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A Technique to Artificially Infest Beech Bark with the Beech Scale, *Cryptococcus fagisuga* (Lindinger)

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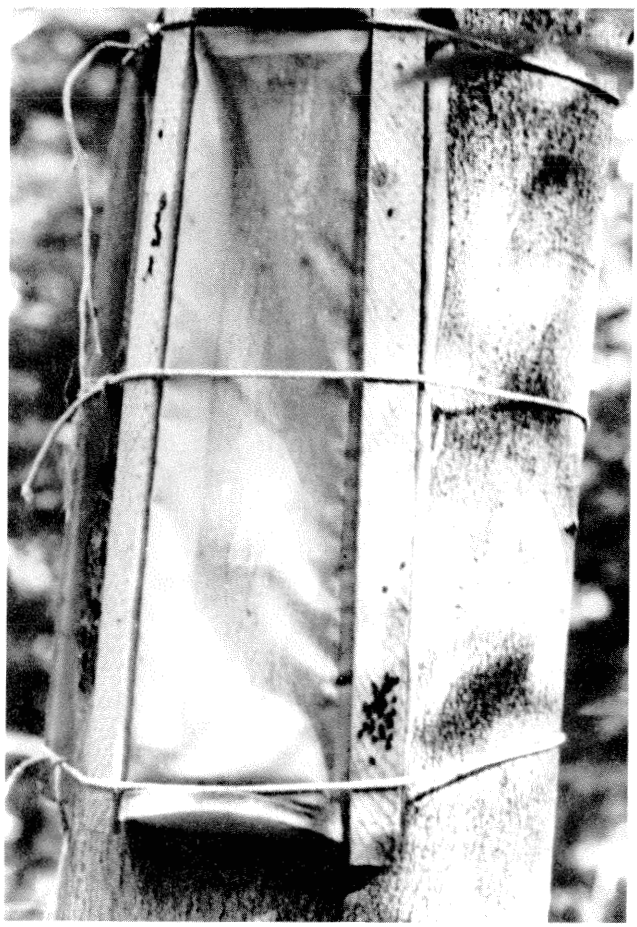

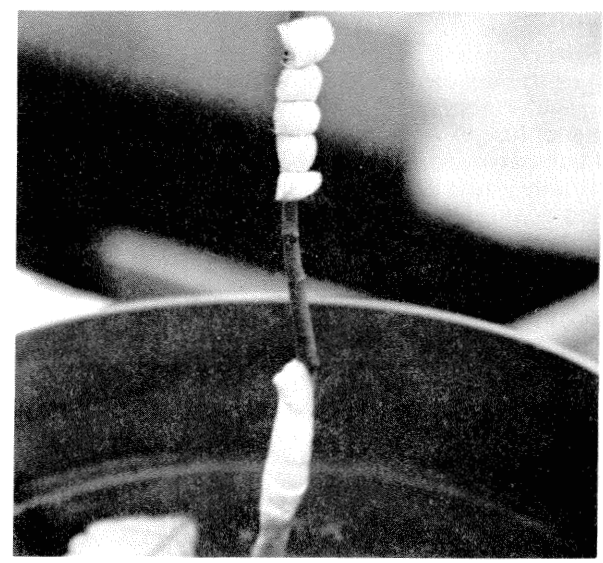

The Author

David R. Houston obtained a B.S. degree in forestry from the University of Massachusetts, a M.F. degree in forest pathology from Yale University, and a Ph.D. in plant pathology from the University of Wisconsin. Dr. Houston has been employed by the USDA Forest Service since 1961. He is now principal plant pathologist at the Northeastern Forest Experiment Stations's Forest Insect and Disease Laboratory in Hamden, Connecticut, and is project leader of a team conducting research on dieback and decline diseases in the Northeast. From May 1975 to May 1976, Dr. Houston studied beech bark disease in the United Kingdom as an exchange scientist with the British Forestry Commission in Farnham, Surrey, English.

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Abstract

Beech bark disease is initiated when bark of beech trees (*Fagus* spp.) is attacked by the beech scale, *Cryptococcus fagisuga* Lindinger. The effects of the insect predispose tissues to bark cankering fungi of the genus *Nectria*. Critical studies of insect-fungus-host interactions had been stymied by the inability to artificially infest beech with the initiating agent. This paper describes a technique using covers of polyurethane foam to initiate or enhance the development of local infestations of the beech scale on both seedlings and large trees.

Beech bark disease is a complex disease of American (*Fagus grandifolia*) and European (*Fagus sylvatica*) beech trees and is initiated by the beech scale, *Cryptococcus fagisuga* (Lindinger) (Ehrlich 1934).

The beech scale is a tiny, wingless sucking insect that infests the bark of beech trees. Reproduction is parthenogenetic, and eggs are laid in June or July. The 0.5-mm-long eggs hatch from mid-August through September, the emerging crawlers locate feeding niches, usually within a few centimeters of where they hatched, insert their feeding stylets, molt, and overwinter as second-stage nymphs (Ehrlich 1934). Feeding resumes in spring, and in June the scales molt once more to become adults. In this stage, they secrete most of the conspicuous white, wool-like wax that covers their bodies. Feeding by the scale renders the bark susceptible to several bark canker fungi of the genus *Nectria* (Ehrlich 1934, Parker 1974, Lohman and Watson 1943). Because the full expression of the disease requires the effects of both agents, studies of host-disease interactions require manipulating each of them. The fungi are relatively easy to work with and can be introduced to bark tissues using standard inoculation techniques (Ehrlich 1934, Parker 1974a, Perrin 1979). Manipulation of the scale has proven more difficult.

European beech trees were infested generally with beech scale by affixing heavily infested bark segments to tree trunks with wire (Houston et al. 1979). However, several years were required for significant population levels to develop.

This paper describes a technique to initiate or enhance the rapid development of local infestations of the beech scale on both seedlings and large trees.

Background

In June 1976, enclosures designed to trap scale parasites were placed over colonies of *C. fagisuga* on beech trees in Maine, Vermont, and Pennsylvania.[1] The frames of the 30- by 60-cm rectangular enclosures were 5- by 5-cm polyurethane foam strips glued at the corners. Covers of nylon mesh screening, 44 strands per inch (17.3/cm), glued to the foam framework completed the enclosures. The traps were oriented in a vertical position with 4-cm-wide wooden laths positioned over the side frames. The traps were held snugly to the trees with ropes (Fig. 1). The foam frame conformed to bark irregularities and prevented the migration of insects into or out of the trap enclosure.

No parasites were detected in subsequent examinations. But, 1 year after installation colonies of *C. fagisuga* had developed in abundance beneath the foam side and top frames (Fig. 2). This unexpected and fortunate observation triggered the following series of studies on the use of foam covers to artificially infest beech trees with the beech scale.

The Studies

Study 1.—Infestation of Seedlings with *C. fagisuga*

Experiment 1. In September 1977, strips cut from a trap frame that bore large numbers of scale eggs and crawlers were used to inoculate 15-month-old beech seedlings in the greenhouse. The 1.3- by 1.3- by 5-cm foam strips were secured in place, eggs toward the stem, with a copper wire wrapped

[1] A cooperative exploratory study with W. E. Wallner, principal entomologist, Hamden, CT.

Figure 1.—A nylon screen-covered enclosure with foam framework placed over colonies of *C. fagisuga*.

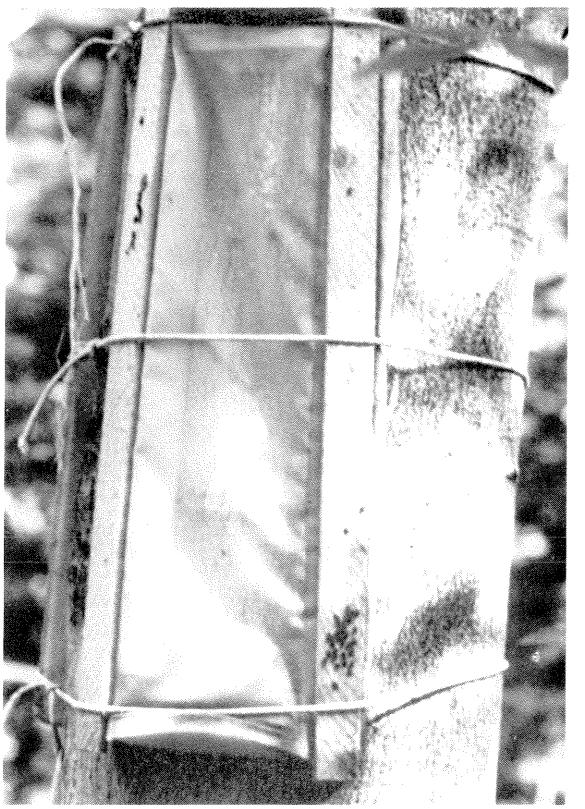


Figure 2.—Colonies of *C. fagisuga* (arrow) developed under the foam framework of the enclosure.

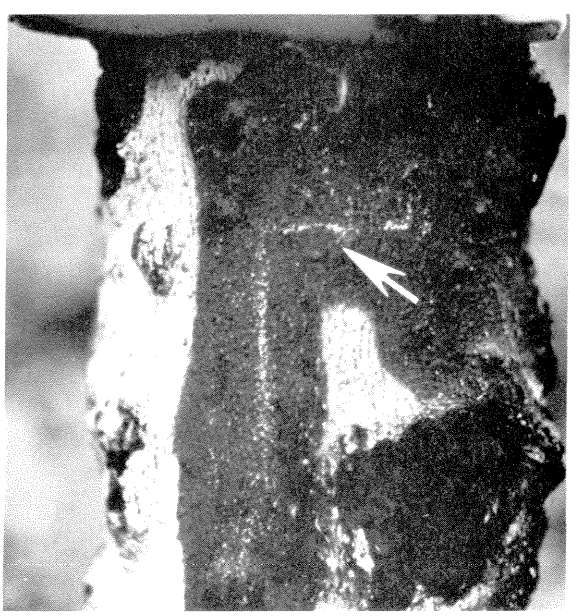


Figure 3.—Strips of the foam frames bearing eggs of *C. fagisuga* held in place on a young beech seedling.

Figure 4.—Colonies of *C. fagisuga* (arrow) developed on the seedling beneath the foam strip. Note the distortion of the stem caused by scale feeding.

spirally around the stem (Fig. 3). Some strips were also wrapped with Parafilm.[2] After 12 months, the coverings were removed and the stems examined.

Results. Successful infestation occurred in all but a few of the 30 seedlings inoculated. The eggs hatched, nymphs established themselves, overwintered, laid eggs, and the new crawlers successfully colonized the bark tissues. In some instances, infestations were very heavy, and the young stems exhibited growth distortions (Fig. 4) similar to the "dimpling" symptom common on some young European beech trees after heavy localized infestation (Parker 1974b). Wrapping with Parafilm did not enhance infestation. The success of this initial experiment using large and undetermined numbers of eggs prompted another experiment using small, discrete numbers of eggs.

Experiment 2. In September 1979, 40 beech seedlings grown for 4 seasons in the greenhouse were inoculated with either 1, 2, 4, 5, 10, or 50 eggs at each of four places chosen to include bark tissues 2, 3, and 4 seasons old. Usually, two inoculations per seedling were made on the oldest tissue. Eleven seedlings planted in 1979 and representing 1-year-old tissue also were inoculated once with either 1, 4, or 50 eggs.

For this and other artificial infestation experiments, eggs were collected and prepared for use as follows: in late June to mid-July heavily infested bark segments were removed from naturally infested trees or from beneath the frames of

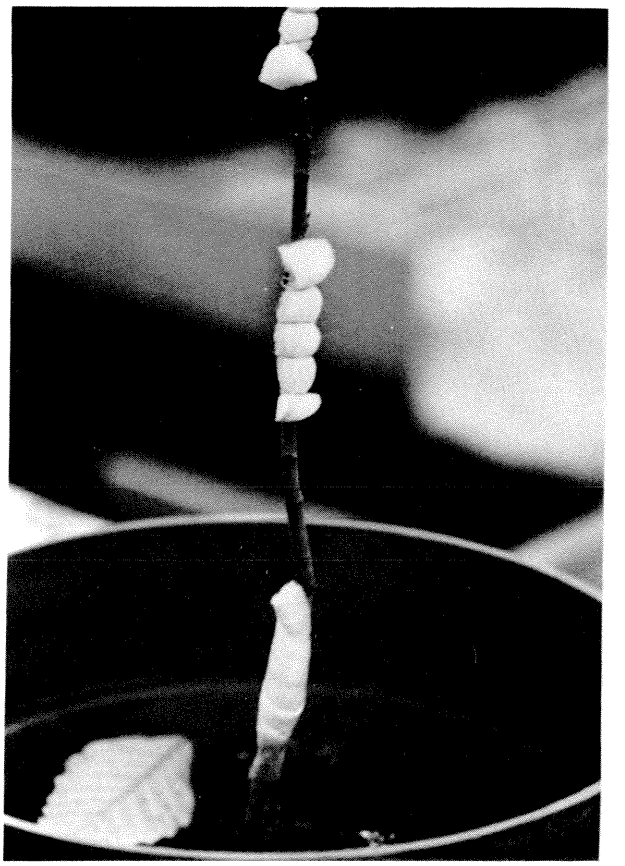
[2] The use of trade, firm, or corporation names in this publication is for the information and convenience of the reader. Such use does not constitute an official endorsement or approval by the U.S. Department of Agriculture or the Forest Service of any product or service to the exclusion of others that may be suitable.

Column 1, last line of first paragraph should read:

at 40°F (4°C) until used.[3]

Figure 5.—Heavy infestation of beech scale beneath foam frames after 3 years. The white is wax secreted by the insect.

Figure 6.—Greenhouse seedling inoculated at various points with specific numbers of *C. fagisuga* eggs.



traps installed 3 years earlier (Fig. 5). The segments of colonized bark were placed in covered plastic cups, kept cool in ice chests, and brought to the laboratory. Eggs were separated from wax and bark debris by gently teasing and tapping masses of egg-laden "wool" and bark fragments over a nylon mesh screen. The eggs were sieved onto the inverted covers of small plastic cups in lots of 50 to 200 and stored in a dessicator over KOH (20 g KOH/100 g H_2O) at 83.8 percent RH at 40°C until used.[3]

The eggs were carefully lifted from the covers with a moistened dissecting needle and placed on the centers of fresh 1.3- by 1.3- by 5-cm polyurethane foam strips each dampened with a drop of sterile water. The strips were affixed to the seedlings with wire as in Experiment 1, and half also were wrapped with Parafilm (Fig. 6). The wrappings were removed and the seedlings were examined in June 1980.

Results. Twenty-two of the 40 seedlings were infested in June 1980. Percentage of infestation increased as more eggs were used:

No. of eggs	*No. seedlings inoculated*	*No. seedlings infested*	*Percentage of infestation*
1	6	1	16.7
2	8	2	25
4	7	3	43
5	7	4	57
10	7	7	100
50	5	5	100

All seedlings inoculated with 10 or 50 eggs per point were infested, whereas only one of the seedlings inoculated with one egg per point was infested. However, among infested seedlings all inoculations were not successful. The relative frequency of the successful infestation among infested seedlings ranged from about 2 percent for 1-egg inoculations to 39 percent for 50-egg inoculations.

[3] Storage conditions recommended by Mr. D. Wainhouse, Forestry Commission, Farnham, England.

Figure 7.—Foam cover in place over natural populations of beech scale.

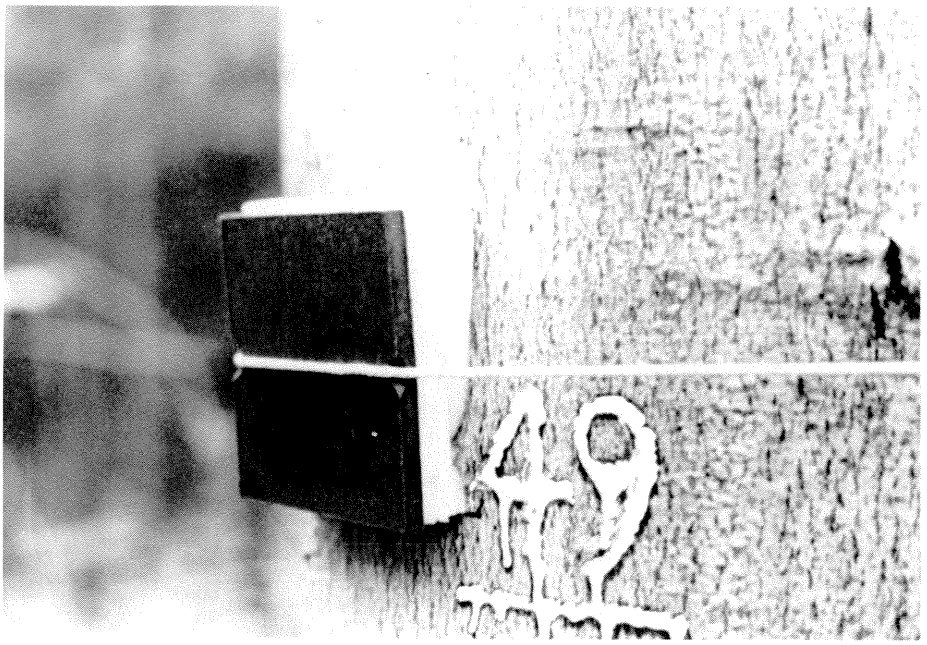
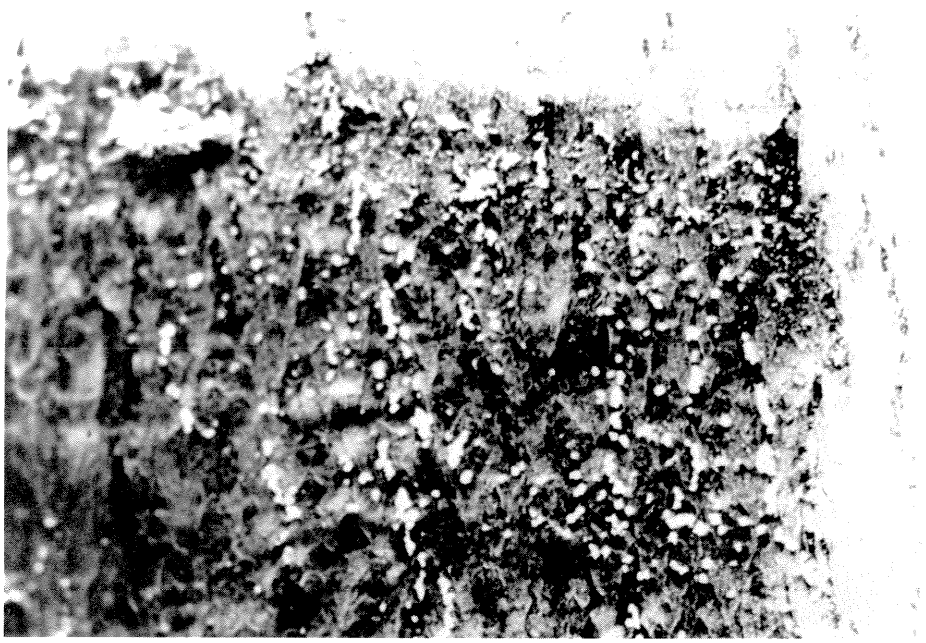


Figure 8.—Population of beech scale increased markedly on bark under foam cover (dark area) 1 year after being installed.



Infestation success was not influenced by age of bark; tissues 2-, 3-, and 4-seasons-old were equally susceptible—25.6, 26.8 and 29.3 percent, respectively. And, 4 of the 11 1-season-old seedlings inoculated became infested: three inoculated with 50 eggs and one inoculated with four eggs. Wrapping with Parafilm was detrimental; fewer seedlings were infested and fewer insects developed with this treatment.

Study 2.—Enhancement of Natural Populations on Larger Forest Trees

Experiment 3. The development of scale beneath the foam trap frames prompted a study to see if foam covers could be used to increase naturally existing populations on large forest trees. In August 1978, 10- by 10- by 5-cm polyurethane foam squares were placed on large, lightly infested beech trees growing in central Vermont. The foam blocks held in place with 10- by 10- by 0.6-cm covers of tempered Masonite,[2] were secured to the tree with rope (Fig. 7). At least four foam squares, usually on four different sides of the tree, were held by each rope. In September 1979, the blocks were removed, and the populations of beech scale beneath them were examined.

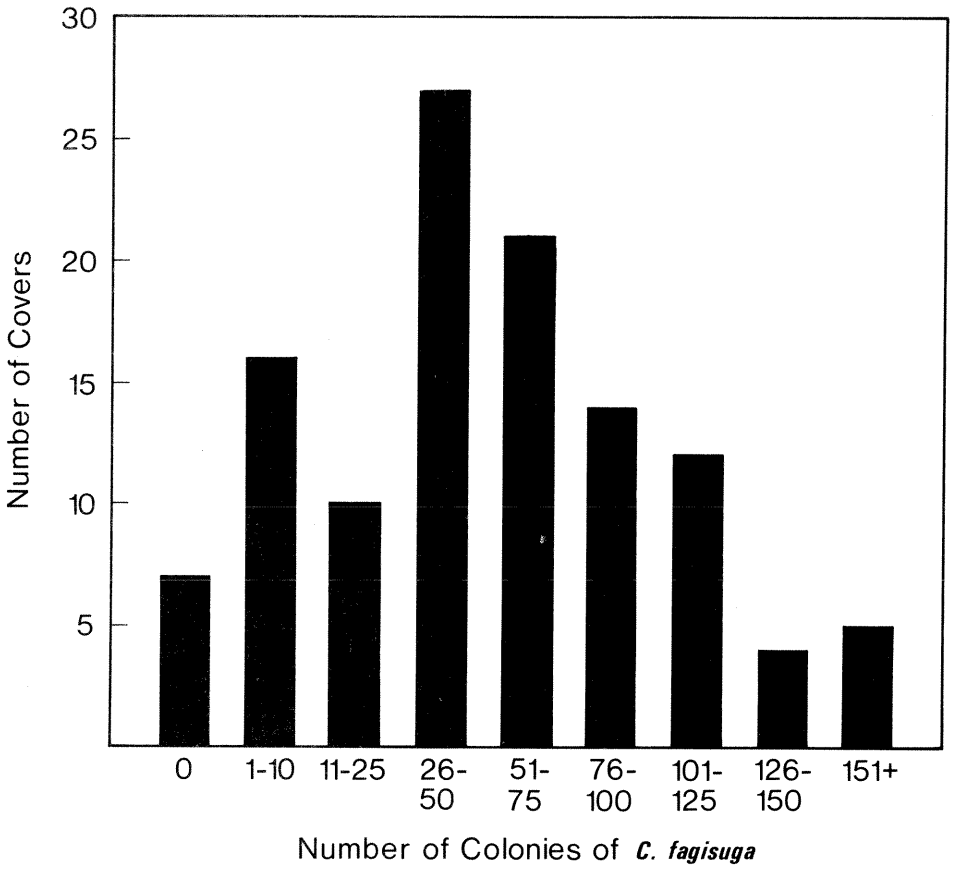
Results. In most instances, more insects had developed beneath the foam covers than on the bark adjacent to them (Fig. 8). Insects were absent beneath some covers located on the lower sides of leaning trees, often in the runnels where water ran down the trunks. These covers were often wet and darkly discolored.

The results of Experiment 3 and Study 1 set the stage for trials to introduce insects to specific locations on previously uninfested forest trees.

Study 3.—Artificial Infestation of Large Forest Trees with *C. fagisuga*

Experiment 4. Foam-Masonite covers identical to those used in Experiment 3 were placed on 28 trees growing in southcentral Connecticut

Figure 9.—Frequency distribution in May 1980 of *C. fagisuga* colonies established under foam covers inoculated with approximately 200 to 300 eggs in July to August 1979.



in July and August of 1979. The four covers per tree were arranged so that two were located on the north and south sides of the tree—one at 1 m and another at 2 m above ground. For two of the trees, an additional cover was placed at each height and randomly assigned to the north or south side. Notches in the Masonite covers facilitated securing the two covers at each height with one rope. Eggs collected from trees in Vermont and New York were prepared as described in Experiment 2. Approximately 200 to 300 eggs were brushed onto the centers of moistened foam blocks and then placed against the trunks. A few trees bearing very sparse natural populations of beech scale were used, but insects were removed before inoculation by scrubbing the bark surfaces vigorously with a stiff-bristled brush. In May 1980, the covers were removed and the individual colonies present were counted.

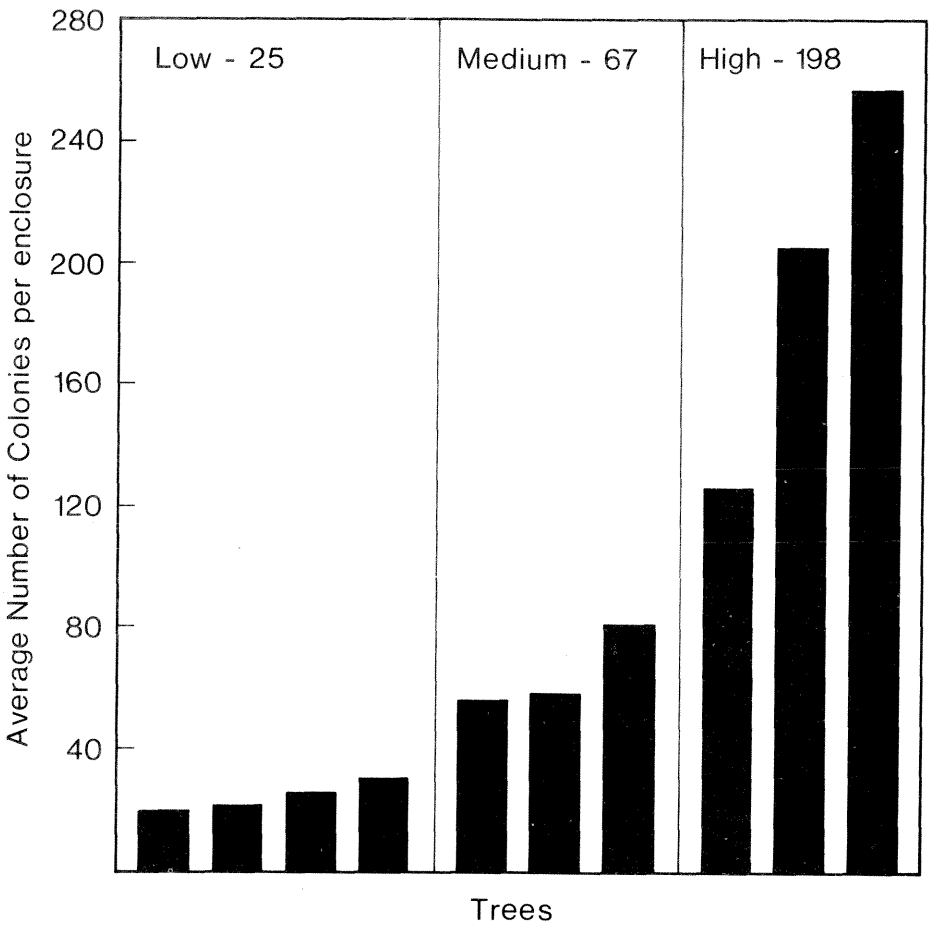
Results. The frequency distribution of colonies beneath covers is given in Figure 9. Seven covers were devoid of insects. As in Experiment 3, these were located in runnels on the tree where stem flow kept them too wet for insects to develop. The average number of colonies that developed beneath infested covers at different heights and aspects was:

Height above ground	North side	South side
1 m	75	59
2 m	69	51

Although results suggest that more scales developed at the lower levels and on the north sides of the trees, the variable number of eggs used initially precluded statistical analysis.

Experiment 5. Ten trees growing in southcentral Connecticut were inoculated in July 1979 by using a foam cover of different design. Cages 25 cm square were constructed similar to the parasite trap enclosures described earlier. A 5- by 5- by 5-cm block of foam was cemented to the center of the nylon screen cover. Approximately 200 to 400 eggs were brushed onto the moistened inner surface of the small, central block and the cages were held securely against the tree. Pressure exerted by the screening held the block against the trunk. The

Figure 10.—Average number of colonies of *C. fagisuga* developing in enclosures on 10 trees arranged by groups of "susceptibility".



cages were placed at three heights on each tree: midcrown, below the crown, and at breast height. Laths were placed over the sides of the foam frame, and each cage was secured snugly against the tree with ropes at the top and bottom. In May 1980, the cages were carefully removed, the insects counted, and the cages then replaced.

Results. In general, infestations developed readily in the enclosures, and no differences in population levels were detected that were related to height on the stem, aspect of the enclosures, or source of eggs. Two cages, one at the highest level and one at the lowest, were devoid of insects. Both of these were in runnels and were wet and darkly stained. In many instances, the populations were not confined to bark beneath the small, central inoculation blocks. Many insects were located beneath the very inner margins of the foam frames. The insects apparently hatched and migrated from the relatively loosely held block to the nearby frames.

Results suggest that different trees varied in their susceptibility or suitability for colonization (Fig. 10). The 10 trees can be separated into three groups based on the average number of colonies per enclosure: for the four trees in the lowest group—25.2, for the next group of three trees—67.4, and for the three trees in the highest group—198.

Discussion

Critical study of the host-insect-fungus interaction of the beech bark disease complex had been stymied by the inability to develop populations of the scale "on demand". This difficulty has been overcome by the successful use of foam covers to enhance naturally existing populations on large trees and to establish new populations on both large and small trees. The foam technique ensures the availability of a continuous and reliable supply of insect-affected tissues.

The relatively high rate at which seedlings were artificially infested,

even when relatively few eggs were used, is encouraging. This indicates that very young tissues can be used for critical studies of insect-host interactions, and also suggests that screening for resistance to scale attack can perhaps can be accomplished at an early age.

Now large forest trees can be challenged with the insect to confirm their apparent resistance. In addition, studies can be conducted to determine the influence of host predisposition on susceptibility to scale attack and to determine the effects of specific infestation densities and durations on host-bark changes and susceptibility to *Nectria*. Also, studies of biological control of both the scale and *Nectria* can proceed now that specific levels of infestation can be achieved.

The success of this technique can be ascribed to the physical protection afforded the scale from its predators and from such adverse environmental factors as rain, wind, and alternate freezing and thawing. In addition, the snug-fitting foam covers serve to prevent dispersion of the crawlers.

Foam covers also exclude light from the photosynthetically active bark tissues. In nature, populations of scale often develop first beneath "covers" of lichens and mosses, beneath large branches, or in other shaded refuges such as bark fissures or callus margins of wounds. And in many sites, populations are highest on—sometimes restricted to—the north sides of trees. Whether the scale succeeds beneath covers because it is protected or because the bark is rendered susceptible by the physiological effects of shade, or both, is not yet known. Studies are underway to clarify these relationships because they have a bearing on approaches to control.

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