Fig. 1. Ascus showing first-division segregation of the spore abortion factor. Fig. 2. Ascus showing second-division segregation of the tan-spored factor. Fig. 3. Ascus resulting from a cross involving the two mutant strains. (×500)

tion factor are phenotypically alike regardless of whether they carry the wild-type or mutant factor at the tan-spored locus.

The use of this organism in genetic studies affords certain definite advantages. It is easily cultured in Petri dishes containing an agar medium of yeast extract and cellulose (*3*). The time required for one generation to mature is between 10 and 14 days. A single plate of paired cultures forms many fruiting bodies, each of which contains hundreds of asci. Since these asci can be scored merely by direct examination, the results of 500 meioses have been counted in one 3-hr period. Another advantage of this method is that the autonomous effects of single genes upon single haploid cells (the ascospores) may be observed without the complications of dominance and recessiveness. Furthermore, the ascospore pattern in each ascus is a visual and orderly replica of meiosis and, as such, is a valuable aid in the study of segregation and crossing over, eliminating the necessity for laborious ascus dissection. It is obvious from the evidence obtained with the use of these mutants that brachymeiosis does not occur in *A. stercorarius*.

A fuller presentation of these data will be published in the near future.

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Oak Wilt Fungus Labeled with C^{14}

Paul F. Hoffman and Bert M. Zuckerman

Section of Applied Botany and Plant Pathology, Illinois State Natural History Survey, Urbana

Since the fungus *Endoconidiophora fagacearum* Bretz, causal agent of oak wilt, lives parasitically only in the woody conducting tissues under the bark of a diseased tree, its progress from a point of infection to distant parts of a tree can be traced only with laborious difficulty The fungus produces, when grown *in vitro*, a toxic substance that is capable of causing tomato and oak cuttings to wilt. Tagging either the fungus, as has been done by Wheeler (*1, 2*) with other fungi, or the toxic substance with a radioactive element, without altering the pathogenicity of the fungus or the nature of the toxin, would afford better opportunity to study the disease experimentally (*3–5*).

Uniformly C^{14}-labeled sucrose (*6*) with a specific activity of 2.64 μc/mg was used in the preparation of 1- and 2-ml lots of a sucrose-asparagine-yeast extract medium (*7*) having activities of 10, 20, and 30 μc/ml, unlabeled sucrose being used to make up the 20-g/lit sucrose ratio in each lot. These lots, each in cotton-stopped, 10-ml Erlenmeyer-shaped flasks, were planted after sterilization with tiny wefts of mycelium and spores.

These cultures were then placed in moist chambers and incubated at 27° to 30°C. After having developed for periods of 7 to 56 days, the new mats of fungus were taken from the flasks, laid on filter paper, and washed with a stream of sterile water until no radioactivity (*8*) could be detected in the wash water. From the washed fungus, transplants were made to unlabeled agars in Petri dishes and allowed to develop into new colonies. Mycelium and spores from the peripheries of these colonies were used in subsequent tests.

Verification that the fungus had become labeled during its growth on the C^{14}-containing media was obtained by autoradiography. Bits of fungus taken from colony peripheries were placed on glass microscope slides in drops of water, in drops of unlabeled liquid medium, or on thinly spread potato dextrose agar and allowed to grow for periods of 36 to 144 hr. Also, to obtain closely appressed growth, transplants were made to DuPont 600 P. T. cellophane spread on potato dextrose, chestnut meal, and water agars in Petri dishes. Before autoradiographs were made, the fungus was killed by heat at 80°C and the preparations were dried for several hours over P_2O_5. Autoradiographs of these preparations were made on Eastman No-Screen X-ray emulsion and on several Ilford nuclear-track emulsions (*9*)—NTB2, NTC2, NTD1, NTE1, and NTG5—ranging from 25 μ to 200 μ in thickness.

Well-defined autoradiographs of germinated spores were obtained on NTG5 emulsion 100 μ, and of mycelium and spores on Eastman No-Screen X-ray emulsion (Fig. 1). The two most satisfactory methods of

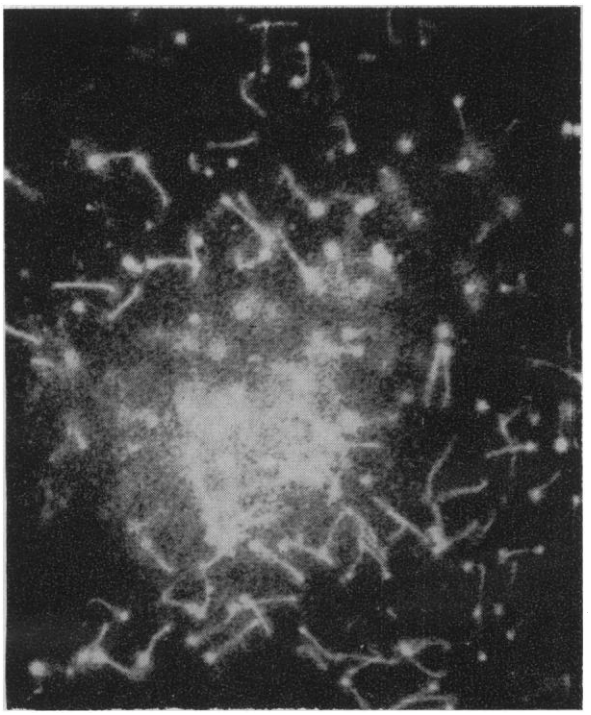

Fig. 1. Autoradiograph produced on Eastman No-Screen X-ray emulsion by germinated spores of *Endoconidiophora fagacearum* Bretz taken from a colony labeled with C^{14}. (×9.5)

preparation were to grow the fungus for periods of 24 to 48 hr in a drop of liquid medium on a glass slide and to grow it on cellophane spread over agar.

When planted on unlabeled agars in Petri dishes, the labeled fungus grew normally, even after it had been grown for 56 days on the 30-μc/ml medium. Macroscopically, colony characters were like those of the unlabeled parent; microscopically, measurements of 200 labeled and 200 unlabeled conidia revealed no significant differences between the two; and significant differences were not apparent in percentage of germination, rate of germination, and appearance after germination (Table 1).

In 3 days, a labeled strain of the fungus developed on the 30-μc/ml medium, and an unlabeled compatible strain, upon being crossed on unlabeled agar, produced perithecia and ascospores that were not different in any morphological character from perithecia and ascospores produced by unlabeled compatible strains. Exuded masses of ascospores lifted from the perithecial beaks contained detectable radioactivity when measured in a Q-gas scaler (*10*) and, upon being inoculated into red oak (*Quercus ruba* L.) seedlings in the greenhouse, produced typical wilt symptoms (*11*) in 47 days. It appears that transference of C^{14} labeling into the ascospores occurred. Possibly, then, radioactive labeling may be useful also in studying the life-history and genetics of the fungus.

Injection of suspensions of labeled conidia into red oak and live oak (*Q. virginiana* Mill.) seedlings was followed by typical wilt symptoms 20 days after inoculation. With symptom-bearing leaves from seedlings of both kinds of oak, autoradiographs were obtained on Eastman No-Screen X-ray emulsion in 20 days. By monitor (*12*), no radioactivity in the stems of the seedlings could be detected through the bark, but at the ends of cut stems readings of 30 to 40 counts/min above background were obtained, and at upper and lower leaf surfaces readings of 20 to 30 counts/min above background were obtained.

When grown in the 20-μc/ml medium, the fungus produced a substance that caused wilting of both tomato and oak cuttings like the wilting caused by the substance which the fungus produces in unlabeled media. That the two substances were the same was indicated further by the fact that filtrates from both labeled and unlabeled cultures gave the same Rf value, that of an as yet unidentified organic acid, on paper chromatographs.

It is not known whether the radioactivity detected by autoradiograph in the leaves and by monitor in the stems and leaves of inoculated oak seedlings indicated the presence of the fungus, its toxin, or some other metabolite. But the experiments show that, within the activity range used, mycelium, conidia, and probably ascospores can be labeled with C^{14} without altering the morphology or pathogenicity of the oak wilt fungus or changing the effect or nature of the toxin.

Work currently under way and planned for the future is concerned with more accurate determinations of the amount of labeling possessed by the fungus

Table 1. Germination at 23°C of unlabeled and C^{14}-labeled conidia of *Endoconidiophora fagacearum* Bretz of the surface of water agar.

Petri dish	Spores	At 24 hr			At 36 hr		
		Number counted	Percentage germinated*	Secondary sporulation	Number counted	Percentage germinated*	Secondary sporulation
1	Unlabeled	200	60.0	None	200	80.0	By about 25% of all germinated spores in each dish
2	Unlabeled	200	65.5	None	200	75.5	
3	Labeled	200	72.5	By 1 germinated spore	200	86.0	
4	Labeled	200	69.0	None	200	78.0	

* Differences are not significant at the 5-percent level.

spores, the manner in which ascospores become labeled, and the isolation and identification of exometabolites produced by labeled strains of the fungus.

References and Notes

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3. J. E. Kuntz, C. H. Beckman, and A. J. Riker, *ibid.* **42**, 13 (1952).
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6. We are indebted to R. F. Nystrom, University of Illinois Radiocarbon Laboratory, for supplying the labeled sucrose and for technical advice.
7. Per liter: KH_2PO_4, 0.5 g; $MgSO_4 \cdot 7H_2O$, 0.25 g; asparagine, 1.0 g; yeast extract, 1.0 g; sucrose, 20.0 g; distilled water, q.s.
8. Dried drops of the last wash water failed to show evidence of radioactivity in autoradiographs after 30 days or more of exposure.
9. Robert A. Reitz, of the University of Illinois Betatron Laboratory, supplied the emulsions and developed certain autoradiographs.
10. The average differences above background of the counts obtained with ascospore masses indicated odds of 9 : 1 that the difference was not due to chance alone.
11. The oak wilt fungus was reisolated from seedlings inoculated with labeled ascospores and conidia; but, probably because of attenuation of the labeling, no significant radioactivity readings were obtained from the isolations.
12. Gross determinations of C^{14} activity were made with a G-M monitor having sensitivity of 10^{-2} to 10^{-3} μc. Where specified, they are given as counts per minute above background and are considered significant.

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Waxy Constituents of the Saw Palmetto, *Serenoa repens* (Bartr.) Small

Edward A. Wilder and Eugene D. Kitzke

Basic Research Department,
S. C. Johnson & Son, Inc., Racine, Wisconsin

Serenoa repens (Bartr.) Small (*1*), commonly called the saw palmetto and often incorrectly called the scrub palmetto, is the most abundant palm in the United States. It occurs in wide distribution in the southeastern and southern states, ranging from South Carolina to the Florida Keys and along the Gulf Coast to Louisiana (Fig. 1).

Although considered somewhat of a weed because it is so ubiquitous, it was found useful in industrial application during World War II when it yielded an accepted cork substitute that could be processed from the soft tissue of the stem (*2*). Other potential uses include tannin source (*3*), fibers, wallboard, and paper.

Because the world market can always appreciate a new hard vegetable wax to supplement existing commercial waxes, such as carnauba (*Copernicia cerifera*), candelilla (*Euphorbia antisyphilitica*), ouricuri (*Syagrus coronata*), sugar-cane wax (*Saccharum officinarum*), and others, recent attention was given to the saw palmetto as a source of wax. In previous field observations, we had noted the waxy bloom.

The leaves used in this study were collected in southern Florida. They were sun-dried, using the technique commonly practiced by harvesters of carnauba in Brazil. The wax was removed from the leaves by brushing off the free-flaking wax first and then by solvent, extracting the entire leaf with heptane to determine total extractables. The free-flaked wax was essentially the same in character as the solvent-extracted wax. The characteristics of the sample of wax examined are as follows:

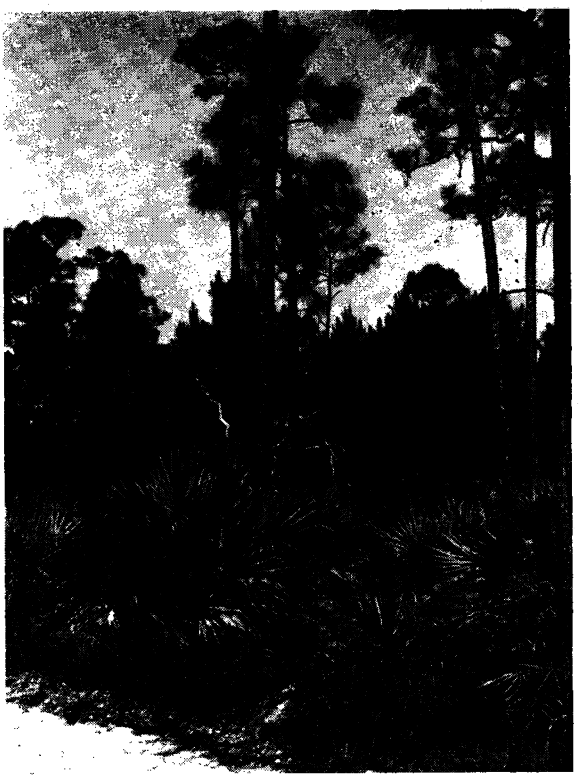

Fig. 1. Saw palmetto undergrowth in a slash pine (*Pinus caribaea* Morelet) woods. This is a common ecological pattern.

Acid number	16.3
Saponification number	101.5
Melting point	81.2°C
Iodine number	9.8
Needle penetration, 100 g/5 sec	< 1

The wax is hard, brown in color, and somewhat resinous in appearance. Considering its qualities for possible use in naphtha-type polish products, it has a precipitation temperature of 98°C, which unfortunately is unusually low for a hard vegetable wax. A gel formation that occurs in naphtha containing 18 percent solids is firm, although it is grainy and has poor solvent retention. The wax contains about 13 percent of resinous material, of which 6.5 percent is soft and tacky and can be removed by leaching the powdered wax with cold (25°C) acetone, and 6.5 percent is hard resinous material that is insoluble in boiling isopropanol. Although this 13 percent of resinous material is lower than what one finds in candelilla wax (20 percent) the near absence of resinous material in carnauba is a better criterion of a good vegetable wax.