Termite Attractant from Fungus-Infected Wood

Abstract. Field observations suggested that subterranean termites might follow a concentration gradient of attractive material to find decaying wood. Laboratory cultures of the brown rot fungus, *Lenzites trabea* Pers. ex. Fr., on pine blocks formed a material attractive to the eastern subterranean termites, *Reticulitermes flavipes* (Kol.) and *R. virginicus* Banks, and a Costa Rican termite, *Nasutitermes columbicus* (Holmgren). Such a potent termite attractant may be useful in termite surveys and control.

The diet of subterranean termites consists primarily of wood infected by fungi. Such fungi may be either beneficial or detrimental to the growth of the insects (*1*). Observations of the eastern subterranean termite, *Reticulitermes flavipes* (Kol.), suggested that a fungus or fungal product might also help the termites locate decaying wood. Shelter tubes built by this termite were found on buildings and trees in Sheboygan, Wis. Such tubes on the bark of living trees invariably led directly to a dead, decaying branch stub. The possibility that the termites followed a concentration gradient of attractive material to find the decaying wood led to laboratory studies on the relationship of termite to fungus.

Eight species of fungi associated with wood decay or termites (*2*) were cultured, and the attractancy of the products to *R. flavipes* was assayed. Culturing was accomplished with soil bottles containing a thin strip of wood called the feeder block. The bottles were inoculated with fungi and then incubated at 80°F at 70 percent relative humidity until the feeder blocks were covered with mycelium (*3*). Autoclaved (15 lbs, 15 min) blocks (¾ by ¾ by 2¾ in.) of western pine sapwood, *Pinus monticola* Dougl., were inserted in the bottles on top of the feeder blocks, and the cultures were incubated an additional 15 or 20 days. Next the test blocks were removed and marked at the upper limit of mycelial growth. Some of the blocks could be demarcated into three regions as follows: (i) a basal region covered by older woolly mycelium, (ii) a mid-region covered by cottony mycelium, and (iii) a top region with no visible mycelium. The mycelium was then brushed off, and each block was cut into sections according to the marked regions.

The blocks (either subsections from fungus-infected wood or control blocks from soil bottles not inoculated with fungi) were placed on moist sand in a large plastic container. Termites (250 of mixed castes excluding macropterous forms) were scattered over the sand in this multiple-choice situation. Within 2 minutes the termites clustered primarily around the basal portion, or woolly mycelium region, of blocks infected with *Lenzites trabea* in each of three replicated containers. Smaller groups of insects gathered around the basal portions of a few of the other blocks infected with other species of fungi. However, the basal portion from blocks infected with *L. trabea* was much more attractive than portions of the other blocks were. After 1 hour the greatest congregation of termites in the three boxes still occurred around the basal portion of the blocks infected with *L. trabea.*

For further studies a more refined bioassay technique for the attractive material was developed. Small paper pads as used in antibiotic assay (12.7 mm in diameter) were placed in a dish 5.3 cm in diameter. The basal portion of the block infected with *L. trabea* was macerated in distilled water to obtain an aqueous extract. A 0.03-ml portion of the extract was placed on one pad, and 0.03 ml of water was placed on another pad in the dish. When 20 termites were introduced they congregated within 30 sec on the pad containing the extract of wood infected with *L. trabea.* This response occurred despite the fact that the termites were in the light and exposed to desiccation. Neither water nor organic solvent extracts of sound wood or of *L. trabea* mycelium grown on malt agar medium elicited any response similar to comparable extracts from the infected wood. Further studies showed that the aqueous extract from 1 g of dry wood could be diluted to 6 liters with distilled water, and 0.03 ml was still adequate for attraction of the termites. Partial purification of the attractive material has been effected. The attractant was extracted from the aqueous material with ether. The ether-soluble materials were then chromatographed on Florisil columns with a benzene-ether elution gradient. The attractant eluted at about a 95:5 benzene-ether mixture. At this stage in the purification, the termites respond to 0.1 μg of the resulting colorless oil.

The aqueous extract from wood infected with *L. trabea* was also found to be highly attractive to other earth-dwelling termites, such as mixed castes, excluding macropterous forms, of *R. virginicus* Banks, and for nasute and worker forms of a Costa Rican termite, *Nasutitermes columbicus* (Holmgren).

The potential uses of such an attractant in survey, control, and research have been considered in a recent review on the general subject of insect attractants (*4*). Similar uses might be made of this termite attractant from fungus-infected wood (*5*).

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2. Forest Products Laboratory cultures: (534) *Lentinus lepideus* Fr.; (617) *Lenzites trabea* Pers. ex Fr.; (697) *Polyporous versicolor* L. ex Fr.; (698) *Poria monticola* Murr.; and (71316) *Polyporous gilvus* Schw. ex Fr. *Penicillium* sp., *Aspergillus* sp., and *Spicaria* sp. were repeatedly isolated from termites.
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5. This report was approved for publication by the director of the Wisconsin Agricultural Experiment Station. This project involved the cooperation of and support by the department of entomology of the University of Wisconsin, the city of Sheboygan, and the Forest Products Laboratory of the U.S. Department of Agriculture Forest Service, Madison, Wis. This work was supported in part by the research committee of the Graduate School from funds made available by the Wisconsin Alumni Research Foundation.

16 March 1961

Initiation of Flower Buds in Rhododendron after Application of Growth Retardants

Abstract. Vegetative terminals of azalea (*Rhododendron* spp.) plants of varying ages initiated flower buds promptly after application of the chemical growth retardants, tributyl-2,4-dichlorobenzylphosphonium chloride (phosfon) and (2-chloroethyl) trimethylammonium chloride (CCC), as soil drenches. This response occurred under environmental conditions which prevented or limited flower bud initiation in untreated plants. Normal flowering followed exposure of the treated plants to dormancy-breaking cool storage.

Application of the growth retardants phosfon (tributyl-2,4-dichlorobenzylphosphonium chloride) and CCC [(2-chloroethyl) trimethylammonium chloride] caused suppression of vegetative growth and prompt initiation of flower buds in azalea, *Rhododendron* spp. These responses did not depend on

minimum age or size of plant, specific photoperiod, or temperature. In the garden azaleas normally initiate flower buds under the high temperatures and relatively long photoperiods of late summer. Bud development continues for several months. Flowering can be accelerated by exposure of the plants to temperatures of 5° to 10° or 12°C for about 4 to 6 wk. Plants grown continuously in the greenhouse initiate flower buds and bloom irregularly throughout the year. Azaleas are reported to be day-neutral (*1*), although long photoperiods promote shoot growth (*2*). Recent work (*3*) suggests that very long photoperiods delay flower initiation in some cultivars of azalea.

Cuttings of *R. obtusum* (Lindl.) Planch., cultivar Coral Bells, were propagated 25 March 1960. The plants were grown in 3-in. pots on natural days in a greenhouse maintained at a minimum night temperature of 15° to 16°C. A terminal flower bud produced on each cutting resulted in physiological pruning and the production of a whorl of new shoots. Later these shoots were pruned by removal of the apical 1 to 2 cm in order to stimulate development of additional vegetative shoots. On 6 October, when the new shoots were 8 to 10 cm long, four lots of plants were treated by adding 79, 197, or 395 mg of CCC or 66 mg of phosfon per plant (all dissolved in 50 ml of water and applied as a soil drench). Half of the plants in each lot were held at a temperature of 12° to 13°C from 9 November until 21 December. To prevent leaf drop during this cool storage the plants were lighted 12 hr daily with incandescent filament bulbs (150 watts) suspended 3 ft over the plants. The appearance of untreated and phosfon-treated plants which remained in the greenhouse and of similar ones which received the cool storage is shown in Fig. 1. Exposure to vernalization resulted in limited flowering of the untreated plants and stimulation of a new flush of growth. Plants treated with phosfon and CCC (not shown) produced a cluster of flower buds in place of further vegetative growth. Vernalization stimulated these buds to develop into flowers. Later new vegetative shoots developed at rates inversely proportional to the concentration of the applied retardant.

Earlier trials were made with CCC in June, July, and August of 1960 with plants of five azalea cultivars propagated in 1959. Without exception, application of CCC at rates of 200 ml containing 1580 mg per 5-in. azalea pot, and 250 ml containing 1975 mg per 6-in. pot, suppressed further vegetative growth and induced flower buds to form earlier than they did in untreated plants. The latter produced new shoots at random during the summer. All treated plants bloomed after 4 wk of exposure to a temperature of 10°C. Flowering of treated plants did not occur without exposure to lowered temperatures.

Later trials were made with CCC and phosfon during the winter of 1960–61 with plants of Coral Bells and Alaska cultivars. These plants were lighted 4 hr nightly with 100-watt incandescent filament bulbs to stimulate growth. Unpruned plants continued vegetative growth and those pruned produced new vegetative shoots, whereas those treated with the retardants initiated flower buds at every terminal.

Wittwer and Tolbert (*4*) reported that CCC induced early flowering of tomato. Triiodobenzoic acid is known to induce early formation of flower buds in tomato (*5*), but it is not effective on many other indeterminate plants.

Treatment of azaleas with the two growth retardants removed limitations of plant size and environment for flower initiation. In effect they acted like flower-inducing hormones (florigens), but this is not necessarily the

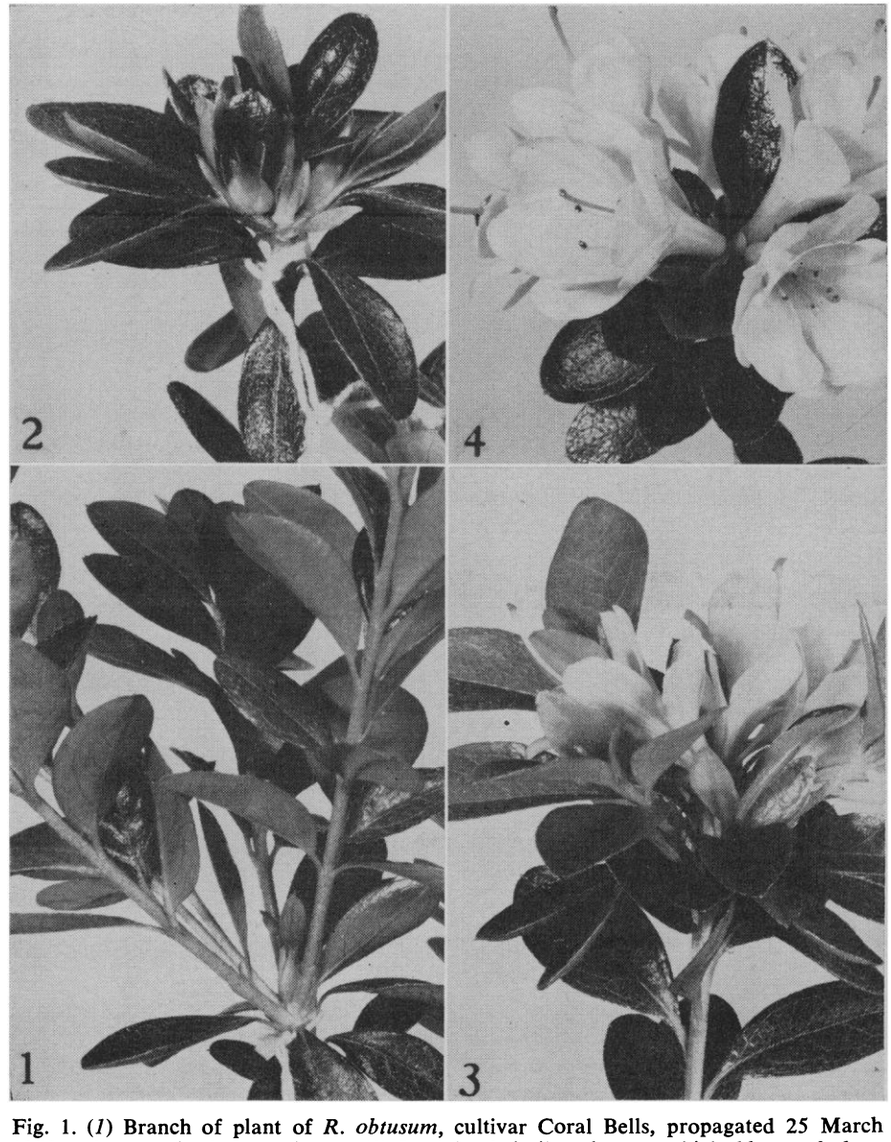

Fig. 1. (*1*) Branch of plant of *R. obtusum*, cultivar Coral Bells, propagated 25 March 1960 and grown in the greenhouse. (*2*) Branch of similar plant to which 66 mg of phosfon was added 6 October. (3) Branch of similar plant that was grown in the greenhouse until stored at a temperature of 12° to 13°C from 9 November until 21 December and then returned to the greenhouse. (*4*) Branch of plant like (*3*) to which 66 mg of phosfon was added 6 October. Photographed 30 January 1961.

explanation. The substances restricted the growth of the plants, thus presumably altering their metabolism and creating conditions conducive to flower initiation. Growth suppression should result in accumulation of photosynthate which may favor flower initiation, but the action mechanism is probably more involved since pruning of the plants also caused temporary growth restriction but did not result in flower initiation after growth was resumed. The potential importance of controlling flower initiation is obvious and points the way to further investigations (*6*).

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23 February 1961

Inhibiting Effect of Tobacco Smoke on Some Crystalline Enzymes

Abstract. Tobacco smoke absorbed in phosphate buffer at neutral *p*H inhibits irreversibly the enzymes rabbit muscle glyceraldehyde-3-phosphate dehydrogenase and yeast alcohol dehydrogenase, whereas lactic dehydrogenase and glutamic dehydrogenase are not inhibited. A transient inhibition of beef liver catalase occurs. Indirect evidence suggests that the observed enzyme inhibition is caused by peroxides present in the smoke.

In spite of the current interest in the biological effects of smoking, almost no work seems to have been done on the biochemical effects of tobacco smoke. The present finding that tobacco smoke is capable of inhibiting various enzymes may therefore be of interest.

The main-stream smoke from cigarettes, cigars, or pipe tobacco was sucked through 5 ml of phosphate buffer at *p*H 7.4 in a gas absorption flask, and one volume of the smoke solution was subsequently mixed with an equal volume of the enzyme to be tested. In Fig. 1, the effect of cigarette smoke on the SH-enzyme rabbit muscle glyceraldehyde-3-phosphate dehydrogenase is shown. In these experiments the smoke from one nonfilter cigarette was used. It is apparent that the smoke solution is capable of reducing the activity of this enzyme by approximately 65 percent in the course of 30 min. When a 1000-fold molar excess of cysteine was added, a moderate reactivation (approximately 15 percent) was obtained. Similar results were found with cigars or pipe smoke.

Ordinary cigarette filters (cotton-cellulose) were unable to reduce the enzyme inhibition. On the other hand, when the smoke was inhaled and then blown through the absorbing buffer, the inhibition was strongly decreased. The small inhibition obtained in this case (approximately 15 percent) was completely reversed by the addition of cysteine. Thus, the data suggest that the inhibition is caused by two factors. The factor responsible for the irreversible inhibition is apparently removed or destroyed by inhalation.

Similar experiments were carried out with yeast alcohol dehydrogenase, lactic dehydrogenase, glutamic dehydrogenase, and beef liver catalase. Yeast alcohol dehydrogenase ($1.5 \times 10^{-7}M$) was inhibited by approximately 50 percent in the course of 30 min. Lactic dehydrogenase and glutamic dehydrogenase were unaffected. With catalase ($7 \times 10^{-7}M$) a transient inhibition was observed, and its maximum (about 20 percent) occurred after 30 min incubation with the smoke solution. The inhibition was spontaneously and completely reversed within 1 hr.

Previous experiments in this laboratory have demonstrated (*1*) that, when serum albumin is irradiated with x-rays and subsequently added to unirradiated solutions of the SH-enzymes here studied, both rabbit muscle glyceraldehyde-3-phosphate and yeast alcohol dehydrogenase are slowly and irreversibly inhibited, whereas neither lactic nor glutamic dehydrogenase is affected. The inhibition observed under these circumstances has been shown to be due to radiochemically formed peroxides (*2*). The striking similarity between the latter results and those reported above suggested the possibility that the present results might be explained by the presence of peroxides in smoke. Ingram (*3*) has demonstrated the presence of free radicals in smoke condensate, and conceivably, peroxides may be formed upon the reaction of such free radicals with oxygen. However, attempts to determine peroxides by standard micromethods were unsuccessful. The smoke solution formed precipitates with the reagents of the ferric-thiocyanate method (*4*) and the titan-sulfate method (*5*). With the iodine method (*6*) no peroxides could be demonstrated, even when bottle hydrogen peroxide was added to the smoke solution. The possibility that the added peroxide was dissipated in reactions with constituents of the smoke solution was excluded by the findings that the addition of bottle hydrogen peroxide led to the expected increase in the inhibition of the rabbit muscle dehydrogenase, and that catalase readily abolished this additional inhibition. Presumably iodine formed in the oxidation by peroxides is consumed in reactions with unsaturated hydrocarbons in the smoke solution.

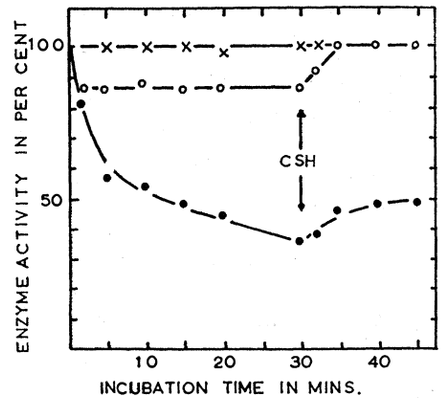

Fig. 1. Inhibition of rabbit muscle glyceraldehyde-3-phosphate dehydrogenase by cigarette smoke. The enzyme ($10^{-6}M$) in $0.067M$ phosphate buffer, *p*H 7.4, at 0°C was mixed with an equal volume of a buffer solution of cigarette smoke. Cysteine (CSH) in a final concentration of $5 \times 10^{-4}M$ was added after 30 min. Solid circles: cigarette smoke absorbed directly in buffer; open circles: cigarette smoke inhaled prior to absorption in buffer; crosses: control (buffer).

Since chemical methods did not provide any direct proof for the existence of peroxides in the smoke solution, some experiments have been performed in order to obtain indirect evidence for the role of peroxides in the enzyme inhibition by tobacco smoke. Thus, when different compounds were added to the absorbing buffer *prior* to the smoking procedure, it was found that thiols in high concentration ($5 \times 10^{-8}M$) abolished completely the inhibition of the rabbit muscle dehydrogenase, whereas ethylene diaminetetraacetic acid had only a moderate effect. Furthermore, when the smoke solu-