1 Fries, N., Trans. Br. mycol. Soc. *88* (1987) 1.
2 Fries, N., Serck-Hanssen, K., Dimberg, L. H., and Theander, O., Exp. Mycol. *11* (1987) 360.
3 Sjöholm, R. T., Acta Acad. åbo. Ser. B. *37* (1977) 1.
4 Bjurman, J., and Fries, N., Physiol. Pl. *62* (1984) 465.
5 Enzell, C., and Theander, O., Acta chem. scand. *16* (1962) 607.
6 Moser, M., Die Röhrlinge und Blätterpilze, 4.th ed., in: Kleine Kryptogamenflora, vol 2 B/2. G. Fischer Verlag, Stuttgart, New York 1978.
7 Miller, O. K. Jr, Mushrooms of North America. Dutton and Co. Inc, New York 1977.
8 Fries, N., Proc. Indian. Acad. Sci., Plant Sci. *93* (1984) 205.
9 Fries, N., Mycotaxon *18* (1983) 345.
10 Fries, N., and Birraux, D., Experientia *36* (1980) 1056.
11 Birraux, D., and Fries, N., Can. J. Bot. *59* (1981) 2062.

0014-4754/88/11-12/1027-04$1.50 + 0.20/0
© Birkhäuser Verlag Basel, 1988

Flux of singlet oxygen from leaves of phototoxic plants

M. R. Berenbaum and R. A. Larson[a]

Department of Entomology and [a]Institute for Environmental Studies, University of Illinois, Urbana (Illinois 61801, USA)
Received 9 June 1988; accepted 10 August 1988

Summary. Detached leaves of *Zanthoxylum americanum* and *Pastinaca sativa*, plants known to produce phototoxins, generate singlet oxygen when illuminated by a xenon arc lamp that simulates sunlight. Other species tested did not produce detectable amounts of singlet oxygen. Calculations of the rate of production of singlet oxygen indicate a flux of up to 4×10^{12} molecules $cm^{-1}\,s^{-1}$. This level is sufficiently high to induce damage in the cells of organisms near the leaf surface. Photodynamic action may thus provide for plants an evolutionary advantage in the form of preemptive protection against predators without tissue loss.
Key words. Phototoxicity; singlet oxygen; *Pastinaca sativa; Zanthoxylum americanum;* plant defense.

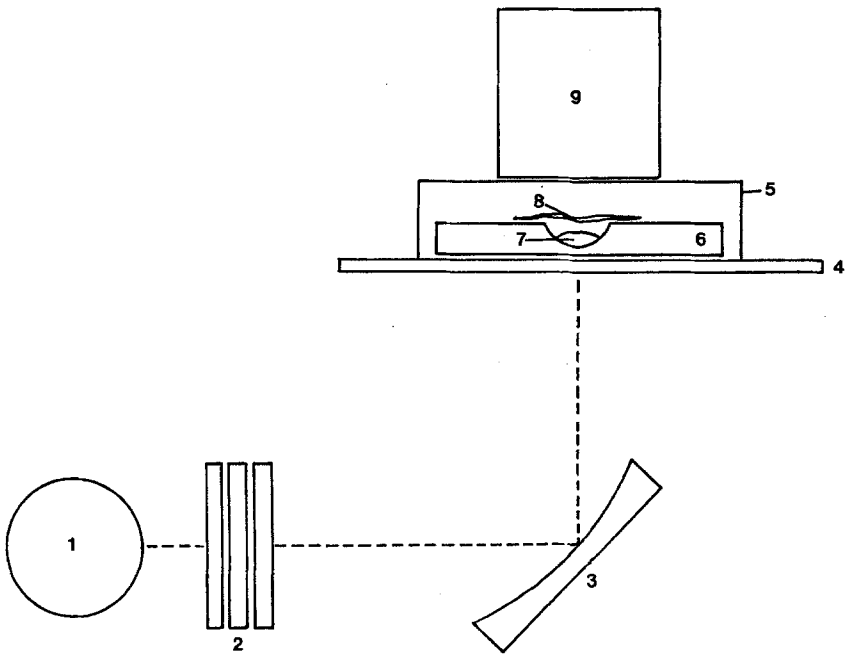
At least eight distinct classes of plant chemicals are photoactive, that is, capable of absorbing sunlight energy to increase their toxicity to living organisms[1]. The efficacy of these photoxins against a diverse array of plant pathogens and herbivores has given rise to the suggestion that these chemicals function in defending plants against potential enemies[2–4]. Many photosensitizing plant chemicals are present primarily in epidermal tissues[5] and thus are in close contact with the atmosphere, making feasible energy transfer from their photochemically excited states to atmospheric oxygen to form singlet oxygen. Singlet oxygen is reactive toward some constituents of DNA, cell membranes, enzymes, and other essential biomolecules[6,7]; because of its long lifetime in the gas phase (roughly 1000 times greater than in the liquid phase), it can diffuse through air for distances of several millimeters to react with substrates in solution[8]. Singlet oxygen generated at a leaf surface could potentially persist long enough to interact with invaders of the phylloplane such as fungal spores, bacteria, yeasts, and plant-feeding arthropods and their eggs. In this study, we document for the first time the presence of singlet oxygen at distances 1–2 mm from the surfaces of leaves of some phototoxic plants and its apparent absence on or near the surface of nonphototoxic plant leaves.

A modification of a previously reported method[8] was used to generate singlet oxygen in the gas phase; an intact plant leaf was secured over the well of a microscope slide containing 100–200 µl of a 2×10^{-4} M aqueous solution of furfuryl alcohol (FFA), a reactive acceptor of singlet oxygen, such that its surface was 1–2 mm above the droplet. The leaf was illuminated from beneath using a mirror to reflect the beam of a 100 W xenon arc lamp (filtered through water, pyrex, and cellulose acetate filters to remove infrared and nonsolar UV wavelengths) up through the underside of the glass slide (fig.). The UV (320–400 nm) intensity on the slide was approximately 300–400 $\mu W/cm^2/min$; while this intensity is relatively low for solar exposure (although typical of overcast or winter conditions), evaporation of the droplet due to increasing temperatures became a problem at higher lamp outputs. At timed intervals, 20 µl samples of FFA were taken and quantified by high-pressure liquid chromatography[9]. Benzyl alcohol (1.8 mM) was used as an internal standard. Initial observations of plants were made after 15 min of illumination. Five species were tested (table 1); of these species, only *Pastinaca sativa* (wild parsnip) and *Zanthoxylum americanum* (prickly ash) showed a decline in FFA concentration of 10% or greater. These results are consistent with the chemistry of these plants; wild parsnip foliage contains high levels of phototoxic furanocoumarins[10] and prickly ash leaves contain furanocoumarins as well as the phototoxic furanoquinoline and β-carboline alkaloids[11,12]. In solution, many of these compounds are known to produce singlet oxygen[13,14]. Although furanocoumarins are reported to occur in *Citrus sinensis*[12], no loss of FFA was observed in experiments with its leaves.

Three species were examined further for time intervals of 45–80 min. From 4–6 determinations were made for each sample. Some loss of FFA relative to the benzyl alcohol standard occurs over the assay interval (table 2); therefore, only regressions with slopes significantly greater than that of the control (0.0009) were considered meaningful. Slopes of FFA loss against time in minutes were significant and negative for both wild parsnip (slope = −0.007, r = 0.92) and prickly ash (slope = −0.002, r = 0.99). A second experiment with a different prickly ash leaf gave a steeper slope (−0.008, r = 0.90) which due to high variance was marginally nonsignificant (p = 0.096). To test whether factors other than singlet oxygen (e.g., plant volatiles) were causing FFA depletion, well slides were set up with wild parsnip leaves as before except that the xenon lamp was left off for the 80 min

Table 1. Changes in peak height of furfuryl alcohol after 15 min of exposure to xenon arc lamp (330–400 $\mu W/cm^2/min$)

Plant species	Peak height relative to time zero (avg. of 2 replicates with SD)
Pastinaca sativa	0.915 (0.007)
Zanthoxylum americanum	0.795 (0.077)
Liquidambar styracifolium	1.040 (0.042)
Ailanthus altissima	1.060
Citrus sinensis	1.090
Glass slide only	1.005 (0.077)



Apparatus for illumination of leaves. 1, xenon lamp; 2, filters (water, Pyrex, cellulose acetate); 3, mirror; 4, Pyrex plate; 5, Petri dish; 6, well slide; 7, test solution; 8, leaf; 9, ice-filled beaker.

Table 2. Regressions between peak height of furfuryl alcohol and length of exposure time to UVA and leaf surfaces

Treatment	*r*	*slope*	*p*
Slopes significantly different from zero			
Pastinaca sativa	0.92	−0.007	0.030
Zanthoxylum americanum	0.99	−0.002	0.006
FFA/benzyl alc.	0.96	−0.0009	0.002
Slopes not significantly different from zero			
Citrus sinensis	0.56	<0.001	0.20
	0.56	0.001	0.44
Pastinaca sativa (abaxial surface)	0.45	>−0.001	0.55
	0.65	>−0.001	0.35
Pastinaca sativa (no light)	0.13	>−0.001	0.86
	0.59	>−0.001	0.22

sampling period. For these leaves, no detectable depletion of FFA occurred (two replicates: $r = 0.59$, $p = 0.22$; $r = 0.13$, $p = 0.86$,), suggesting that singlet oxygen was indeed responsible for the observed disappearance of FFA.

When the abaxial (lower) parsnip leaf surface was positioned over the FFA and illuminated, no depletion was observed (two replicates: $r = 0.45$, $p = 0.55$; $r = 0.65$, $p = 0.35$,). It is possible, since furanocoumarin synthesis is induced by light[10], that these compounds tend to accumulate in the upper surfaces of the leaf over time; singlet oxygen production would accordingly be greater on the upper surface.

Assuming first-order kinetics, the half-life of FFA can be calculated as 41 min and 54 min (2 replicates) for prickly ash and as 154 and 231 min for wild parsnip (2 replicates). To calculate the flux of singlet oxygen from the surface of the leaf, equations given by Haag and Hoigné[9] and Midden and Wang[8] were used to establish that, for the case of *Zanthoxylum*, assuming a leaf thickness of 0.1 mm, approximately 4×10^{12} singlet oxygen molecules/cm^2/s were evolved. This value is comparable to the surface flux (5×10^{12} molecules/cm^2/s) measured for the synthetic dye, rose bengal, immobilized on silica gel[15].

Due to the appreciably longer lifetime of singlet oxygen in air versus that in aqueous or organic solutions, its steady-state concentration near illuminated surfaces of phototoxic plants may be considerably higher than inside plant tissues. Singlet oxygen production by photosensitizers in the interior of plant tissues is likely to be as hazardous to the plant itself as it is to potential plant enemies; thus, plants possess multiple protective systems for quenching singlet oxygen, including carotenoid pigments, α-tocopherol, and ascorbic acid[16]. From the perspective of plant defense against herbivores and pathogens, however, these protective substances presumably reduce the efficacy of phototoxins when they are ingested or contacted. In contrast, production of singlet oxygen at leaf surfaces ensures transfer of a toxicant to potential enemies without the intervention of protective quenchers and, moreover, can effect toxicity without rupture or damage of leaf tissue. The photoovicidal effects of several plant chemicals[17,18] may be attributable at least in part to cell-damaging effects of gas-phase singlet oxygen[19–21]; preventing egg hatch would be another way to reduce herbivore populations without sacrificing leaf tissue. This preemptive protection against predation could conceivably confer a selective advantage over plants which must suffer tissue damage in order to deter enemies and may account for the widespread occurrence of biosynthetically distinct phototoxins among vascular plant families[22].

Acknowledgments. This work was partly supported by a U.S. Department of Agriculture competitive research grant (# 87CRCR12374) and by a Guggenheim Foundation fellowship (to M.R.B.). We thank Walter Eisenberg and Karen Marley for discussions and assistance.

1 Downum, K. R., and E. Rodriguez, J. chem. Ecol. *12* (1986) 823.
2 Berenbaum, M. R., Science *201* (1978) 532.
3 Champagne, D. E., Arnason, J. T., Philogène, B. J. R., Moran, P., and Lam, J., J. chem. Ecol. *12* (1986) 835.
4 Ashkenazy, D., Kashman, Y., Nyska, A., and Friedman, J., J. chem. Ecol. *11* (1985) 231.
5 Downum, K. R., and Nemec, S., Light Activated Pesticides, Eds J. Heitz and K. R. Downum. Am. chem. Soc. Sympos. Ser. *339* (1987) 281.

6 Larson, R. A., CRC Crit. Rev envir. Control *8* (1978) 197.
7 Spikes, J. D., and Straight, R. C., in: Light Activated Pesticides, Eds J. Heitz and K. R. Downum. Am. chem. Soc. Sympos. Ser. *339* (1987) 98.
8 Midden, W. R., and Wang, S. Y., J. Am. chem. Soc. *109* (1983) 4129.
9 Haag, W. R., and Hoigné, J., Chemosphere *13* (1984) 631.
10 Zangerl, A. R., and Berenbaum, M. R., Ecology *68* (1987) 516.
11 Waterman, P. G., Biochem. systemat. Ecol. *3* (1972) 149.
12 Murray, R. D. H., Mendez, J., and Brown, S. A., The Natural Coumarins. Wiley, New York 1982.
13 Poppe, W., and Grossweiner, L., Photochem. Photobiol., *22* (1975) 217.
14 Chae, K. H., and Ham, H. S., Bull. Korean chem. Soc. *7* (1986) 478.
15 Krishna, C. M., Lion, Y., and Riesz, P., Photochem. Photobiol. *45* (1987) 1.
16 Knox, J. P., and Dodge, A. D., Phytochemistry *24* (1985) 889.
17 Kagan, J., and Chan, G., Experientia *39* (1983) 402.
18 Kagan, J., Kolyvas, C. P., and Lam, J., Experientia *40* (1984) 1396.
19 Eisenberg, W. C., Taylor, K., and Schiff, L. J., Experientia *40* (1984) 514.
20 Schiff, L. J., Eisenberg, W. C., and Taylor, K., Mutat. Res. *142* (1985) 41.
21 Dahl, T. A., Midden, W. R., and Hartmann, P. E., Photochem. Photobiol. *46* (1987) 345.
22 Towers, G. H. N., and Champagne, D. E., in: Light Activated Pesticides, Eds J. Heitz and K. R. Downum. Am. chem. Soc. Sympos. Ser. *339* (1987) 231.

0014-4754/88/11-12/1030-03$1.50 + 0.20/0
© Birkhäuser Verlag Basel, 1988

Instructions to Authors

Experientia is a monthly journal for life sciences devoted to publishing articles which are interdisciplinary in character and which are of general scientific interest. Considered for publication will be hitherto unpublished papers that fall within the following categories:

Reviews (one-man and multi-author reviews)
Mini-reviews (1–2 printed pages)
Full Papers
Short Communications (1–2 printed pages, published within 3 months of acceptance)

Papers reporting on work that is preliminary in nature, or wherein animal experiments have been conducted without the appropriate anesthesia, will not be accepted.
Manuscripts (including all tables and figures) must be submitted in *triplicate* and must be in *English*. *Title pages* should bear the author's name and address (placed directly below the title), a brief *abstract* (of approximately 50 words for short communications) mentioning new results only, and a listing of *key words*. *Footnotes* must be avoided. *Tables*, and then *figures*, are to follow the body of the text and should be marked with self-explanatory captions and be identified with the author's name. All *data* should be expressed in units conforming to the Système International (SI). *Drawings* are to be on heavy bond paper and marked clearly in black. *Photographs* should be supplied as glossy positive prints. Please note that we use two different systems for citing references. 1. For *Review Articles*, references should be arranged alphabetically and be numbered. Within the text, literature should be referred to by number and, where indicated, by author. The references should contain full journal article titles and the first as well as the last page of the article cited. 2. For *Short Communications*, *Full Papers* and *Mini-reviews*, an abbreviated bibliography is requested and references should be listed chronologically. Please consult a current issue of Experientia or inquire at the editorial office for details on form.

Authors are requested to specify into which discipline their papers fall:

1. Anatomy, Physiology
2. Biochemistry and Biophysics
   Metabolism
   Neurobiology
   Pharmacology
3. Endocrinology
4. Cellular Biology
   Molecular Biology
   Immunology
5. Genetics, Developmental Biology
6. Ethology, Ecology
   Natural Product Chemistry

All incoming manuscripts are acknowledged immediately. Authors will be notified of the editorial board's publishing decision once their manuscripts have been evaluated by a minimum of two field experts. Fifty reprints of papers accepted for publication will be sent to authors free of charge; additional reprints may be ordered.
Manuscripts and all communications to the editors should be addressed to:

Experientia
Birkhäuser Verlag
P.O. Box 133
CH-4010 Basel/Switzerland
Tel. 061 73 53 00