopy and leaf level 2-methyl-3-buten-2-ol fluxes from a ponderosa pine plantation // Atmospheric Environment, 34, 3535-3544, 2000.

51. *Gray D.W., Lerdau M.T., Goldstein A.H.* Influences of temperature history, water stress, and needle age on methylbutenol emissions // Ecology, 84, 765-776, 2003.

52. *Gray D.W., Goldstein A.H., Lerdau M.T.* The influence of light environment on photosynthesis and basal methylbutenol emission from *Pinus ponderosa* // Plant, Cell & Environment, 28, 1463-1474, 2005.

53. *Gray D.W., Goldstein A.H., Lerdau M.T.* Thermal history regulates methylbutenol basal emission rate in *Pinus ponderosa* // Plant, Cell & Environment, 29, 1298-1308, 2006.