FRONTIERS
of PLANT SCIENCE

SPRING 1976

Fifty-year-old sprouts of chestnut clustered around the long-since decayed parent stumps. The sprouts were cut and yielded chiefly ties and lumber with some poles. Voluntown, Connecticut, 1910.

THE CONNECTICUT AGRICULTURAL EXPERIMENT STATION NEW HAVEN

Biological Control of Blight May Revive the Chestnut

By Richard A. Jaynes

We have evidence that an altered form of the chestnut blight fungus can inactivate the virulent form.

Few people have first-hand knowledge of what the American chestnut once meant in New England and the Appalachian mountains because this stalwart forest tree was destroyed a half century ago by the chestnut blight fungus (*Endothia parasitica*).

Chestnut was valuable because of its versatility. The wood resisted decay and found uses from cribs and coffins to fences and fuel. Railroad ties, mine timbers, and utility poles were commonly chestnut. The nuts were a staple food for wildlife, were eaten by the farmer and his children, and were feed for fattening hogs in the fall. In 1910, chestnuts grew on about 40% of the forested land in Connecticut.

The disease, discovered in New York City in 1904, is presumed to have arrived from Asia on imported nursery stock. The blight spread approximately 20 miles a year. It was first seen in Connecticut in Stamford in 1907. Most of Connecticut's large chestnut trees were killed by 1920, but the Lebanon chestnut, the last survivor of the native trees, did not die until 1936.

The Station has never found a truly resistant tree or seedling in this state. All trees that have been reported as such either belonged to a different species or were survivors that finally died. Chestnuts persist today through a succession of sprouts from the stumps of diseased trees.

G. P. Clinton, who was Station botanist when the blight hit Connecticut, suspected and hoped, up to his death in 1937, that the blight organism would lose its virulence. He was encouraged in this view by the loss of virulence of cultures grown in the laboratory for 20 years. Unfortunately, the "outdoor" blight showed no similar loss of its ability to girdle chestnut trees. But what did not happen naturally may now come from research.

Although biological control of a plant disease may sound like science fiction, we have evidence that an altered form of the chestnut blight fungus can inactivate the virulent form.

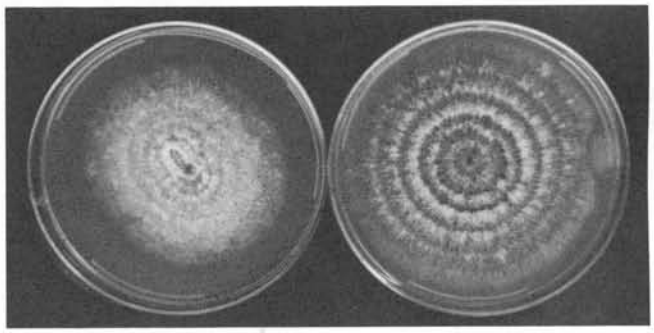
Our original less-virulent strain was sent to us by two French scientists, Grente and Sauret, who isolated it from stands of susceptible European chestnuts where they had noticed a remission of disease. Grente called the new form "hypovirulent" because of its less virulent growth on trees normally susceptible to the fungus. In pure culture, the hypovirulent strain has a lighter color and develops the normal orange pigment of the virulent strain more slowly (Fig. 1).

First experiments with the French hypovirulent fungus were confined to the greenhouse, and other precautions were taken to ensure that the new fungus did not escape into the wild until more was known about it. We grew the fungus aseptically in the laboratory in dishes containing a gelatinous (agar) medium. Then we placed plugs of agar containing the fungus into small wounds made in the bark of chestnut seedlings.

These tests showed that the hypovirulent (H) strain of the fungus grew slowly when placed in an artificial wound, while the normal virulent (V) strain grew rapidly and formed a canker. If the two were placed adjacent to each other, the canker was restricted. The effectiveness of the French H strain in the first test was not clear. However, by using isolates recovered from that experiment and crossing them with other American V strains, we recovered H strains that quickly limited growth of American V strains.

After 2 years of testing in the greenhouse and on small American chestnut seedlings at Lockwood Farm, 12 woodland plots were established in the fall of 1974 and the spring of 1975. Each plot contained 25 native sprout clumps and each clump had one or more stems l

Fig. 1. Differences in appearance of the hypovirulent blight fungus (left) and the virulent strain (right).



inch in diameter or larger. Half the plots were untreated.

In the other six plots, cankers were treated by removing 9 mm plugs of bark from the horizontal extremities of cankers and placing in the holes similar-sized plugs of potato dextrose agar containing the H strain. These inoculation sites were covered with masking tape to prevent drying. The first inoculations were made in the fall of 1974, and subsequent inoculations were made during the 1975 growing season when new infections were noted.

After an initial lag of 1 to 2 months, most of the cankers inoculated with H stopped growing and began to heal. Indeed, the growth of 86% of the treated cankers was arrested compared to only 5% for the untreated cankers.

Whether the H strain will establish itself and spread to developing cankers is yet to be determined. Since this has apparently occurred in Europe, we anxiously await the results in our forest plots. We have also sprayed spores of the H strain on natural cankers to see if inoculation by this means will work as well as the agar plugs.

Much needs to be learned about the biology of the H strain in the field. Since it grows slower and produces fewer spores, we assume that at least in Europe where it is established it maintains itself by growing on other organic matter, perhaps on the litter on the forest floor.

The mechanism of hypovirulence at the cellular level is also under intensive study. There is evidence that a mycovirus, a kind of virus found only in fungi, may be responsible; various approaches are being used to confirm this, including gel electrophoresis, electron microscopy, and serology.

Should the hypovirulent strain prove to be a practical control of the chestnut blight fungus in the United States, it has potential advantages over the use of blight-resistant trees or fungicides. It should be less expensive than a chemical control, and it should allow us to take advantage of the persistent chestnut sprouts that already exist in the woodlands.

Many American chestnuts need only protection from blight and some release from competing vegetation to begin the restoration. If native sprouts are used instead of seedlings, reestablishment of chestnut stands could be speeded by 5 to 10 years.

Regardless of the ultimate success with wide-scale control of the chestnut blight fungus, the results have already opened up a whole new area of inquiry: can hypovirulent forms of other serious fungal pathogens be found or generated to control other plant diseases?

Although the direction of present chestnut research was not anticipated until recently, early research afforded intimate knowledge of the host and the parasite and prepared us to take advantage of this new approach to disease control.

Chestnut Blight Research at the Station

The discovery and subsequent observations of the spreading blight drew diverse interpretations. Station Botanist, G. P. Clinton, blamed the seriousness of the blight on adverse weather that had weakened the trees. In 1908, Clinton said, "While the trouble is no doubt a serious one, we are inclined to believe that its power of spreading and the likelihood of its annihilating all the trees of infected regions have been over-emphasized."

At this same time, H. Metcalf, of the U. S. Department of Agriculture, declared: "Unless something now unforseen occurs to check its spread, the complete destruction of the chestnut orchards and forest of this country, or at least the Atlantic states, is only a question of time." Metcalf was right.

Between 1920 and 1936, chestnut research in Connecticut took a back seat to other pressing agricultural problems. Even P. L. Anderson, who was in charge of the Windsor laboratory from 1925 to 1953 did not find time for chestnut, although he had earlier conducted classical studies in Pennsylvania on the biology of the blight organism.

In 1936, however, the annual report of the Station stated: "Experiments were more extensive than previously because of the cooperation of Dr. A. H. Graves of the Brooklyn Botanic Garden in New York." Some of his first work with chestnut was traveling the rural dirt roads of Massachusetts by motorcycle to survey for the spread of the blight fungus.

It was not until 1929, however, when he was over 50, that Graves began breeding chestnuts, doing most of the work on his own time and on his own land. Dr. Graves enlisted the aid and advice of Donald F. Jones, chief of the Genetics department. Blight-resistant species and hybrids proliferated at the Lockwood Farm of the Station.

In 1947, a Yale graduate student, Hans Nienstaedt, came to the Station to study chestnut. After he completed his dissertation on blight resistance, he took charge of the chestnut research. Graves continued as an active participant and consultant, donating the chestnut plantation he owned on the Sleeping Giant to the State.

Recently, John Puhalla and Neil Van Alfen have made important contributions. Present participants include Richard Jaynes, Sandra Anagnostakis, Peter R. Day, and John Elliston. Now primary attention is being given to biological control of the chestnut blight.

R. A. Jaynes

Physics and Insect Pheromones

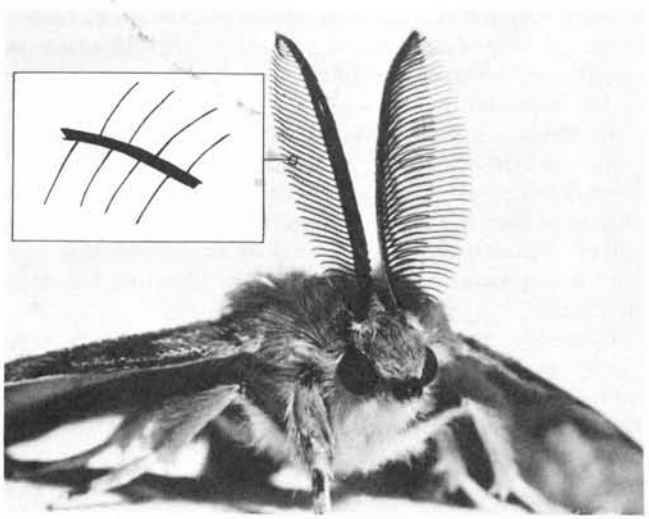
By Donald E. Aylor

Pheromones are volatile chemical odorants that are released into the air by insects to communicate information, often over large distances. Once emitted, a pheromone depends upon the wind for its transmittal. Insects use pheromones in many ways: for example, they may signal danger or they may attract male insects to mate with virgin females. This attractiveness is especially important for some insects like the gypsy moth because the female does not fly, and their procreation depends largely upon the ability of the female to "call" a male for mating.

Since many insect pheromones have been identified and synthesized by biochemists, the prospect of using synthetic pheromones to jam communications and thus disrupt insect mating is an attractive means of biological control currently under study at this Experiment Station and elsewhere. Effective use of pheromones requires a basic understanding of the behavioral responses of insects to the amount of pheromone as well as an understanding of its physical dispersion and dilution by the atmosphere.

The response of the moth depends on the concentration of pheromone (g/cm^3) in the air filtering through its antennae (Fig. 1). Pheromones are biologically active at such low concentrations that even our most sensitive analytical instruments are overtaxed. The sensitivity required is equivalent to finding a single special kernel of wheat in all of the wheat kernels harvested in the United States in 1 year. Since our ability to measure is meager, it is particularly important to estimate pheromone concentration from a knowledge of the amount of material released and of atmospheric mixing processes, or turbulence. The physicist's role is to predict this aerial concentration. The challenge is great because dilution by the atmosphere does not proceed in an orderly way.

Fig. 1. A male gypsy moth showing antennae. Fine hairs (inset) act as efficient filters of odorous molecules from the air.



We must know the instantaneous peak concentration in the air rather than the average.

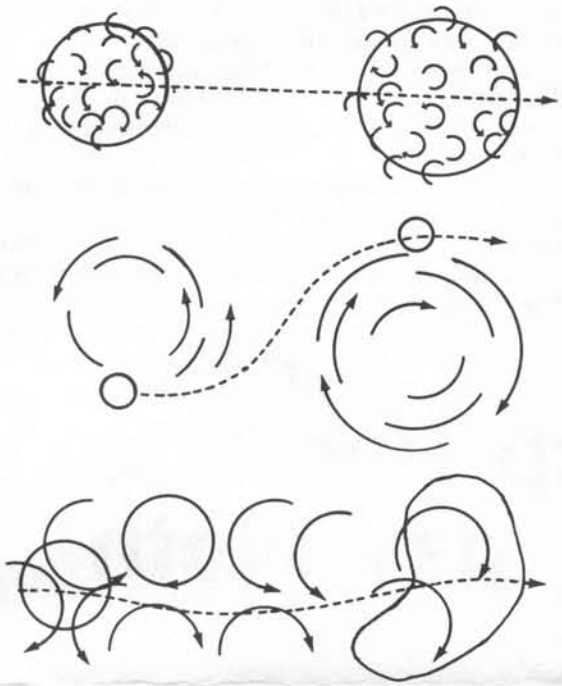
Turbulent movements of the wind appear to be random and as such defy prediction in detail. Fortunately, however, if many repeated observations of wind are made, and these readings are averaged, a pattern begins to emerge. Such an orderliness often results from averaging large numbers of "chance" events, as when we repeatedly toss a coin. While we have no way of predicting the outcome of an individual coin toss, we can expect that, after many tosses, about half the outcomes will be heads and about half will be tails.

A similar averaging allows us to predict the concentration during a suitably long time of a substance emitted into a wind whose average characteristics do not change in time or space. This averaging gives a smooth distribution of concentration in the air that can be described mathematically. For diffusion in the atmosphere, such a smooth pattern usually emerges only after averaging for 10 or 15 minutes.

By averaging the wind, however, we have sacrificed detail for simplicity. To decide if our method of averaging the pheromone concentration over time has sacrificed too much, we must consider how an insect senses and responds to pheromone. Insects sense the absorption of the chemical pheromone on sites within their external antennae (Fig. 1). Electrophysiological studies indicate that the response is regulated by the rate of pheromone molecules hitting active sites on the antennae. Therefore, both the concentration of pheromone and the rate of air movement past the antennae are important because they combine to give dosage.

To select a suitable averaging time for the atmospheric problem we need to know how quickly a gypsy moth responds to a given amount of pheromone. The time course of a sensation to a given stimulus has received considerable study, particularly in man. The general course is a brief period when the sensation to a

Fig. 2. Idealized dispersion (redrawn from Meteorology and Atomic Energy, D. H. Slade, editor). In eddies smaller than the puff, it grows slowly and concentration decreases slowly (top). Eddies larger than the puff transport it intact along a serpentine path, and concentration is reduced little (middle). Finally, eddies about the size of the puff tear it apart and rapidly diminish concentration (bottom). In the atmosphere all of these processes occur together.



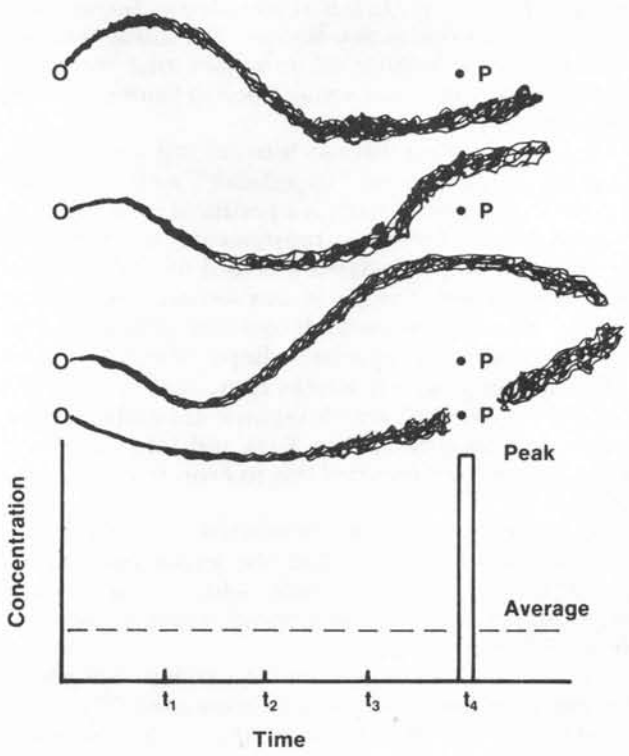
constant stimulus increases sharply with increasing exposure time (temporal summation), followed by a period that is usually longer when the sensation slowly decreases (adaptation).

Although man can communicate his sensation to levels of loudness, brightness, or smell directly to an experimenter, information about insects must obviously be obtained indirectly. These indirect studies are generally of two types: electrophysiological, in which nerve endings are probed with tiny electrodes to measure the electric signals induced by the pheromone; and behavioral, in which an excited state of the moth, such as rapid beating of wings induced by pheromone, is observed. The electrophysiological studies show that enough electrical impulses for response are triggered within about a half second of exposing the antennae to pheromone, while behavioral studies show that moths respond within about 2 seconds of exposure. Because this response is fast, we must know the brief maximum concentration in the air rather than the average discussed above. This requires a deeper understanding of turbulence than the mere averaging I indulged in above.

A fundamental feature of turbulence is that it consists of parcels of fluid or eddies ranging from a fraction of a centimeter to many meters in size, all undergoing simultaneous random movements. Such a chaotic pattern is visible in the turbulent wake of a boat. An idealized picture of how a cloud of pheromone is dispersed by eddies of various sizes is shown in Fig. 2. Eddies much larger than the puff of pheromone essentially transport the puff as a whole and cause it to meander in a serpentine way, while eddies much smaller than the puff tend to redistribute the pheromone within the puff but dilute it little. Eddies that are about the same size as the puff reduce the concentration most effectively. The familiar looping of a smoke plume from a tall chimney when the air near the ground is warmer than the air aloft offers a good illustration of meandering.

Meandering due to large eddies is a major cause of differences between maximum and average concentrations. Referring to Fig. 3, we see that a sampler continuously inhaling air at position P will sometimes sample the cloud and sometimes it will not. Earlier, when we spoke of average concentration, we included the times when the plume missed the sampler, thus adding zero material to the total and reducing the average concentration. Clearly, since a moth senses the brief maximum pheromone concentrations that it will encounter at a given distance from the source, those "zeros" are meaningless and thus we move our sampler with the serpentine movements of the plume, seeking the maxima.

Fig. 3. Successive schematic "snapshots" of a smoke plume coming from point O and being sampled at point P. Sometimes the plume passes through P, but at other times it misses P altogether.



For experiments with gypsy moths in the forest we accomplished this by releasing fluffy seeds that drifted with the wind and thus outlined the invisible pheromone plume. At various distances from a source of pheromone we moved male moths in screen cages in the plume. We detected the presence of pheromone by observing the rapid and ample wing-fanning that is a characteristic response of the male to pheromone. Thus we have considered diffusion relative to the plume rather than relative to a fixed position in space.

Again, for a wind that is uniform in time and space, we can predict how this dilution relative to the puff center will proceed. Recalling the random nature of turbulence and our coin tossing example, we know that we must again speak of averages, but now, however, our average excludes the large eddies responsible for meandering. By considering diffusion relative to the plume, we predict maximum concentrations near the source that are about 25 times greater than averages relative to a fixed place on the ground.

The importance of relating maximum pheromone concentrations to moth behavior will now be illustrated. Pheromone-baited traps have proven useful for monitoring the increase of insects and thus improving the efficiency of pesticide applications. For an accurate census the insects must land on them. Just as a high concentration can change an insect's behavior from direct upwind flight to a zigzag, still higher concentrations apparently make some insects slow down and land. Such a landing, if premature because pheromone concentrations were too high outside the trap, would obviously cause an underestimate of the insect populations.

It remains for entomologists to define insect behavior in terms of the level, or concentration, of pheromone in the air. Then, the methodology outlined here can help them to dispense the proper amount to achieve the desired results.

Improving a Wasp Parasite to Control the Gypsy Moth

By Ronald M. Weseloh

Releasing *Apanteles* could increase the parasite's effectiveness, while avoiding the problems caused by hyperparasites.

The gypsy moth, *Lymantria dispar,* has been decreasing since 1971, but it has always become less abundant after outbreaks. We are continuing research into its control because we anticipate that the gypsy moth will again become a major pest in Connecticut, as it has in the past.

One encouraging approach to control is the use of parasites. The recent experience with the elm spanworm in Connecticut is a pertinent example. The elm spanworm, *Ennomos subsignarius,* together with the gypsy moth, defoliated a record 655,107 acres in 1971. But within 2 years, it was virtually eliminated from Connecticut woodlands by a tiny, stingless wasp.

The reproductive capacity of the parasite, *Ooencyrtus ennomophagus,* which attacks eggs, is so great that it was able to overtake the elm spanworm and cause the outbreak to collapse. Harry Kaya and John Anderson of the Station staff reported this in *Frontiers 24:*(2) May 1972.

But one major difference between the elm spanworm and the gypsy moth is that the gypsy moth is an imported insect. Unfortunately, when it was accidentally released in the United States, all of its parasites were left behind in Europe.

To fill this void, a number of parasites have been successfully introduced into this country. These are listed in Table 1. These parasites already do some good, but unfortunately, as 1971 demonstrated, they are not as effective as the parasite that eliminated the elm spanworm.

I have been studying gypsy moth parasites in hopes of finding ways to make them more effective. The one that I have been working with most is less than a quarter-inch long. It is a stingless wasp called *Apanteles melanoscelus,* which is shown in Fig. 1.

Table 1. Imported parasites that attack gypsy moths in North America.

Parasite	Type	Host Stage	Generations per Year
Anastatus disparis	Wasp	Egg	1
Ooencyrtus kuwanai	Wasp	Egg	2-4
Apanteles melanoscelus	Wasp	Young larva	2
Phobocampae disparis	Wasp	Young larva	1
Exorista larvarum	Fly	Larva	Numerous
Compsilura concinnata	Fly	Larva	Numerous
Parasetigena silvestris	Fly	Larva	1
Blepharipa pretensis	Fly	Larva	1
Brachymeria intermedia	Wasp	Pupa	1-2

Apanteles has two generations a year. Adults emerge from overwintering cocoons around the first of May and lay eggs in young gypsy moth larvae. When the larval parasites are fully developed, they burrow through the sides of the dying caterpillars to spin white cocoons around themselves. In early to mid-June the second generation adults that emerge from these cocoons attack intermediate-sized caterpillars. The progeny that develop in these caterpillars spin cocoons and hibernate in crevices on tree trunks from July to May when the cycle begins again.

Last summer I found that *Apanteles* females cannot readily lay eggs in moderately large caterpillars. This is because the parasite cannot easily get through their long hairs. The large caterpillars also thrash about as they are attacked, often shaking the wasps off. But even if the parasites lay eggs in the younger caterpillars, most of their offspring cannot develop fast enough to emerge as adults before the older caterpillars are too large for them. As the second generation of *Apanteles* finds only a few late-developing small caterpillars of the gypsy moth that have developed late and are small enough to be attacked successfully, the percentage of gypsy moths parasitized is often less in June than it is in May (Fig. 2).

It might be possible to overcome this problem by selecting a strain of the parasite that can successfully attack large caterpillars, or one that develops fast enough to attack younger, and thus smaller caterpillars.

Fig. 1. Adult *Apanteles melanoscelus*.

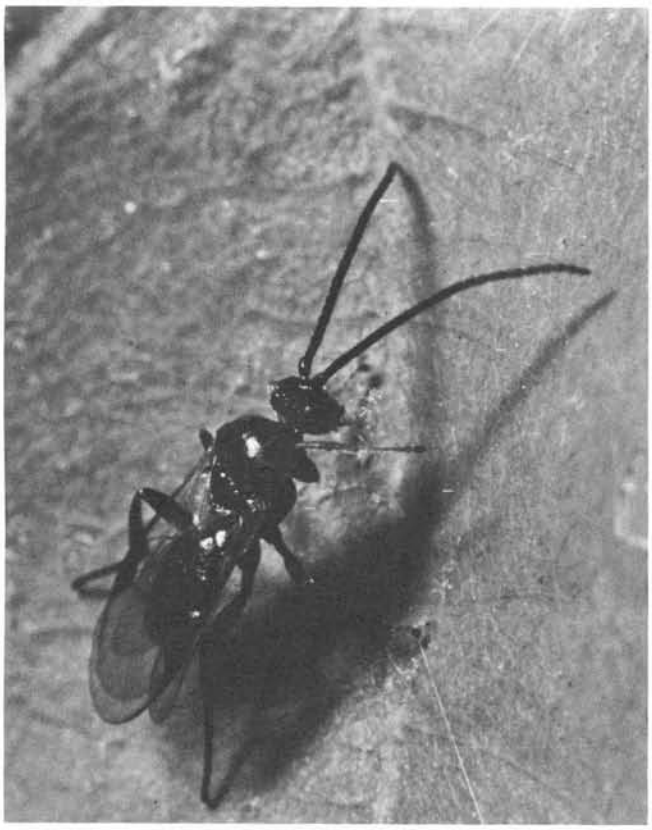
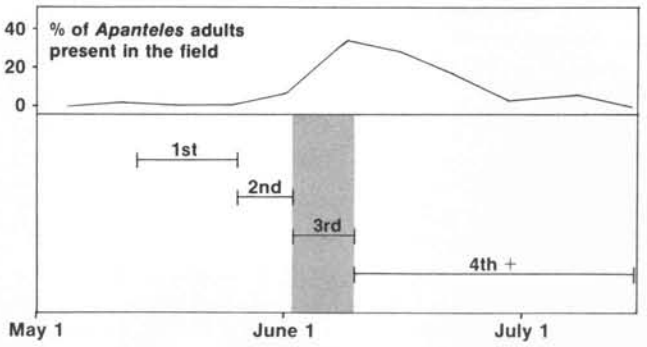


Fig. 2. Relation of *Apanteles melanoscelus* adult abundance to seasonal abundance of gypsy moth instars. The section of the figure to the left of the shaded area shows when almost all hosts are acceptable to *Apanteles*. To the right of the shaded area hardly any hosts are acceptable, and in the shaded area only some hosts are acceptable. Bracketed lines represent periods when 50% or more of the caterpillars are in the instars indicated.



Other *Apanteles* strains from Europe or Asia may already have these characteristics.

Even if *Apanteles* could successfully attack large caterpillars, however, there would still be another problem: The overwintering cocoons of *Apanteles* are heavily attacked by other parasites. About 36 species of these hyperparasites attack *Apanteles*, primarily during July. These parasites of a parasite are called hyperparasites. Since hyperparasites can destroy over 90% of the *Apanteles* in cocoons, it is indeed remarkable that *Apanteles* is as abundant as it is.

Currently I am studying ways to overcome these hyperparasites directly. Because there are many of them, and the interactions between them and their host are so complex, progress is slow.

The indirect solution of rearing large numbers of *Apanteles* for release early in the spring when gypsy moths are hatching may be more promising.

For two summers John Anderson and I carried out release experiments. We reared 18,000 *Apanteles* in the laboratory on gypsy moth larvae fed with an artificial diet of wheat-germ, agar, and other ingredients. Adult female *Apanteles* were introduced into the containers to lay eggs in the caterpillars. Eventually the *Apanteles* larvae emerged to form cocoons which were placed in areas where gypsy moth caterpillars were found.

Caterpillars were collected weekly to find how many were parasitized by *Apanteles*. Where cocoons were set out, we found *Apanteles* in up to 44% of the gypsy moth larvae as compared to 0 to 10% where no parasites were released.

This experiment shows that releases could increase the effect of *Apanteles* while avoiding problems caused by hyperparasites. Unfortunately it is difficult in the laboratory to rear the number that would be needed for practical control because the parasites will grow readily only on gypsy moth caterpillars. To overcome

Continued on Page 8

THE CONNECTICUT
AGRICULTURAL EXPERIMENT STATION
NEW HAVEN, CONNECTICUT 06504
Paul E. Waggoner
Director
PUBLICATION
PENALTY FOR PRIVATE USE, $300

An Equal Opportunity Employer

POSTAGE AND FEES PAID
U.S. DEPARTMENT OF
AGRICULTURE
AGR 101

U.S.MAIL

THIRD CLASS
BULK RATE

Improving a Wasp Parasite

Continued from Page 7

this, I have been searching for an artificial diet for *Apanteles*. A substitute will be difficult to find because the parasite naturally lives in a specialized environment, constantly bathed in gypsy moth blood.

However, I have been successful in getting young *Apanteles* to grow somewhat in glass vials containing gypsy moth blood. If these *Apanteles* will develop in insect blood, they will probably also develop in nutrient solutions of vitamins, amino acids, minerals, sugars, and lipids that are especially formulated to grow insect tissue.

As part of this work, I have found that *Apanteles* females recognize particular characteristics of gypsy moth larvae. These include hairiness and one or more chemicals in the integument or skin. *Apanteles* should be induced to lay eggs in an artificial host having these characteristics, and if it were filled with the proper nutrients, young parasites might grow without gypsy moth caterpillars.

Even if this were possible, however, it would not be feasible to release the parasites everywhere gypsy moths occur. We would probably have to restrict releases to where gypsy moth numbers are likely to increase drastically and keep the wasps from flying out of the area where they were released. A way of keeping the parasites in the endangered place may come from another study in which I have found that *Apanteles* intensely examine silk produced by gypsy moth larvae. It is clear that a chemical attractant is involved because *Apanteles* will not examine silk from other caterpillars, or gypsy moth silk that has been washed. If this attractive chemical can be isolated and then sprayed into an area, the *Apanteles* may search intensely and remain where they are released.

These studies have shown why *Apanteles* cannot adequately control the gypsy moth and have pointed out one way to improve it somewhat. I am optimistic that other improvements are possible.

Frontiers of Plant Science published in May and November, is a report on research of The Connecticut Agricultural Experiment Station. Available to Connecticut citizens upon request.
Vol. 28 No. 2 Spring, 1976 ISSN 0016-2167 Paul Gough, *Editor*