Table 2. Effect of night interruptions on senescence of debudded *Xanthium* plants. 9H, Inductive photoperiod consisting of 9 hours of light followed by 15 hours of darkness; 9I, 9-hour-interrupted noninductive photoperiod.

Photoperiod	Mean days to death*	Range in days to death
9H	57.3	56 to 61
9I	80.5	70 to 107

* Average number of days from start of photoperiodic treatment to death of the plant. Ten plants per treatment.

served in intact fruiting plants (that is, dehydration of the upper portion of the shoot and severe petiole epinasty at the time of fruit ripening, and basipetal pattern of necrosis of the entire shoot following bur maturation) even though they completely lacked the capacity to produce reproductive structures. Both intact and debudded plants were dead after 9 to 10 weeks of short-day treatment. Debudded plants that were kept under long-day conditions, however, took nearly twice as long to die (13 to 17 weeks); intact long-day plants were still alive and vegetative at this time.

To find out whether the time of death of the plant was determined by time of debudding or actual photoperiodic treatment, a second experiment was conducted in which the time of debudding was varied. Three sets of 42-day-old plants were placed under differential photoperiodic treatment (9H and 20H); one set was not debudded, another was debudded at the initiation of differential photoperiodic treatment, and the third set was debudded 16 days later. As before, plants given continuous photoinductive treatment (9H) died considerably sooner than those kept under continuous nonphotoinductive conditions (20H), regardless of the time of debudding (Table 1). In debudded plants receiving short-day treatment (9H), the mean number of days to death was nearly identical even though one group was debudded 16 days after the other. These results show that whether or not there was a difference in the production of a floral stimulus in plants debudded before and after the beginning of photoinductive treatment (*11*), there was no significant difference between treatments in the number of days to death. The fact that time of debudding had no effect on time of death of the photoinduced plants suggests that the metabolic consequences of photoperiodic induction were of greater importance in determining longevity in these plants than were the changes created by the removal of terminal and lateral buds. The time of death of plants exposed to long-day conditions, however, was influenced greatly by debudding and time of debudding. It is, therefore, quite apparent that bud removal had a significant effect on the longevity of these plants.

In order to establish whether the difference in time of death observed between debudded plants grown under a 20-hour photoperiod and those exposed to a 9-hour photoperiod was caused by a difference in total light energy (that is, total hours of illumination), an experiment was conducted in which long-day conditions were mimicked by using a 9-hour-interrupted (9I) noninductive photoperiod. This consisted of 8 hours of natural light plus a 1-hour light interruption midway through the dark period, since this is also the most effective time for preventing floral induction in *Xanthium* (*12, 13*). In both treatments the plants received a total of 9 hours of illumination (of equal energy value). Although most studies on flowering in *Xanthium* have utilized 16- to 20-hour photoperiods as control conditions (*13*), the advantage of minimizing possible differences in aging caused by differences of photosynthetic origin by using short-day and long-day conditions of the same duration seems obvious. As in previous experiments debudded plants exposed to long-day conditions (9I) died later than those given short-day treatment (9H) (Table 2).

More work must be carried out before the exact role of photoperiodic induction in plant senescence is established, but it is clear from these findings that the development of flowers and fruits is not the primary cause of death in certain short-day annuals. The feasibility of utilizing debudded plants and night-break conditions to investigate problems of aging in photoperiodically sensitive plants has been established, and this should allow a complete reexamination of the role of mobilization forces in plant senescence.

DONALD T. KRIZEK
WAYNE J. MCILRATH*
BENITO S. VERGARA†
Department of Botany, University of Chicago, Chicago, Illinois 60637

References and Notes

1. H. Molisch, *The Longevity of plants* (transl. and published by E. H. Fulling, New York, 1938); A. C. Leopold, *Plant Growth and Development* (McGraw-Hill, New York, 1964); E. Wangermann, *Handb. Pflanzenphysiol.* **15** (pt. 2), 1026 (1964).
2. S. Austin, *Science* **78**, 363 (1933).
3. J. A. Lockhart and V. Gottschall, *Plant Physiol.* **36**, 389 (1961).
4. O. V. S. Heath, *Symp. Inst. Biol.* **6** (1957), p. 9.
5. K. Mothes and L. Engelbrecht, *Flora Jena* **139**, 1 (1952).
6. A. C. Leopold, *Science* **134**, 1727 (1961); ———, E. Neidergang-Kamien, J. Janick, *Plant Physiol.* **34**, 570 (1959); J. Janick and A. C. Leopold, *Nature* **192**, 887 (1961).
7. C. E. Olmsted, *Bot. Gaz.* **112**, 266 (1951).
8. P. F. Wareing, *Physiol. Plant.* **7**, 261 (1954); I. D. J. Phillips and P. F. Wareing, *J. Expt. Bot.* **10**, 504 (1959); C. F. Eagles and P. F. Wareing, *Physiol. Plant.* **17**, 697 (1964).
9. D. J. Osborne, *Discovery* **24**, 31 (1963); D. J. Osborne and M. Hallaway, *New Phytol.* **63**, 334 (1964).
10. B. S. Vergara and W. J. McIlrath, *Bot. Gaz.* **122**, 96 (1960).
11. D. J. Carr, *Physiol. Plant.* **6**, 680 (1953); R. G. Lincoln, K. A. Raven, K. C. Hamner, *Bot. Gaz.* **119**, 179 (1958).
12. F. B. Salisbury and J. Bonner, *Plant Physiol.* **31**, 141 (1956).
13. F. B. Salisbury, *The Flowering Process* (Pergamon Press, New York, 1963).
14. Supported in part by grants to W.J.M. from NSF (G-4018 and G-21412) and from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago. D.T.K. gratefully acknowledges a NSF predoctoral fellowship.
* Present address: Graduate School, Northern Illinois University, DeKalb 60115.
† Present address: International Rice Research Institute, Los Baños, Laguna, Phillipines.

11 October 1965

Myristicin in Cigarette Smoke

Abstract. *The pharmacologically active aromatic ether, myristicin, was isolated from the smoke of commercial cigarettes. The compound was identified by spectrometry (infrared, ultraviolet, and mass) and gas chromatography. The amount of myristicin in smoke is relatively low, and its contribution, if any, to the physiological action of cigarette smoke is unknown.*

The presence of myristicin (5-allyl-2, 3-methylenedioxyphenyl methyl ether) in cigarette smoke has been demonstrated (*1*). This compound has biological activity and is believed to be responsible, at least in part, for the narcotic effect of nutmeg oil (*2*).

Myristicin was found in the fraction of the neutral substances soluble in nitromethane. This fraction was obtained from 1 kg of smoke condensate (equivalent to 50,000 cigarettes) by the separation procedure described (*3*). Chromatography on silicic acid of the components of the nitromethane soluble fraction yielded a fraction eluting with a mixture of *n*-hexane and benzene (1:1) which had an infrared

spectrum showing aromatic, hydroxyl (or amino), and carbonyl groups. This fraction, on alumina, was resolved further; a subfraction was eluted with a mixture of petroleum ether and benzene (1 : 1) which, on gas chromatographic analysis (2.4 m by 6.4 mm column of 20 percent SE-30 on Chromosorb W, 180°C for 15 minutes, and programmed at 8°C per minute to 265°C), showed three peaks emerging at 13, 26, and 34 minutes, respectively.

The infrared spectrum of the component eluting at 13 minutes was suggestive of an aromatic ether. Absorption at 9.57 μ indicated an ether group, and peaks at 3.45, 10.69, and 13.85 μ revealed a possible methylenedioxy-substituted benzene ring (*4*). Strong absorption at 6.12 μ showed *exo* unsaturation which was substantiated by bands at 10.08 and 10.92 μ (vinyl group). Aromatic absorption was apparent at 12.09 μ (one adjacent free hydrogen on the aromatic ring) and in the usual range, 3.25 to 3.40 μ. The absence of absorption at 6.29 μ indicated the lack of conjugation of the vinyl group with the aromatic ring. The ultraviolet spectrum confirmed the presence of aromaticity (broad absorption at 260 to 295 mμ). The mass spectrum showed a parent peak at 192 and major fragment peaks at 161 (loss of -OCH_3) and 165 (substituted tropylium ion). Comparison of all spectra with those of authentic myristicin (*5*) showed identical characteristics except for two extraneous peaks (mass/charge 153, 194) in the mass spectrum of the unknown; these peaks were probably derived from a minor contaminant structurally related (for example allyl 2,6-dimethoxyphenyl ether) or dissimilar (as a substituted indole) to myristicin. Indoles and carbazoles have been isolated from the fraction containing myristicin in the original column chromatography. Similar retention times were obtained with the isolated substance and authentic myristicin on 20 percent Apiezon L on Chromosorb W (2.4 m by 6.4 mm column operated at 275°C; retention time, 7 minutes). In addition, co-chromatography of the isolated substance and authentic myristicin on SE-30 gave a single peak. The level of myristicin in cigarette smoke is at least 0.64 μg per cigarette based on the observed recovery, which was undoubtedly not quantitative.

The nitromethane-soluble fraction of smoke is of special interest since it contains the major carcinogenic polynuclear aromatic hydrocarbons of smoke and has significant physiological activity. In addition to the carcinogens this fraction contains a number of other aromatic compounds, such as benzyl benzoate and benzyl cinnamate; certain heterocyclic aromatic compounds (indoles, carbazoles, etc.) have also been demonstrated (*6*). However, myristicin appears to be unlike these recently isolated compounds in that some distinct pharmacological activity has been attributed to it. Although controversy exists as to whether the physiological effects of nutmeg oil (nauseau, tachycardia, cyanosis, stupor, and others) are due exclusively to myristicin (*2*), it appears safe to conclude, on the basis of available biological data (*2*), that myristicin has some degree of toxicity and produces some neurological effects on administration. Also, it should be noted that myristicin is an analog of safrole, which is regarded as a low-grade hepatic carcinogen for rats (*7*). Whether the low level of myristicin in cigarette smoke contributes to the overall physiological effect of smoke is unknown.

Since commercial American cigarettes contain flavoring additives, including natural oils and resins, the possibility exists that myristicin, as well as the other benzyl esters in smoke, is derived from this source rather than the tobacco leaf. Myristicin has been isolated from the oil of several species, and the benzyl esters are common constituents of many natural oils and resins (*8*).

IRWIN SCHMELTZ, R. L. STEDMAN
J. S. ARD, W. J. CHAMBERLAIN
Eastern Utilization Research and Development Division, USDA, Philadelphia, Pennsylvania 19118

References and Notes

1. I. Schmeltz, R. L. Stedman, W. J. Chamberlain, D. Burdick, *J. Sci. Food Agr.* **15**, 774 (1964).
2. A. T. Weil, *Econ. Bot.* **19**, 194 (1965).
3. I. Schmeltz, R. L. Stedman, W. J. Chamberlain, *Anal. Chem.* **36**, 2499 (1964).
4. K. Nakanishi, *Infrared Absorption Spectroscopy; Practical* (Holden-Day, San Francisco, 1962), p. 36.
5. We thank Dr. A. T. Shulgin, Dow Chemical Co., for this sample and an infrared spectrum of authentic myristicin.
6. I. Schmeltz, R. L. Stedman, W. J. Chamberlain, C. D. Stills, *Abstr., Tobacco Chem. Res. Conf. 19th Lexington, Ky., 1965*; manuscript in preparation.
7. E. L. Long, A. A. Nelson, O. G. Fitzhugh, W. H. Hansen, *Arch. Pathol.* **75**, 595 (1963) [from *Biol. Abstr.* **45**, 60335 (1964)].
8. W. Karrer, *Konstitution und Vorkommen der Organischen Pflanzenstoffe* (Birkhäuser, Basel, 1958), pp. 100, 375.
9. We thank C. J. Dooley, R. L. Miller, C. D. Stills, and C. Leander for assistance.

9 November 1965

Homograft Target Cells: Contact Destruction in vitro by Immune Macrophages

Abstract. *Specific adherence of immune macrophages to monolayers of target cells is a passive phenomenon which represents only the first step in the mutually destructive interaction of immune macrophages and target cells. A specific hemagglutinin, responsible for specific adherence, was eluted from well-washed immune macrophages by heat treatment. The nature of the events in the interaction subsequent to adherence are unknown, but apparently demand the biosynthetic activities of the immune macrophage.*

Peritoneal macrophages from C57B1/Ks mice (*1*) immunized against antigens of A/Jax mice cause specific "contact destruction" in vitro on monolayers of specific target cells derived from A/Jax mice and C_3H mice (*2*). Our results established that: (i) the first step in the interaction is specific adherence of essentially all of the immune cells to the target cell monolayer, (ii) both the macrophage and the target cell are destroyed in the interaction, and (iii) normal peritoneal macrophages treated with specific humoral isoantibody derived from immune donors do not interact with target cells to produce cell destruction (*2*).

In our studies, immune macrophages were collected, and a standard suspension containing 20×10^6 cells per milliliter was prepared in tissue culture medium (*2*). The preparations were diluted in the medium to give suspensions containing 20, 15, 10, 5, 1, 0.5, and 0.1×10^6 cells per milliliter. A drop of each cell suspension (0.05 ml) was placed at each of two separate sites on pure monolayers of A/Jax fibroblasts or L-cells. After 48 hours the monolayers were stained by the plaque-staining method (*2*). The results from two separate experiments in which four sites were used for each concentration of immune overlay cells are reported.

An overlay of 50,000 immune cells caused full plaque formation. However, macroscopically evident partial plaques were produced with 25,000 immune cells, and microscopically evident plaques were produced with as few as 5000 immune cells. Ninety-eight percent of the cells that became detached from the plaque area were nonviable.

Starch-induced macrophages from