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SURVEY FOR POTENTIAL BIOLOGICAL CONTROL AGENTS FOR *MYRICA FAYA* IN THE AZORES AND MADEIRA

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ABSTRACT

Myrica faya (firetree), an aggressive alien shrub or tree infesting Hawaiian forests, is native to the Azores, Madeira, and the Canary Islands. In 1984 Donald Gardner and Charles Hodges, plant pathologists, traveled to those regions in the North Atlantic Ocean to search for natural enemies (of *M. faya*) which could be utilized as biocontrol agents. The results of their study were published in a previous CPSU/UH technical report.

A second exploratory trip, discussed in the present report, was conducted to the Azores and Madeira in 1987. Gardner and Hodges were accompanied by George Markin, an entomologist, to search for, collect, and return for testing potential biocontrol insects and disease agents. During the second trip, the previously discovered fungi were collected, as were several insect species. Of the fungi, *Ramularia destructiva* and *Nectria galligena* were returned under permit to Hawai'i, and *Cryphonectria* sp. was returned to North Carolina State University for further study. Of the more than 20 insect species found on *M. faya*, *Phyllonorycter myricae*, *Carposina atlanticella*, an unidentified geometrid, an unidentified shoot tip borer, and an unidentified staminate flower feeder were returned to quarantine in Hawai'i for further study.

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INTRODUCTION

In 1984, Donald Gardner, a National Park Service (NPS) research scientist, and Charles Hodges, then with the U. S. Forest Service (USFS), spent 3 weeks in the Azores, Madeira, and Canary Islands in search of potential biocontrol agents for <u>Myrica faya</u> (Hodges and Gardner, 1985). This tree, commonly known as firetree or fayatree in Hawai'i, has become an aggressive weed threatening native habitats since its introduction to Hawai'i in the early 1900s (Whiteaker and Gardner, 1985). Firetree is native to the above island groups in the North Atlantic Ocean.

Several insects and pathogenic fungi were noted which appeared to have promise as biocontrol agents. Specimens and cultures of two fungi--<u>Nectria</u> sp. (Ascomycetes) (now identified as referrable to <u>N</u>. <u>galligena</u>) associated with large stem and branch cankers, and <u>Ramularia destructiva</u> (Fungi Imperfecti), which causes a shoot blight--were collected and stored in the USDA Plant Pathogen Containment Facility at Fort Detrick, Maryland. United States Department of Agriculture, as well as Hawaii State Agriculture Department regulations prohibit introduction of unidentified organisms except to a certified quarantine facility. However, prior to the trip it was not possible to anticipate which pathogenic fungi would be encountered during exploration. For this reason, permits could not be obtained to bring these fungi directly to Hawai'i for testing. Although several insects were seen feeding on <u>M</u>. <u>faya</u> during the 1984 trip, no living material was collected since it was again not possible to anticipate permitting specifications. A report on the observations made during the 1984 trip, along with historical aspects of previous attempts at biocontrol of <u>M</u>. <u>faya</u>, has been published (Hodges and Gardner, 1985).

More recently, permits were obtained to bring <u>R</u>. <u>destructiva</u> and <u>Nectria</u> sp. into Hawai'i for evaluation in the laboratory as biocontrol agents for firetree. An approved insect quarantine facility, located at Hawaii Volcanoes National Park (HAVO), has also been completed since the 1984 trip, which permits testing of introduced insects in Hawai'i. In order to collect living insects and additional fungus cultures, another trip was made to the Azores and Madeira during May and June, 1987. The research team consisted of Donald Gardner, NPS plant pathologist; George Markin, USFS entomologist; and Charles Hodges, retired USFS forest pathologist, now associated with North Carolina State University. Local personnel, who were generous in their friendliness and cooperation, included Mr. Silva, director, and several other officials of the Servicos Florestais (Forest Service) of the Azores. These personnel aided with transportation and in providing direction and information on firetree sites. Also, Dr.

Victor H. Forjaz, a faculty member of the university at Ponta Delgada, and Dr. Martin, the biology department chairman of the same university, were helpful in arranging possible cooperative efforts for the future. Dr. Martin is himself engaged in the study of biocontrol.

Generally the same areas in the Azores and Madeira, determined previously as probably the best sites for productive use of time, were visited as in 1984. In the Azores, the islands of Sao Miguel, Terceira, Pico, and Faial were again visited. These had been previously determined as the locations with the best stands of firetree. During the current trip, however, additional sites on the western side of Terceira and the eastern side of Pico were surveyed. None of these new sites was particularly productive, however, since they are largely agricultural and M. faya is found only as individual plants along roadsides and hedgerows.

On Madeira, areas adjacent to the cross-island highway from Ribeira Brava to Sao Vicinte were surveyed for the first time. This proved to be an excellent area with M. faya present from about 2,250 feet elevation on the southern slope (according to previous reports, the plant is present only on the northern slopes) to sea level on the northern slope. Considerable area of laurel forest[1] containing M. faya, a secondary canopy component, is present on the north slope along this highway. On the south slope between 2,300 and 2,800 feet is a relatively large pine plantation on cleared laurel forest. Numerous young M. faya originating from resprouting stumps occur in the plantation, many of which were attacked by R. destructiva and several insects.

Details of the climate, geography and plant communities in the Azores and Madeira were given in the report of the previous trip (Hodges and Gardner, 1985) and will not be repeated here.

OBSERVATIONS

Diseases

No new pathogenic organisms were found during the current trip, but further observations were made on those discovered previously. As before, symptoms of the most common and widespread disease observed were stem and branch cankers (Hodges and Gardner, 1985: Fig. 17). Also, as before, a species of Nectria was consistently associated

[1]consisting principally of four native tree species in the Lauraceae family--Laurus azorica, Ocotea foetens, Persea indica, and Apollonias barbujana

with the cankers, often fruiting profusely at the canker margin. The fungus was collected and cultures obtained from 14 locations in the Azores and Madeira, including all islands visited in the Azores.

No species of Nectria has been reported previously from M. faya. Although morphological and cultural characters do not fully correspond to known species, U. S. Department of Agriculture specialists in the genus Nectria now consider this fungus to be referable to N. galligena which is usually associated with canker diseases. This pathogen is considered the most serious parasite among several species of Nectria known to attack a large number of hardwood trees and to be widely distributed in Europe and North America (Alexopoulos and Mims, 1979). Nectria galligena causes the European canker disease, in which the bark and vascular tissues are severely disrupted and the tree becomes girdled. Also, bark cankers are frequently the sites of entry for other pathogens and insects, and may predispose the tree to mechanical injury such as wind breakage. Since no other associated tree species were observed with Nectria cankers, the N. galligena attacking firetree may represent a race or form specific to this host.

Shoot dieback, caused by Ramularia destructiva (Hodges and Gardner, 1985: Fig. 19) was again found in most areas visited but damage was extremely variable, being heavy in some locations and relatively light in others. Although R. destructiva, like other leaf or shoot-infecting pathogens, probably is not capable of causing immediate death of the plant itself, the destruction of shoots observed on heavily-infected plants was considered to have a significant potential impact on the growth and vigor of the plant. However, as noted previously (Hodges and Gardner, 1985), there appears to be considerable resistance within populations of M. faya to this fungus, with heavily and lightly infected plants often occurring side by side. The effectiveness of this fungus as a biocontrol agent will depend to some degree on the susceptibility of the Hawaiian populations of M. faya. Collections and cultures of the fungus were made in 13 locations during the trip.

During the 1984 trip, two collections of a Cryphonectria sp. (Ascomycetes) associated with stem cankers were made on the islands of Terceira and Pico. The fungus could not be further identified because only the imperfect state was present. During the current trip, a special effort was made to find additional collections. The fungus was found on all islands visited in the Azores and in Madeira. However, it was not especially common and in at least two cases was present with Nectria on the same canker. Although this infection characteristic could indicate that the Cryphonectria fungus is a secondary invader, subsequent testing, discussed under RESULTS AND

DISCUSSION, demonstrate its pathogenic potential. Cultures of Cryphonectria were obtained in six areas and a permit may be requested for testing it in Hawai'i. Although the fungal material is not plentiful, a few specimens were found with the perfect state which should allow it to be identified to species.

Insects

Because previous visits to the native habitats of firetree were made by F. Bianchi in 1955 and by N. L. H. Krauss in 1960 and 1962, also for the purpose of discovering potential biocontrol agents, no unreported species of insects attacking this plant were expected to be found. The previous investigators had conducted extensive surveys of the insects associated with *M. faya* and had identified over 50 species (Krauss, 1964). However, while Krauss and Bianchi both made numerous shipments of potential biocontrol agents to quarantines in Hawai'i, none could be adequately propagated for testing and none was released. Eventually, a small moth, *Strepsicrates smithiana*, was collected from the related plant *M. cerifera* (southern waxmyrtle) in Florida, and introduced to Hawai'i with the hope that it would also attack *M. faya* (Weber, 1957). In Hawai'i this insect has been found only on *M. cerifera*, and with negligible impact (Davis and Krauss, 1962). It has not been found in nature attacking *M. faya*. *Myrica cerifera* occurs at lower elevations in the Panaewa area on the Island of Hawai'i.

With regard to insects, the current visit to the native habitats of firetree was made for the following purposes:

1. To collect larvae or pupae of the more abundant and promising insects, and raise them to adults which could be used for obtaining positive identifications. Many of the insects collected by Bianchi and Krauss were either never identified, identified only tentatively, or may have subsequently undergone taxonomic changes.

2. To select the most promising potential biocontrol agents for further work, based on direct observations in the field on the feeding behavior and biology of the insects associated with *M. faya*.

3. To collect and transport small colonies of the most promising insects to quarantine in Hawai'i and attempt their propagation. If rearing techniques could be developed, to begin colonies for use in host testing to determine their suitability for release in Hawai'i.

Limited time restricted the amount of field work that could be conducted, so collections were primarily from easily obtained foliage, flowers, and fruit of shrubs and small trees. Observation on mature trees and a search for boring insects that might attack larger branches, trunks, and roots was not attempted. Insects were found by direct observation, by beating branches onto a spread cloth, or by collecting bouquets of branches and maintaining them enclosed in plastic bags for several days. A collection of frass in the bags indicated the occurrence of insect feeding. Once collected, insect larvae were placed in petri dishes and fed the appropriate part of the plant, or placed on a small bouquet of foliage and enclosed in a plastic bag. Observations were made daily to determine feeding behavior and rates of development, as well as to obtain pupae, cocoons, or any parasites that might emerge. Much of the rearing was done in the field, but due to the nature of the trip, large numbers of larvae or pupae were shipped to the HAVO quarantine facility. Observations and attempts at rearing the insects were continued at that facility with M. faya foliage collected in Hawai'i.

RESULTS AND DISCUSSION

Diseases

Two of the fungi (Ramularia destructiva and Nectria galligena) isolated from diseased firetree tissue were cultured on artificial growth media and the cultures returned under permit to Hawai'i for testing. Newly developed foliated twigs collected from Hawaiian firetree populations were maintained under controlled laboratory conditions with cut ends in water. They were inoculated by spraying with aqueous spore suspensions of R. destructiva. However, to date no evidence of infection has been observed after several inoculation trials. Symptom manifestation would be expected to occur within the observation period if host tissue were susceptible; however, no information is available on the developmental characteristics of this disease.

Mycelial tissue of N. galligena was placed under cut bark of firetree twigs likewise maintained in the laboratory with the cut ends in water. Although some discoloring of wood has been observed, to date this infection has not resulted in the potentially disruptive cankers evident on firetree in its native habitats. It is possible that cankers exhibiting the type of advanced development observed in the field may have resulted from an extended period of slow development.

Cultures of Cryphonectria sp. from the Azores were returned to North Carolina State University for testing on M. faya stem sections from Hawai'i. Although results are still preliminary, this fungus shows the best evidence, among the tree pathogens observed and collected in the native habitats, of ability to attack firetree from

Hawai'i. This would indicate that this unidentified species of _Cryphonectria_ is not merely a secondary invader but is a virulent pathogen on this host. The potential of this pathogen for virulence is exhibited by the closely related fungus _C._ (=_Endothia_) _parasitica_, which was responsible for the well documented and severe decimation of stands of American chestnut throughout the eastern U. S. in the early 1900s. This former dominant tree species of eastern hardwood forests was reduced to a minor understory shrub and the European chestnut was threatened with extinction by the chestnut blight disease (Alexopoulos and Mims, 1979). Another member of the genus _Cryphonectria_, _C. cubensis_, causes a serious canker disease of _Eucalyptus_ spp. in many tropical areas of the world (Hodges et al., 1986).

Insects

During the survey, over 20 insect species were found feeding directly on _M. faya_. The more common insects with feeding behaviors indicating potential as biocontrol agents were:

Phyllonorycter myricae, a leaf roller. This small moth (Deschka, 1976) was the most common insect found on _M. faya_ at all locations in both the Azores and Madeira (Fig. 1). The first instar larva begins feeding as a leaf miner on the underside of the leaf and eventually forms a small chamber on the leaf edge by rolling the leaf inward. When approximately half grown, the larva migrates from the leaf edge roll to a new leaf where it begins webbing the leaf tip back and underneath to form a cylinder, then seals the cylinder ends to form a small chamber in which it completes feeding (Fig. 2). While the larva attacks two leaves in its life cycle, its feeding is seldom extensive enough to kill either, but results in 50 - 80% destruction of the photosynthetic surface. Furthermore, wounding allows pathogens to enter and may trigger early leaf drop.

The impact of a single larva may seem insignificant; however, if _P. myricae_ can be freed from its natural enemies when released in Hawai'i, allowing development of higher populations than were observed in the native habitats, continued defoliation of all new foliage could eventually result in severe stress or outright killing of the plants. Observations indicate that the insect can complete its life cycle in approximately 2 months and has a very high reproductive rate. Preliminary sampling also indicated that in the field natural mortality of the larvae was quite high (88%) and was caused in part by attack by an unidentified parasitic Hymenoptera, pathogens, and an unidentified predator that chewed into the leaf roll and consumed the larva. These observations indicate that the insect, if removed from its enemies, should be capable of very rapid population increase if successfully established in a new area.

Lepidoptera: Geometriidae. This unidentified looper was the second most widely distributed insect encountered. Newly hatched larvae, approximately 3 mm long at eclosion, grew to 3 - 3.5 cm just before pupation (Fig. 3). The larva undergoes several different changes in colors and markings during its growth, which range from a light gray at eclosion to green, then to yellowish brown, and darker brown at pupation. Adults (Fig. 4) are brownish moths with a wing span of 4 - 5 cm. The insect has several generations per year and completes a life cycle in 60 - 80 days. In the field, larvae are solitary and seldom feed on a single leaf for more than a few hours before moving. However, when confined to a cage, a single larva can readily defoliate a 6-inch shoot of foliage while completing its development.

Carposina atlanticella (Carposinidae), a fruit feeding moth. The small (4 - 5 mm), reddish larvae of this moth bore into the developing fruit and consume the seed (Fig. 5) (Hodges and Gardner, 1985). Attacks are easily visible since an attacked fruit is marked by a collection of dark frass at the feeding entrance. Several larvae attacking the same fruit cluster can readily destroy 90% or more of all seeds. On the islands of the Azores visited, firetree was in early to mid stages of flowering with almost no fruit present. Fruit feeders were therefore not observed in most locations, with the exception of one location on the west end of the island of Faial where fruit had already set. On Madeira, fruit was 50 - 90% developed and *C. atlanticella* was found at all sites sampled. In observations made in the laboratory, caterpillars which finished feeding spun a tight, thick cocoon adhering closely to the bark of a small stem and camouflaged it by covering it with a layer of frass particles. The larvae remained inside the cocoons through the remainder of the summer and following winter. It is presumed that in the field the adult would emerge in the spring. This indicates that this species has a single generation per year which is synchronized with the flowering peak of *M. faya*.

Shoot tip borer. The small caterpillar of this unidentified moth is similar in size and appearance to the larva of *C. atlanticella* described above. However, instead of attacking the fruit this insect mines the terminal 5 - 10 cm of the developing shoot (Fig. 6) (Hodges and Gardner, 1985). The entrance hole is located either at the tip of the shoot or somewhere along its side and is usually marked by a collection of ejected frass. Tips which have been fed in for an appreciable length of time can be recognized by the wilting or dead leaves. No sign of this insect was seen in the Azores, although several branch tips that may have been killed the previous year were noted. The shoot tip borer is probably present in the Azores, but was not encountered so early in the season in that region, which is north of Madeira.

On Madeira, the insect was found at all locations visited, but while widespread, it was never very abundant and a search of up to 30 minutes might reveal only one or two larvae. Only in the vicinity of Ribeiro Frio could the larvae be easily found. Even at this location, however, attack occurred on only 1 - 2% of the available shoots. This low population was unexpected since during previous visits (Hodges and Gardner, 1985; Krauss, 1964) insects were much more abundant. Attempts to rear this insect were made by cutting the infected shoots, placing the cut ends in water and covering them with plastic bags. However, survival was very poor and few larvae were successfully transported to Hawai'i. Those that did complete larval development and spun cocoons were still prepupa 4 months later, indicating that this insect also has a single generation per year and needs some environmental factor to break diapause.

Staminate flower feeder. At the locations in the Azores where *M. faya* was in full flower in early June, adults of this small, unidentified moth (length 4 mm, wingspan 8 mm) were common flying around and resting on staminate plants (Fig. 7). The small (3 - 4 mm) reddish larvae were found at all locations sampled (sea level - 1,200 ft.) feeding inside the developing, or open, staminate flowers. Larvae returned to Hawai'i spun a small, white elongated cocoon in the dried remains of the staminate flower and emerged as adults in 2 - 3 weeks. Adults showed no inclination to attack shoots containing staminate flowers, but usually hid in the plant or old flowers. The insect is therefore suspected of having a single generation per year, to overwinter as an adult and to have a life cycle which is tightly synchronized with the occurrence of staminate flowers in spring.

Other insects. A large number of other insects was observed feeding on firetree, including several other Lepidoptera larvae, another small leaf mining Lepidoptera, several aphids and leaf hoppers, a large weevil, and several small weevil species. Many of these insects may be general plant feeders, but it is presumed that at least some of them are highly specific feeders associated only with *M. faya*. These insects represent a large pool of potential biocontrol agents which should be investigated. Establishment of complete identities of all heretofore unidentified insects on *M. faya* from the Azores, Madeira, and the Canary Islands, resulting from previous as well as current exploration, is an essential part of the biocontrol program. Any information in the literature concerning the life histories, feeding behavior, and host specificity of these species can thereby be utilized.

CONCLUSIONS

Observations of the current trip support the results of the 1984 exploration (Hodges and Gardner, 1985), which indicated that in comparison to other plant species, relatively few pathogens appeared to attack M. faya in its native habitats. The inability thus far to achieve infection with R. destructiva on firetree from Hawai'i may indicate the resistance among Hawaiian populations that was evident in the Azores. If this degree of resistance exists in Hawai'i, the effectiveness of R. destructiva as a biocontrol agent would be highly questionable.

Likewise, the apparent lack of virulence of N. galligena may indicate resistance in Hawai'i to this fungus. However, it is also possible that the process of canker formation is slow and that more time is needed to obtain positive results. Such longer-term testing would require use of potted plants maintained under quarantine conditions. Although attempts are made to minimize the effects of artificial conditions as much as possible, the controlled laboratory environments required for pathogenicity tests with foreign organisms may also affect the expression of certain diseases such that they do not resemble their development or symptoms in the field (D. Gardner, pers. obser.). Studies on the identity and pathogenicity of the Cryphonectria fungus are continuing.

Based on the studies of the early exploratory entomologists and the results of the 1987 trip, it is obvious that M. faya in its native regions supports a large and diverse community of insects, many of which appear to have evolved a life history highly synchronized with that of their host plant. According to preliminary indications, therefore, many of these insects should be sufficently host-specific to be ideal as biocontrol agents. Colonies of two insect species (P. myricae and the Geometrid looper) have been successfully established in the HAVO quarantine facility and will be used in host testing to determine their feeding specificity. The other three candidate insects were successfully brought to Hawai'i but did not complete their development. They remained in diapause as prepupae or adults. Identifying the environmental conditions necessary to break diapause will be necessary before laboratory colonies can be established. Additional work is needed on the complex of the other M. faya insects encountered in the native regions. In particular, a detailed survey is needed to determine any stem and root boring insects which might be present. Several foliage feeders have already been identified, as well as a shoot tip, a fruit, and a flower feeder. These observations indicate presence of a complex that has evolved in close association with this plant. It is therefore likely that other parts of the plant not examined to date, (i.e. root, stem) also have associated insects.

ACKNOWLEDGEMENTS

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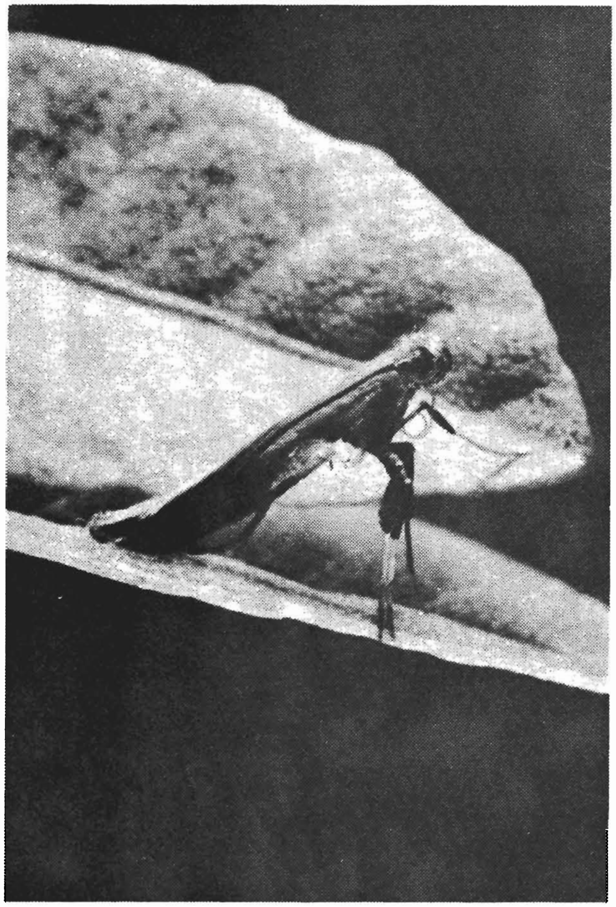
Fig. 1. Adult of the firetree leaf roller, <u>Phyllonorycter myricae</u>, in typical resting position. Males and females are the same color and size, with a body length of 1 cm. Adults are active diurnal flyers and the female lays eggs scattered on the undersurfaces of new <u>Myrica faya</u> leaves.

Fig. 2. The larva of <u>Phyllonorycter myricae</u> has rolled the leaf tip into a cylinder, then sealed together the ends. Discoloration is from larval feeding from inside the leaf roll. The outer cuticle of the leaf will remain untouched until the larva has completed its development and is ready to exit the leaf chamber to pupate.



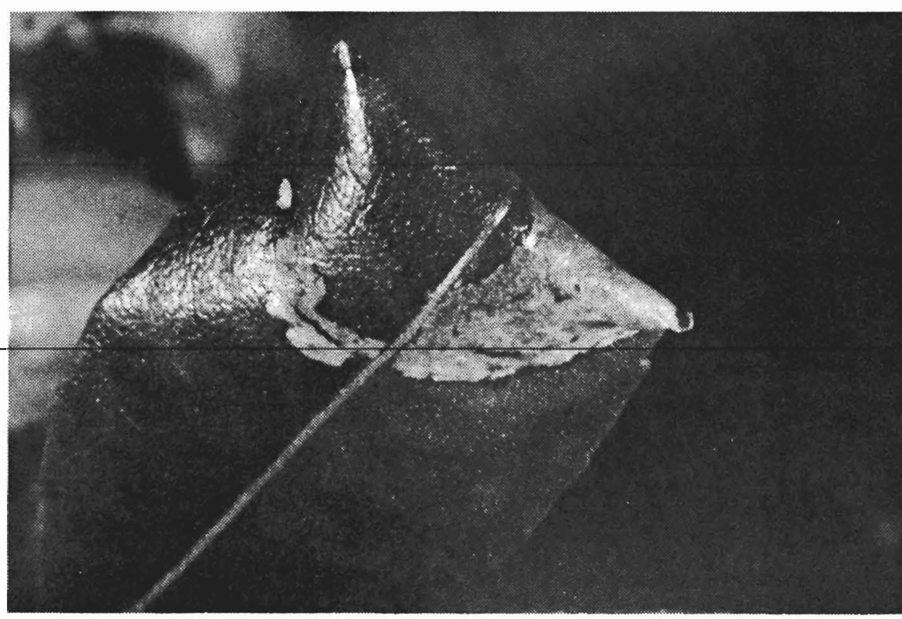


Fig. 3. Late instar larva of a Myrica faya looper. This larva is approximately 3 cm long and almost ready to pupate. The larva is highly polymorphic and variable in color and shade.

Fig. 4. Female of the Myrica faya looper. The body length is 1.5 cm. This moth contains a long extendable ovipositor with which she can deposit egg masses of 25 - 100 eggs in cracks in the bark of branches or trunks. In her lifetime she may lay 200 - 300 eggs.

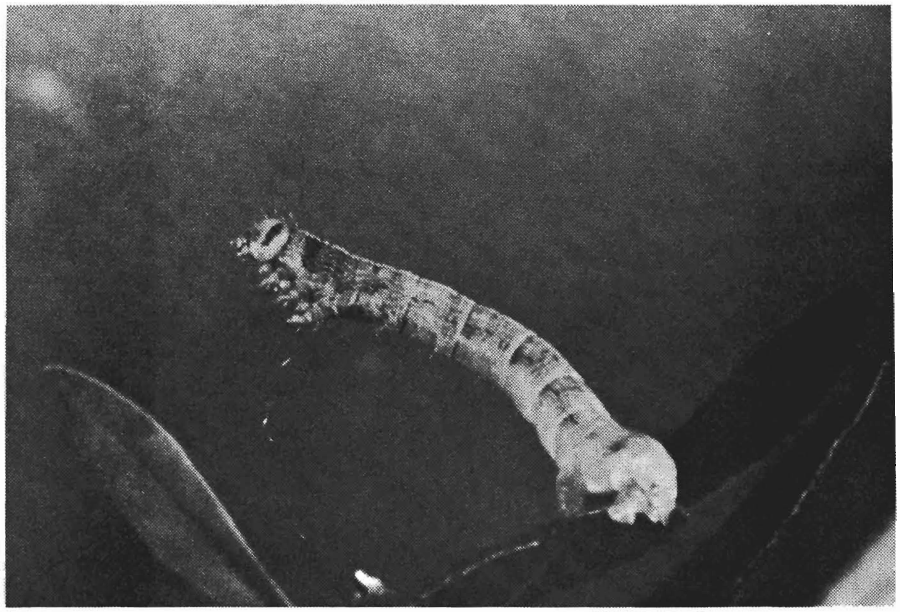


Fig. 5. Mature larva of the fruit feeder <u>Carposina atlanticella</u> on developing <u>Myrica faya</u> fruit. The reddish color of the young fruit closely matches that of the larva. The larvae were seldom found in mature fruit that had turned black, but limited their attack to green to light red maturing fruit.

Fig. 6. Shoot tip of <u>Myrica faya</u> opened to show the unidentified tip mining larva and its feeding damage. The larva is mature and approximately 8 mm long.

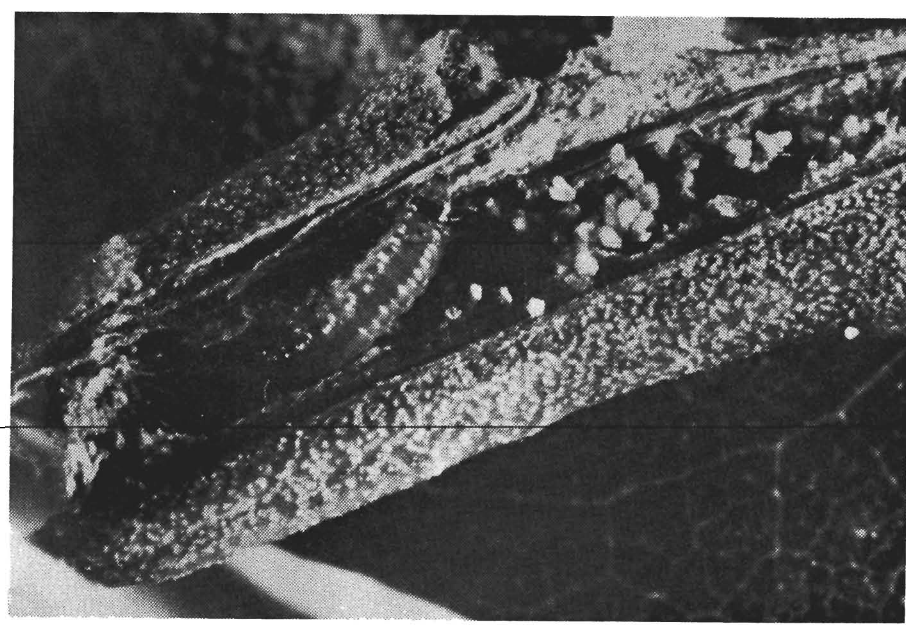


Fig. 7. Larvae of this small moth (length 3 mm) were found attacking the staminate flowers of *Myrica faya*. Adults are active diurnal flyers with a distinct whitish cast to their wings and body.