# Gypsy Moth Larval Necropsy Guide

## Laura M. Blackburn and Ann E. Hajek

## Abstract

Since the early 1900s, a number of parasitoids have been released for classical biological control of the introduced destructive forest insect, *Lymantria dispar* (gypsy moth), in North America. During this time, two pathogens were accidentally introduced. These pathogens and several of the parasitoid species are now commonly found in North American gypsy moth populations. The aim in creating this guide was to provide laboratory techniques for distinguishing between two common pathogens, the fungus *Entomophaga maimaiga* and the gypsy moth multiple nucleopolyhedrovirus, and provide illustrations and images for adults, puparia, and cocoons of established gypsy moth parasitoids commonly found in the larval or pupal stages of gypsy moth in North America. Gypsy moth collection and rearing techniques are also reviewed, and a technical glossary and summary table highlighting the affected life stage by gypsy moth parasitoids in North America are included in this guide.

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## Cover

North American range of the gypsy moth as of 2017, showing suitable habitat and some common pathogens and parasitoids of the gypsy moth. Photo credits from left to right: *Entomophaga maimaiga* conidia on cadaver (photo by Ann Hajek, Cornell University, used with permission), gypsy moth larva killed by the viral pathogen LdMNPV (photo by Ruth Plymale, Ouachita Baptist University, used with permission), *Blepharipa pratensis* (photo by Christophe Quintin; CC BY-NC 2.0 Generic), *Parasetigena silvestris*-parasitized gypsy moth larva (photo by Gyorgy Csoka, Hungary Forest Research Institute, via Bugwood.org).

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Gypsy moth defoliation of hardwood trees along the Allegheny Front near Snow Shoe, Pennsylvania, in July 2007. Photo by Dhalusa (CC BY-SA 3.0), via Wikimedia Commons.

# INTRODUCTION

Gypsy moth (*Lymantria dispar*) is a major pest of deciduous trees in the northern hemisphere. Native to Eurasia, this species was introduced to eastern Massachusetts by 1869 (Forbush and Fernald 1896), and despite many efforts to control gypsy moth spread, range expansion continues. Gypsy moth now occurs from Canada (southern Ontario, southern Quebec, southwestern New Brunswick, and southwestern Nova Scotia), south to North Carolina, and west to Wisconsin, Illinois, Indiana, Ohio, and West Virginia (Fig. 1). Since the early 1900s, intensive efforts have been made to introduce biological control agents against the gypsy moth, including 34 parasitoids, 1 predator, and 5 pathogens (Fuester et al. 2014). Successful establishment of parasitoid introductions has been limited, as has the effects of parasitoids on the regulation of gypsy moth populations (Hajek and van Nouhuys 2016). While parasitoids contribute to the overall mortality of gypsy moth populations, pathogens often play a more vital role.

This guide is intended to assist researchers involved in gypsy moth life table studies. A life table accounts for stage-specific mortality in a study population. Life tables can provide key information on the magnitude of host mortality caused by specific agents, information that is critical to understanding the role of such agents in host population dynamics. Knowledge of the processes driving gypsy moth population dynamics is crucial for understanding gypsy moth outbreaks and management practices.

This publication focuses on the larval stage, where mortality due to natural enemies can be substantial. Death of field-collected larvae can generally be attributed to one of four causes:

- A fungal pathogen: most commonly *Entomophaga maimaiga*
- A viral pathogen: *Lymantria dispar* multiple nucleopolyhedrovirus (LdMNPV)
- Parasitoids: flies and wasps
- Some combination of these agents

Descriptions of the most common pathogen and parasitoid species that cause death in gypsy moths are provided in this document, along with descriptions of some less common agents. Information on identifying selected hyperparasitoids is also included for species commonly emerging from field-collected gypsy moth larvae or pupae. This guide focuses on collecting gypsy moth larvae and pupae, thus only limited information on egg parasitoids is provided. Collection and rearing techniques for gypsy moth larvae and pupae and laboratory techniques for identifying the most likely causes of mortality are also reviewed.
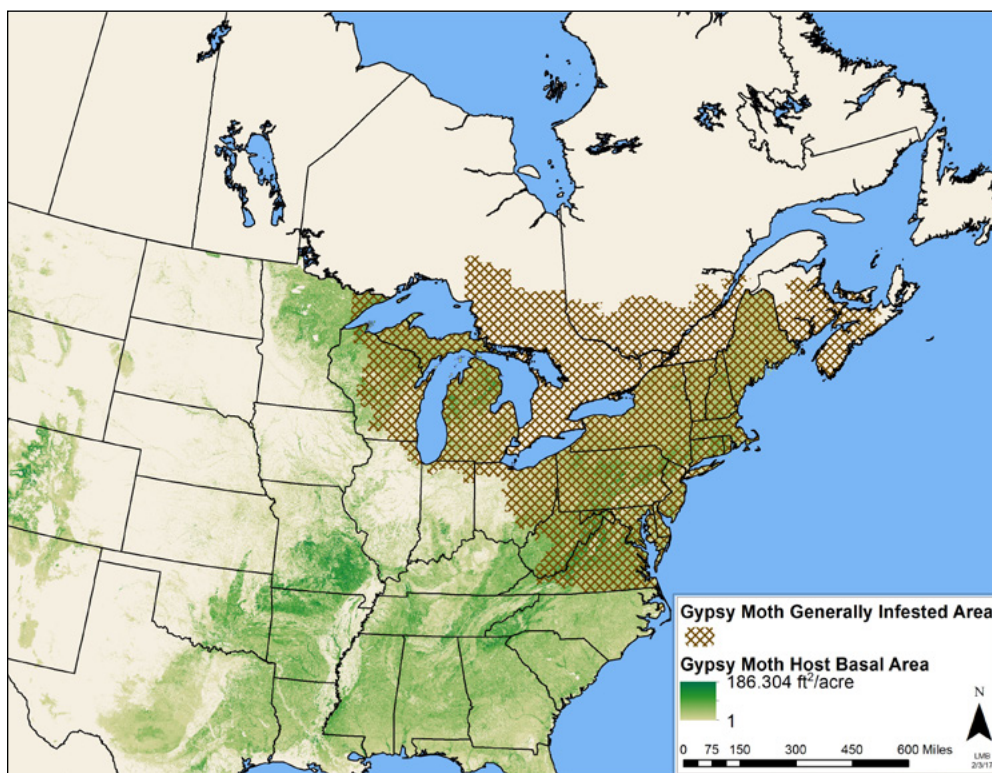


Figure 1.—Range of gypsy moth in North America as of 2017.

**Table 1.—North American gypsy moth parasitoids and hyperparasitoids and affected host life stages. Species are included in order of host stages initially parasitized.**

| Parasitoid | Host stages affected | |
|---|---|---|
| | Initial parasitization | Exit |
| Hymenoptera, Eupelmidae: *Anastatus japonicus* Ashmead | eggs | eggs |
| Hymenoptera, Encyrtidae: *Ooencyrtus kuvanae* (Howard) | eggs | eggs |
| Hymenoptera, Ichneumonidae: *Phobocampe unicincta* (Gravenhorst) | early instars | middle instars |
| Hymenoptera, Braconidae: *Cotesia melanoscela* (Ratzeburg) | early, middle instars | middle instars |
| *Hymenoptera, Braconidae: *Aleiodes indiscretus* (Reardon) | early, middle instars | late instars |
| Diptera, Tachinidae: *Compsilura concinnata* (Meigen) | early, middle, late instars | middle, late instars, pupae |
| Diptera, Tachinidae: *Blepharipa pratensis* (Meigen) | early, middle, late instars | late instars, pupae |
| Diptera, Tachinidae: *Parasetigena silvestris* (Robineau-Desvoidy) | middle, late instars | late instars, pupae |
| *Diptera, Tachinidae: *Exorista larvarum* (Linnaeus) | late instars | late instars |
| *Diptera, Tachinidae: *Exorista mella* (Walker) | late instars | late instars |
| *Diptera, Tachinidae: *Lespesia frenchii* (Williston) | late instars | late instars, pupae |
| *Hymenoptera, Perilampidae: *Perilampus hyalinus* Say | late instars | pupae |
| *Diptera, Sarcophagidae: *Agria housei* Shewell | late instars, pupae | pupae |
| Hymenoptera, Ichneumonidae: *Theronia atalantae (*Poda) | prepupae, freshly formed pupae | pupae |
| Hymenoptera, Ichneumonidae: *Pimpla disparis* Viereck | prepupae, pupae | prepupae, pupae |
| *Hymenoptera, Ichneumonidae: *Pimpla pedalis* Cresson | prepupae, pupae | prepupae, pupae |
| *Hymenoptera, Ichneumonidae: *Itoplectis conquisitor* (Say) | prepupae, pupae | pupae |
| *Diptera, Sarcophagidae: *Arachnidomyia aldrichi* Parker | prepupae, pupae | pupae |
| Hymenoptera, Chalcididae: *Brachymeria intermedia* (Nees) | pupae | pupae |
| *Hymenoptera, Torymidae: *Monodontomerus aereus* Walker | pupae | pupae |

*Indicates species that are uncommon or rare parasites of gypsy moths. Many of the species released for classical biological control are not well established or are only established in certain areas.

## PATHOGEN VERSUS PARASITOID

The cause of death in gypsy moth can be narrowed down by considering the life stage at time of death. For example, infections by *Entomophaga maimaiga* and LdMNPV can occur in all larval stages but are usually most prevalent in late instars (Hajek 1994, Hajek and Snyder 1992). Parasitoids are limited by their ability to only develop in specific life stages. Table 1 provides a list of affected life stages for commonly occurring gypsy moth parasitoids.

Physical characteristics of the cadaver also provide useful clues that can aid in identifying the cause of death. Be sure to note distinguishing characteristics such as a sugar coating on larval hairs (Hajek and Roberts 1992), a thin cuticle (Hajek and Roberts 1992), an oozing wound (Campbell 1963), or large white eggs on the dorsum of late instar larvae (Fuester et al. 2014, Ticehurst et al. 1978). When gypsy moth-parasitized larvae or pupae are held in the laboratory, eventually a life stage of the parasitoid may emerge. However, when field-collected larvae die during rearing and no other insect eventually appears in the container with the cadaver, the cause of death will likely be a pathogen.

## COMMON PATHOGENS

### Fungal Pathogen

***Entomophaga maimaiga* Humber, Shimazu & Soper (Phylum Zoopagomycota; Order Entomophthorales; Family Entomophthoraceae)**

Native to Asia, *Entomophaga maimaiga* was deliberately released in the United States in 1910-11 and again in 1985-86 (Nielsen et al. 2005). Though attempts to establish this fungal pathogen in the United States were thought to have failed, epizootics were first seen during the very wet spring of 1989, predominantly in New England (although not near areas of the 1985-86 releases), after which this fungus spread
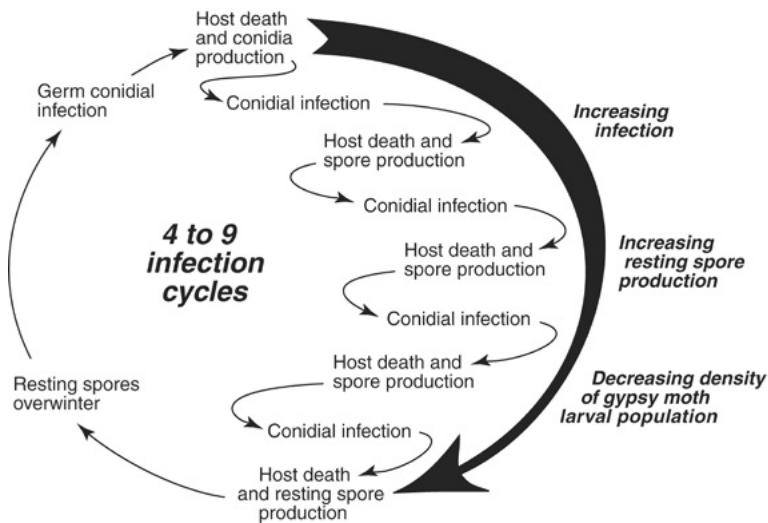
Figure 2.—Multiplicative cycles of *Entomophaga maimaiga* infection during one field season that create epizootics. Models have indicated that during epizootics, four to nine infection cycles can occur during one gypsy moth generation. Illustration from Hajek 2004, used with permission.

rapidly throughout the contiguous gypsy moth range (Andreadis and Weseloh 1990, Hajek 1999, Hajek et al. 1990). Like all fungi, *E. maimaiga* is greatly affected by moisture and temperature. Thriving in cool and wet spring and early summer weather, this fungus is an important source of larval mortality in both low- and high-density gypsy moth populations.

Extensive laboratory and field tests have demonstrated that while *E. maimaiga* can infect some other lepidopteran species in the laboratory, high levels of infection in any species other than gypsy moth have never been reported from the field (Hajek et al.1995, Hajek et al. 1996, Hajek et al. 2000a, Hajek et al. 2004). This fungus can be grown outside of insects (in vitro) in the laboratory under special conditions, but this process is difficult and viable methods for mass production have not been developed.

*Entomophaga maimaiga* produces two kinds of spores (conidia [singular = conidium] and resting spores [azygospores]), but spores are only produced after host death. The type of spore produced is dependent on host age. Infective conidia (asexual short-lived spores) are usually produced from early instar cadavers, while resting spores, and sometimes conidia also, are produced from later instar cadavers and occasionally from pupae (Hajek 1999). Resting spores, produced near the end of the larval season, overwinter in soil and can survive for at least 6-12 years (Hajek et al. 2000b, Weseloh and Andreadis 1997). During May and early June, resting spores germinate and produce infective germ conidia. The conidia (either from cadavers or resting spores) are actively discharged and are

dispersed by the wind, where they infect gypsy moth larvae with which they come into contact. Once they land on a host, the conidia grow through the cuticle and start consuming the living insect. After the fungus consumes the inside of the larva and the larva is dead, under humid conditions conidiophores can grow out through the cuticle. Conidia that are produced from the ends of the conidiophores are shot into the air where they are dispersed and can then infect other larvae. If no host is found, a conidium can germinate and expel a secondary conidium. This infection cycle continues throughout the season. Resting spore production occurs in the fourth to sixth instar larvae, which allows the spores to overwinter and start the cycle again the next year or in subsequent years (Fig. 2).

***Entomophaga maimaiga* conidia**—Conidia are produced externally on infected gypsy moth larvae after they die. Whether an infected larva will produce conidia depends on a variety of factors, including larval age, fungal isolate, and environmental conditions. If conidia are produced from the cadaver, they will be formed within hours to a few days after larval death (Fig. 3A). Conidia are actively expelled from the cadaver, and *E. maimaiga* is spread within surrounding gypsy moth populations during the current season, although some spores may travel longer distances.

Conidia (Fig. 3B) produced by cadavers are clear, pear-shaped spores measuring approximately 20 μm x 25 μm. Conidia are usually produced from cadavers of early instars, which typically are found with the prolegs gripping a twig or branch and the anterior portion of the body at a 90° angle hanging downward compared
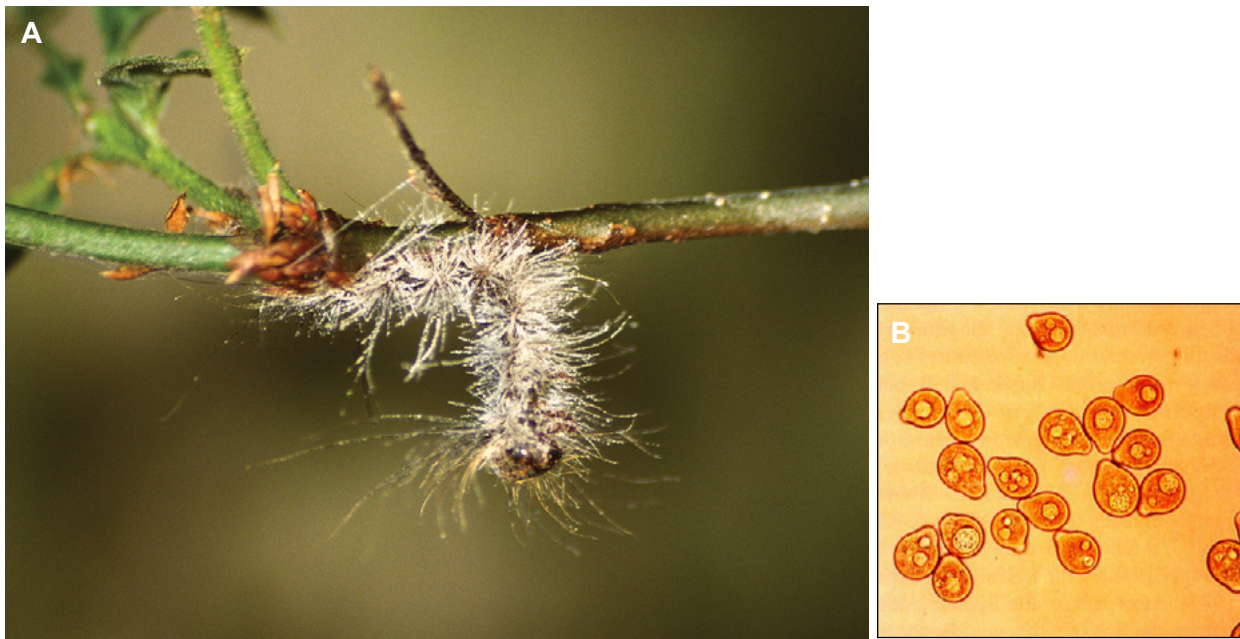
Figure 3.—*Entomophaga maimaiga* conidia on cadavers (A) are produced relatively shortly after the larva dies. Conidia shot off from the cadaver adhere to the larval hairs, making the dark larval hairs appear white. (B) A microscopic view of *E. maimaiga* conidia (20 μm x 25 μm) stained red to improve viewing. Photos by Ann Hajek, Cornell University, used with permission.

with the posterior part of the body (Fig. 3A). Once conidia have been discharged, some spores can be retained on the larval hairs and may appear similar to a sugar coating. The surface of a cadaver from which conidia are being ejected appears moist and white to light brown. After conidial discharge, these wet spores and conidiophores will decompose quickly, and soon there will be little to no indication that they were ever present. Therefore, in order to detect these fungal stages, daily monitoring of larval death and of cadavers (for ~3 days after death) is very important.

***Entomophaga maimaiga* resting spores**—Later in the larval season when gypsy moths are in the fourth- to sixth-instars, *E. maimaiga* will produce thick-walled resting spores inside of cadavers. The bodies of recently killed larvae containing resting spores are soft, and the contents appear to be liquid. Over time, the cadavers become dry and stiff with a firm, but not fragile, cuticle. Cadavers containing resting spores can often be found hanging vertically on tree trunks with prolegs usually extended at 90° from the body (Fig. 4). After some time, the cadavers fall from the trees and disintegrate on the ground. Most resting spores overwinter in the top layers of the soil and germinate during the following year(s) where they infect gypsy moth larvae in successive generations.
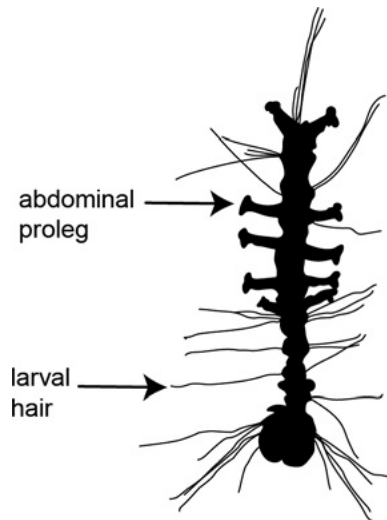


Figure 4.—Symptomatic expression of resting spore-producing cadaver with legs extended and stiff posture. Cadavers of late instars killed by *E. maimaiga* are typically seen in this position on tree trunks during epizootics. Illustration by Laura Blackburn, USDA Forest Service.
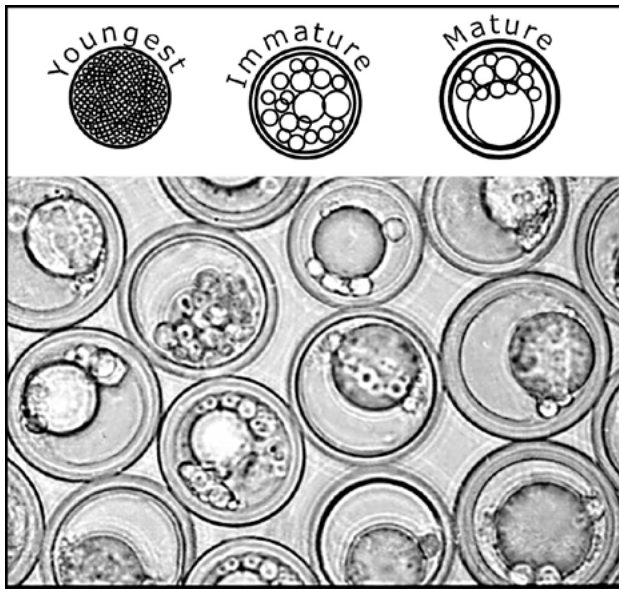
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Figure 5.—Progression of *Entomophaga maimaiga* resting spore maturation following gypsy moth larva death (adapted from Hajek et al. 2008).
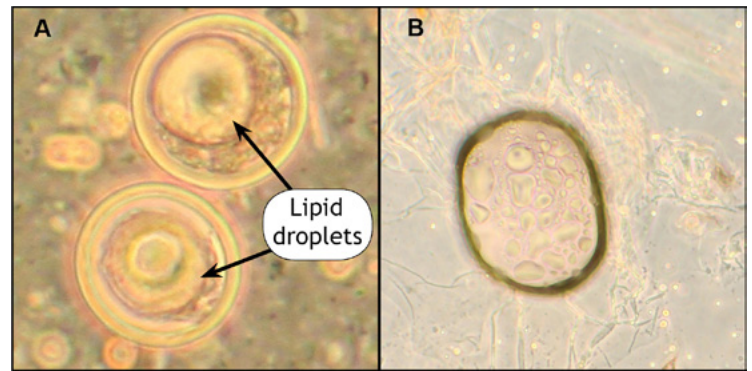


Figure 6.—Mature *E. maimaiga* resting spores (A) are typically found with a thick wall and a single large lipid droplet. Younger resting spores will contain multiple lipid droplets, giving them a granular appearance. However air bubbles under slide covers (B) can easily be mistaken as immature spores. Use the fine focus dial on the microscope to overcome this by simply zooming in and out. The air bubbles will have a thick dark wall. The wall on mature resting spores is thick and white/clear. Photos by Ruth Plymale, Ouachita Baptist University, used with permission.

Resting spores develop from immature to mature spores in the days following larval death (Fig. 5). When first formed, resting spores possess a single thin wall and have a granular interior. As the spores mature, they develop a thickened wall and the granular interior coalesces into a small number of large lipid droplets. Normally, mature resting spores are observed in cadavers of larvae that have died at least several days before, but occasionally immature spores are also still visible at that time. Resting spores are approximately 30 µm in diameter (Fig. 6A) and at times can be mistaken for air bubbles (Fig. 6B).

## Viral Pathogen

### *Lymantria dispar* **multiple nucleopolyhedrovirus (LdMNPV)**

LdMNPV is a highly host-specific virus that infects gypsy moth larvae when they consume foliage contaminated with viral occlusion bodies. This virus most likely arrived in North America before 1907, along with parasitoids being introduced for biological control (Howard and Fiske 1911). Prior to the arrival of *E. maimaiga*, LdMNPV was well known for causing epizootics resulting in the collapse of outbreak gypsy moth populations, but this usually only occurred after populations had reached high densities. On its own, LdMNPV only increases in abundance in high density populations (Doane and McManus 1981). LdMNPV is now found infecting insects in high density gypsy moth populations, but its prevalence is often low, and *E. maimaiga* infections have become much more common, at times leading to the collapse of gypsy moth populations at a variety of densities (Hajek et al. 2015, Hajek and van Nouhuys 2016, Liebhold et al. 2013).

After being ingested by a gypsy moth larva, viral occlusion bodies (environmentally persistent protein packets containing the actual virus) dissolve in the alkalinity of the insect's gut, releasing the virions (infective virus particles). The virions infect the midgut cells and infection subsequently spreads to the rest of the body. The virus replicates in the nuclei of infected cells, forming two types of progeny: budded virus and occluded virus. The budded virus leaves an infected cell and spreads the infection to additional cells within the insect. Toward the end of the infection cycle, new occlusion bodies are made that contain the occluded virus. Subsequently, infected cells burst, releasing the occlusion bodies. This systematic destruction of infected cells eventually destroys the internal organs of the host, leaving the cadaver as a slurry of occlusion bodies and cell fragments. The virus also destroys cells beneath the larval cuticle and produces cuticle-degrading enzymes, making the cuticle very thin and fragile (Miller 2013). After the host dies and the cadaver decomposes, the cuticle breaks and viral occlusion bodies are released into the environment.

Figure 7.—Gypsy moth larvae killed by LdMNPV in the typical upside down (inverted) V-shape. Photo by Ruth Plymale, Ouachita Baptist University, used with permission.

under a compound microscope using phase contrast. (The view in phase contrast has been described as similar to the appearance of the Milky Way on a dark night). While viewing the occlusion bodies at 400x and focusing up and down, the occlusion bodies will often have a dark center and bright halo. To diagnose LdMNPV in cadavers, add potassium hydroxide (1 M KOH), an alkaline solution, to the side of the cover slip, and watch for the dissolution of the occlusion bodies as the KOH moves under the cover slip (Fig. 8B). Refer to the section on pathogen identification for detailed techniques on microscopic examination.

# LESS COMMON PATHOGENS

## Fungal Pathogens

### *Isaria farinosa*–(Phylum Ascomycota; Order Hypocreales; Family Cordycipitaceae)

Long known as *Paecilomyces farinosus*, this entomopathogenic fungus has a relatively broad host range and is found throughout the world in both tropical and temperate zones. It can be isolated from water, air, arthropods, plants, and other fungi. This ubiquitous fungus also infects and kills lepidopteran larvae and pupae and is most commonly found in forest soils with pine (*Pinus* spp.), oak (*Quercus* spp.), poplar (*Populus* spp.), and acacia (*Acacia* spp.) trees (Domsch et al. 1980). It is a moderately fast-growing fungus, beginning with white colonies that turn yellow and form woolly aerial mycelium. The conidia are transparent and circular to fusiform (2-3 µm x 1-1.8 µm). After death, fungal mycelia grow out from infected gypsy moth larvae, often covering cadavers, which can look white and flocculent. See Zimmermann (2008) for

Larvae that die from LdMNPV often hang in an upside down V shape (an "inverted V"; Fig. 7) and have a very thin cuticle. The cadavers may break when collected or before being dissected, so if you have to pull to tear the cuticle apart, it is unlikely that the larva died from LdMNPV alone. A larva killed by LdMNPV alone will have a large number of occlusion bodies present within them (Fig. 8A). The occlusion bodies are 1-10 µm in diameter and are generally spherical but with slightly uneven sides. Occlusion bodies under a cover slip on a microscope slide will appear bright and sparkly
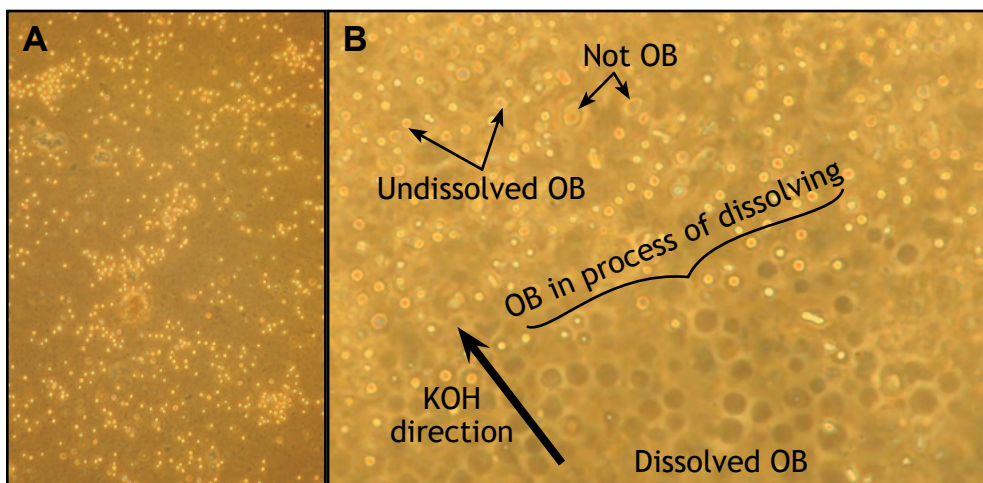


Figure 8.—LdMNPV occlusion bodies (OBs) viewed with phase contrast microscopy at (A) 100x and at (B) 400x while dissolving from the addition of KOH, which was added at the lower right (lower right OBs have dissolved while upper left OBs have not). Photos by Ruth Plymale, Ouachita Baptist University, used with permission.

an in-depth synthesis of information on the biology, ecology, and use of *I. farinosa* in biological control.

Prior to the first reports of *E. maimaiga* in North America, Majchrowicz and Yendol (1973) found *I. farinosa* to be the most common fungal pathogen of diseased gypsy moth larvae and pupae, infecting over 12 percent of a study population in Berks County, Pennsylvania in 1971. After *E. maimaiga* arrived, Hajek et al. (1997) found 4.9-12.2 percent *I. farinosa* infection in gypsy moth larvae from the Mid-Atlantic region, with no association, either positive or negative, between *E. maimaiga* and *I. farinosa*.

### *Beauveria bassiana* (Phylum Ascomycota; Order Hypocreales; Family Cordycipitaceae)

First observed in 1835 in silkworms (*Bombyx mori*) reared for silk production, this fungus causes larvae to harden after death, and white mycelia grows out of the dead insect (Kleespies et al. 2008). This contagious condition, termed "white muscardine," was found to be caused by the fungus *B. bassiana.* This fungus has a wide host range of over 200 insect species, mainly Lepidoptera and Coleoptera (Feng et al. 1994), and it is generally associated with shaded areas such as forests and uncultivated hedgerows (Bidochka et al. 1998, Meyling and Eilenberg 2006). As with *E. maimaiga* and *I. farinosa*, infection is caused by conidia that attach to the host cuticle and germinate in humid environments. The host cuticle is penetrated by germ tubes growing from the conidia. Fungal growth increases upon entering the hemocoel, and the host dies due to depleted hemolymph, nutrients, or toxemia (Khachatourians 1991). When moisture levels are high, *B. bassiana* produces aerial conidia on the cuticle of the host. Diagnostic characteristics include conidiogenous cells that are typically densely clustered or whorled with toothed rachises that extend apically and bear a single conidium per denticle (Humber 1997). The nearly globose conidia are ≤3.5 μm in diameter.

Although *B. bassiana* is common, infections by the fungus are low in prevalence. For example, Majchrowicz and Yendol (1973) isolated *B. bassiana* from 7 percent of dead gypsy moth larvae and pupae collected in Berks County, Pennsylvania in 1971, and infections were rare in a study of four Mid-Atlantic states, after *E. maimaiga* had become established (Hajek et al. 1997). Trials applying *B. bassiana* against gypsy moth larvae in Slovakia and Maryland did not yield control (see Hajek et al. 1997).

# COMMON PARASITOIDS

The term "parasitoid" refers to insects that spend part of their life, usually immature stages, attached to or within an insect host where they feed on and ultimately kill the host. Since 1906, parasitoid species have been introduced from Europe and Asia as part of a large classical biological control program for the gypsy moth. These parasitoids belong to the orders Hymenoptera (wasps) and Diptera (flies). The greatest number of parasitoid species belong to the order Hymenoptera and generally attack immature stages (including eggs) of host insects (Eggleton and Belshaw 1993). Species of parasitoids in the order Diptera have a wide host range and attack all stages of insect hosts except the egg (Eggleton and Belshaw 1993), with members of the family Tachinidae being the most beneficial (Clausen 1972).

Parasitoids and hyperparasitoids of gypsy moth larvae and pupae that are established in North America are described below. For a review of historic (1906-1959) gypsy moth parasitoid releases see Clausen (1978), and for parasitoid releases between 1961 and 1977 see Reardon (1981). A list of introduced Eurasian and North African parasitoids that are known to be established in North America is presented in Fuester et al. (2014). Simons et al. (1979) provide a more exhaustive key to gypsy moth parasitoids and hyperparasitoids (including species that are rare or were released but are not considered established). Keys to gypsy moth parasitoids in specific families are also available: Braconidae (Marsh 1979), Ichneumonidae (Dasch 1971, Gupta 1983), and Tachinidae (Sabrosky and Reardon 1976).

The most commonly encountered parasitoids reared from gypsy moth larvae include *Cotesia melanoscela, Phobocampe unicincta, Compsilura concinnata, Parasetigena silvestris,* and *Blepharipa pratensis* (Hajek and van Nouhuys 2016, Williams et al. 1993). Common parasitoids reared from gypsy moth pupae include *Brachymeria intermedia, Pimpla disparis, Parasetigena silvestris,* and *Theronia atalantae fulvescens* (Fuester et al. 1997). Most of these commonly encountered parasitoids are multivoltine and will transform into adults within the same season that gypsy moth larvae are collected, which simplifies identification. The exceptions include *P. unicincta, B. pratensis,* and *P. silvestris,* univoltine species that overwinter as cocoons or puparia and emerge as adults the following spring.

These three parasitoids, however, are very easy to identify based on their puparia.

Parasitoid assemblages vary with gypsy moth population density. When gypsy moth densities are low, the tachinids *Compsilura concinnata* and *Blepharipa pratensis* contribute more to overall larval mortality (Doane and McManus 1981). When gypsy moth densities are high, the chalcidid *Brachymeria intermedia* causes high levels of mortality (Doane and McManus 1981, Ticehurst et al. 1978). During a gypsy moth outbreak, *Cotesia melanoscela* was found to be most abundant (Williams et al. 1992). After a gypsy moth outbreak, the tachinid *Blepharipa pratensis* can be a great source of mortality (Ticehurst et al. 1978). And 2 years post-outbreak, *Parasetigena silvestris* reaches peak density (Sabrosky and Reardon 1976).

Forest type, ecological relationships, and site characteristics play a major role in the dominance and assemblage of parasitoids. *Exorista larvarum* is more prevalent in the willow-poplar (*Salix* spp.-*Populus* spp.) forest type where its alternate host, the satin moth (*Leucoma salicis*), is present (Sabrosky and Reardon 1976). *Compsilura concinnata* causes gypsy moth mortality in oak forests near orchards where many alternate hosts are available (Sabrosky and Reardon 1976). *Brachymeria intermedia* and *Cotesia melanoscela* prefer dry sites, while *Parasetigena silvestris* and *Phobocampe unicincta* are commonly recovered from mesic sites (Skinner et al. 1993).

## Hymenoptera (Wasps)

### *Brachymeria intermedia* (Nees)

This chalcidid was introduced from Europe and repeatedly released for biological control between 1908 and 1963 (Clausen 1978, Hoy 1976). It is rarely found in low density gypsy moth populations (Williams et al. 1993). This parasitoid prefers open sunny areas (Hoy 1976) and exhibits delayed density dependence, causing high gypsy moth mortality in outbreak populations where defoliation is severe (Ticehurst et al. 1978, Williams et al. 1993). *Brachymeria intermedia* is a pupal endoparasitoid with a bias for male gypsy moth pupae (Fuester and Taylor 1996). Known to be polyphagous, Dowden (1935) notes that this species may also be a hyperparasite, attacking puparia of the tachinid parasitoids *Compsilura concinnata* and *Exorista larvarum*, in the laboratory, though this is thought to be a rare occurrence in the field.



Figure 9.—*Brachymeria intermedia* adult on gypsy moth pupa. Photo by Pedro Barbosa, University of Maryland, used with permission.

Dowden (1935) found that in Europe, *B. intermedia* completes one generation on the gypsy moth and a second generation on other lepidopteran hosts; due to the comparatively delayed host development in New England, this parasitoid likely completes a first generation on other lepidopteran hosts and a second generation on gypsy moth. The adult emerges from a misshapen exit hole, typically between the middle to the anterior end of the host pupal shell (Dowden 1935). The adult female (Fig. 9) overwinters with other mated females found clustered in the litter and under loose bark (Dowden 1935). Adults are small and stout with a large hind femur and a body length of 5 mm (Howard 1889). For a key to *Brachymeria* of the United States and Canada refer to Burks (1960).

### *Cotesia melanoscela* (Ratzeburg)

This braconid parasitoid completes two generations a year. It was introduced to the United States in 1912, specifically for biological control of the gypsy moth (Burgess and Crossman 1929). Discovered in North Africa and India, this oligophagous species thrives in xeric sites (Liebhold and Elkinton 1989, Skinner et al. 1993). While Burgess and Crossman (1929) considered this parasitoid to be one of the most valuable enemies of the gypsy moth, its abundance is limited by host availability and a number of hyperparasitoids (Muesebeck and Dohanian 1927). Females lay from 50-1,000 eggs, depositing a single egg in first or second instar larvae. During egg deposition, the females also insert a virus (Family Polydnaviridae; Genus *Bracovirus*), which prevents molting and suppresses the immune response of the host (Lavine and Beckage 1995, Shelby and Webb 1999, Stoltz et al. 1988, Stoltz and Xu 1990). The small parasitoid larva emerges from a parasitized (generally living) gypsy moth larva
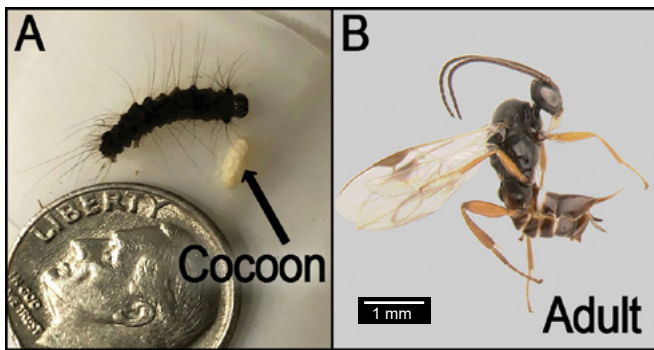
Figure 10.—*Cotesia melanoscela* (A) cocoon with gypsy moth larva and (B) adult. Photos by George Boettner (cocoon), University of Massachusetts and Michael Sharkey (adult), University of Kentucky, used with permission.
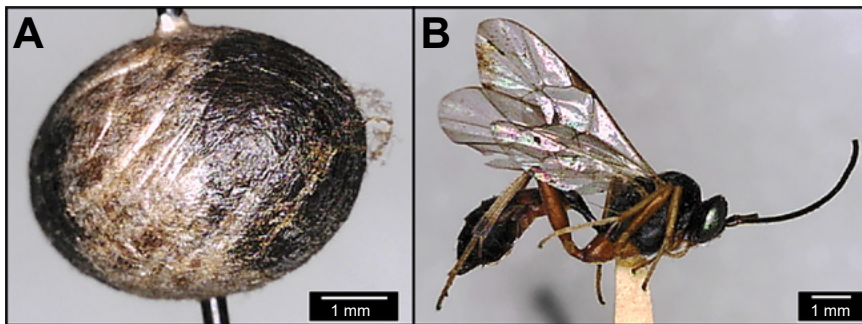


Figure 11.—*Phobocampe unicincta* (A) cocoon and (B) adult. Photos by Yong-Lak Park, West Virginia University, used with permission.

and spins a white cocoon (about the size of a grain of rice) nearby (Fig. 10A). The virus often keeps the gypsy moth larva alive for many days after parasite emergence (Fleming 1992). Therefore, field-collected small instars that live for many days in rearing without molting should be inspected closely for exit holes of *C. melanoscela*. A second generation of wasps will emerge from these cocoons in 4-11 days to attack second or third instar host larvae (Reardon et al. 1973). Again, a parasitoid larva emerges from the parasitized gypsy moth larva and spins a white to yellowish cocoon attached to tree trunks and the undersides of branches. These overwintering cocoons produce adult wasps during the following April or May, when gypsy moth eggs are at peak hatch (Fuester et al. 2014). Adult *C. melanoscela* are shiny and black with a body length of 2.5-3 mm (Fig. 10B). See Crossman (1922) for a detailed description of this parasitoid.

### *Phobocampe unicincta* (Gravenhorst)

*Phobocampe unicincta* is a univoltine, ichneumonid parasitoid released in Massachusetts between 1907 and 1912 to help control the gypsy moth (Clausen 1956). Rates of parasitism are typically low but can be heavier in dense woodlands (Gupta 1983). This parasitoid (historically referred to as *Hyposoter disparis*) is of minimal value for controlling the gypsy moth due to

heavy hyperparasitism, high overwintering mortality, and high mortality of egg and first instar larvae due to phagocytosis by the host immune system (Muesebeck and Parker 1933). This parasitoid oviposits in first and second instar larvae. Females can produce in excess of 1,200 eggs over a period of 5-8 weeks (Clausen 1956). A week after oviposition, the egg hatches inside the host larva, and the larval parasitoid grows while feeding within the host for nearly a month. When the host reaches the fourth instar it dies and the fully grown wasp larva emerges as a large, slimy-looking, whitish-gray larva. The larval parasitoid spins a hardened, dark, oval cocoon of 6 mm x 4 mm on a nearby leaf or twig. When formed, the dark cocoon has a very distinctive light band around the middle (Fig. 11A). This loosely attached cocoon then falls to the ground where pupation takes place and it remains over the winter, to emerge the next spring. Adults have a short thorax with an elongated abdomen. The body length is 4-7 mm (Gupta 1983, Viereck 1911) and the color is black with reddish-yellow legs (Fig. 11B).

### *Pimpla disparis* Viereck

This ichneumonid wasp is a polyphagous endoparasitoid of lepidopteran pupae (Fuester et al. 1989). Native to Asia, this species was released between 1972 and 1987 for biological control of gypsy moth in New Jersey,

Figure 12.—*Pimpla disparis* adults. Photo by Michael Hrabar, Simon Fraser University, via flickr, used with permission.



Figure 13.—*Theronia atalantae fulvescens* adult. Photo by James Lindsey at Ecology of Commanster (CC BY-SA 2.5 or 3.0), via Wikimedia Commons.

Pennsylvania, and West Virginia (Reardon 1981, Schaefer et al. 1989). Since 2000, it has been commonly recovered from coastal Massachusetts and Maine.[1] In the United States its primary hosts are gypsy moth, browntail moth (*Euproctis chrysorrhoea*), bagworm (*Thyridopteryx ephemeraeformis)*, and tent caterpillars (*Malacosoma americanum)*; in Asia it also attacks pupae of fall webworm, *Hyphantria cunea* (Fuester et al. 2014). *Pimpla disparis* is multivoltine, completing a single generation in 25-32 days (Schaefer et al. 1989) and overwintering as a mature larva in the host pupa. The adult parasitoid emerges from the pupal case by chewing a hole from which it exits (Hrabar et al. 2012). Body length is 12-15 mm (Gupta 1983, Viereck 1911). Adults are black with white pubescence, and the hind leg has a black coxa, reddish-yellow femur, a dark tibia and a dark tarsus (Fig. 12).

### *Theronia atalantae fulvescens* (Cresson)

*Theronia atalantae fulvescens* is a primary pupal parasitoid of gypsy moth and a secondary parasitoid of ichneumonids and tachinids. This ichneumonid wasp is a hyperparasitoid of both *Pimpla disparis* and *Brachymeria intermedia* (Fuester et al. 1997, Schaefer et al. 1989). While parasitism rates can be as low as 2 percent in gypsy moth pupae (Howard and Fiske 1911), up to four times as many pupae are stung and killed by *T. atlantae* (Campbell 1963). This species is native, but specimens from Europe have been released in New England prior to 1910 to control gypsy moth (Townes 1940). It is polyphagous and seems to prefer

shady conditions (Campbell 1963) and dense forests (Gupta 1983). This wasp lays a single egg in either a prepupa or a newly formed pupa (Townes 1940). Transformation from egg to adult only takes 14-18 days (Townes 1940). *Theronia atalantae fulvescens* has 2-3 generations a year and overwinters in the host pupa. Adults may be present through the spring and summer, but in the fall only females persist (Townes 1940). Adult females overwinter under the bark of logs suspended across creeks (Dasch 1971). The size of the adult varies depending on the host, with body length ranging from 6 to 13 mm and forewing lengths ranging from 4 to 11 mm (Gupta 1983) (Fig. 13). This species is easily identified by pure orange hind legs.

## Diptera (Flies)

### *Blepharipa pratensis* (Meigen)

Introduced to North America between 1905 and 1933 for biological control of the gypsy moth (Grenier 1988), these tachnid flies are most abundant when host populations are at low to intermediate densities (Ticehurst et al. 1978). This univoltine, oligophagous parasitoid aggregates on gypsy moth-damaged leaves where females lay tiny, microtype eggs. Oviposition typically occurs when gypsy moth larvae are in the late third or early fourth instars (Godwin and Odell 1981, Williams et al. 1992). The eggs hatch after they are consumed by gypsy moth larvae. The maggot bores through the gut wall and into a longitudinal intersegmental muscle where it remains until the gypsy moth larva pupates. Then, the maggot completes its development and leaves its host to form a puparium in the soil where it will overwinter. Puparia are roughly 6-10 mm in length and can be identified by

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[1] Elkinton, J.; Boettner, G. 2015. Personal communication. Professor and lab technician, UMASS-Amherst, Department of Environmental Conservation, 250 Natural Resources Rd., Amherst, MA 01003.
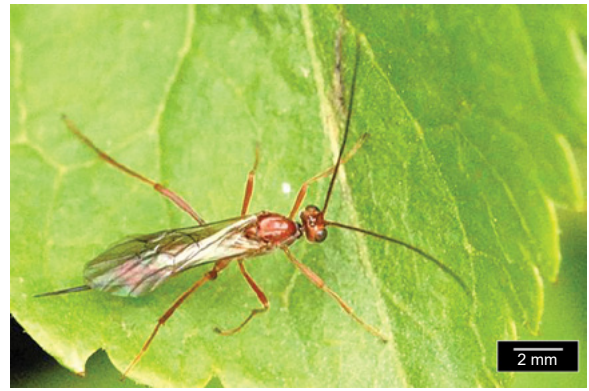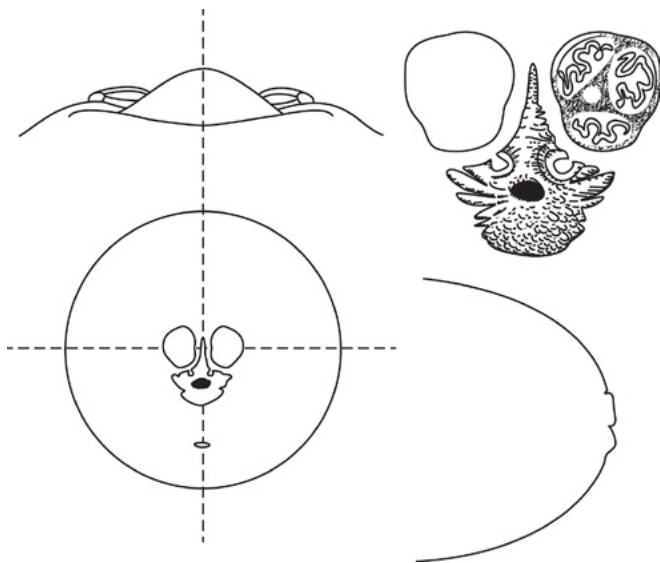
Figure 14.—Line drawing of *Blepharipa pratensis* puparium. Illustration adapted from Figure 69 of Sabrosky and Reardon (1976) and Simons et al. (1979) by Laura Blackburn, USDA Forest Service.



Figure 15.—*Blepharipa pratensis* adult. Photo by Christophe Quintin (CC BY-NC 2.0 Generic).



Figure 16.—Puparium of *Compsilura concinnata*. Photo by © 2010 Malcolm Storey, via www.bioimages.org.uk, used with permission.

the distinctive posterior area of the spiracular plates (Fig. 14), which can easily be seen under a dissecting microscope. The puparium of *Blepharipa pratensis* has a large, prominent subspiracular protuberance with a triangular shaped ridge-like extension dorsally between and ventral to the spiracular plates (Sabrosky and Reardon 1976). Adults (Fig. 15) emerge to mate the following spring and begin oviposition. Adults are 10-14 mm long, by far the largest and most robust of the gypsy moth parasitic flies occurring in North America (Sabrosky and Reardon 1976).

### *Compsilura concinnata* (Meigen)

This generalist tachinid parasitoid was introduced for gypsy moth control as early as 1906 (Culver 1919, Sanchez 1995) and is well established throughout much of the northeastern and north central United States. It is common in low density gypsy moth populations and tends to prefer late instar larvae (Skinner et al. 1993). *Compsilura concinnata* has at least four generations per year, although only one of these generations (and a partial second generation) attacks gypsy moths. This species is a likely culprit in the decline of native giant silk moths of the northeastern United States (Elkinton and Boettner 2004). This fly is ovoviviparous, inserting between one and five larva(e) into the host's midgut or body cavity (Culliney et al. 1992). A larva will complete two molts in roughly 2 weeks, emerging from the host as a white maggot, then forming a smooth, reddish brown puparium (Fig. 16). After about 10 days, adult flies emerge. Gould et al. (1990) demonstrated that this fly can be the dominant parasite of small patches of outbreaking gypsy moth larvae. However, the fly is dependent on rich forest habitats with abundant alternate hosts to complete its multiple generations per year, which limits its ability to respond to gypsy moth numbers in some years.

If rearing field-collected gypsy moth larvae individually in cups, at least one puparium will be found from any larva parasitized by *C. concinnata*, and from this an adult will emerge in a few weeks (Fig. 17). Adult length is 7-8.5 mm (Sabrosky and Reardon 1976), and the puparium is 6.5 mm long (Koch and Hutchinson 2017) with a rounded posterior end in profile. The spiracular plates (Fig. 18), which have straight or very slightly curved slits, are vertical to the long axis of the puparium with little to no protuberance above the surrounding surface (Sabrosky and Reardon 1976).

Figure 17.—*Compsilura concinnata* adult. Photo by Joyce Gross, UCB, via Bugwood.org.



Figure 19.—*Parasetigena silvestris* eggs attached to host, a late instar gypsy moth larva. Photo by Gyorgy Csoka, Hungary Forest Research Institute, via Bugwood.org
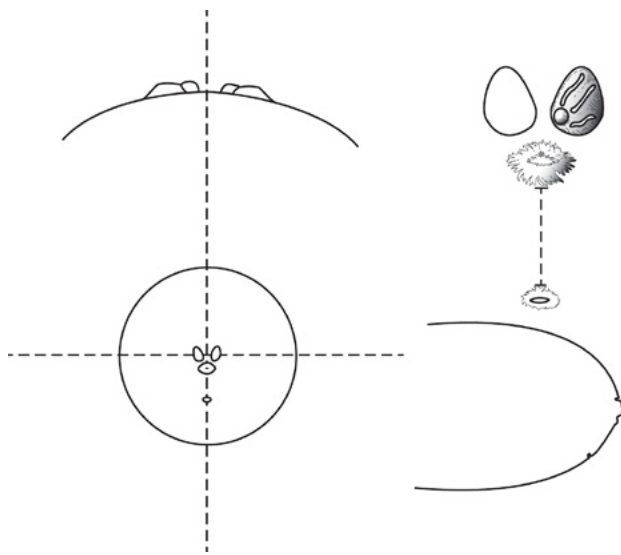


Figure 18.—Line drawing of *Compsilura concinnata* puparium. Illustration adapted from Figure 61 of Sabrosky and Reardon (1976) and Figure 66 of Simons et al. (1979) by Laura Blackburn, USDA Forest Service.
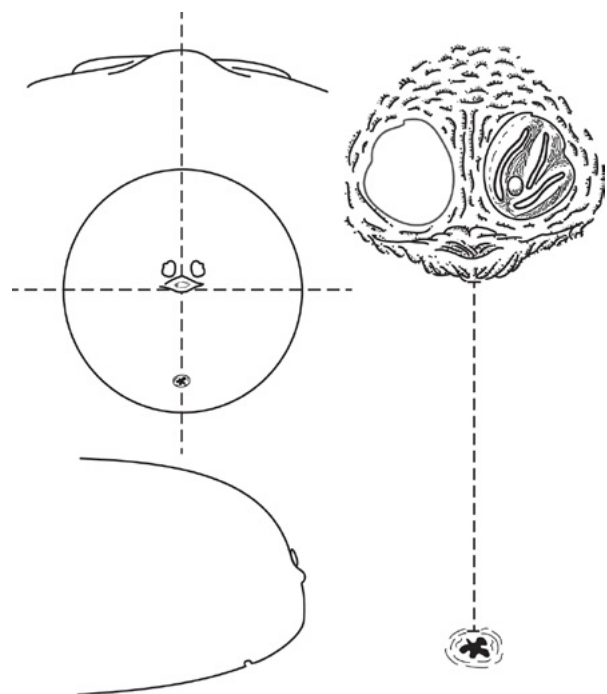


Figure 20.—Line drawing of *Parasetigena silvestris* puparium. Illustration adapted from Figure 53 of Sabrosky and Reardon (1976) and Figure 63 of Simons et al. (1979) by Laura Blackburn, USDA Forest Service.

### *Parasetigena silvestris* (Robineau-Desvoidy)

This tachinid fly is a univoltine, oligophagous larval parasitoid that was introduced for gypsy moth control in 1910 (Kenis and Vaamonde 1998). It is one of the most important natural enemies of gypsy moth throughout its global range (Elkinton and Liebhold 1990, Kenis and Vaamonde 1998), exhibiting peak parasitism when host populations decline following an outbreak. Females lay large, white eggs on the dorsum of middle to late instar larvae (Fig. 19). The eggs hatch after 2 days, and the young maggot enters the host's body, forming a respiratory funnel at the point of entry. This larval stage lasts from 16 to 35 days (Fuester et al. 2014). Then, the larva emerges from the fully-grown larval host, or occasionally from the pupal

host, and creates a puparium to pass the winter in the soil. In profile, the puparium (Fig. 20) has a rounded posterior end that is slightly depressed above the apex, with spiracular plates on the depressed surface above the apex and a bulge between the anus and the subspiracular protuberance (Sabrosky and Reardon 1976). In larger pupae, the distance between the spiracular plates and the anal opening is much longer in *P. silvestris* than in *C. concinnata*, making these species easy to distinguish. Adult flies are 8-12.5 mm long and emerge in May and June (Fig. 21).

12

Figure 21.—*Parasetigena silvestris* adult. Photo by Patrick Derennes, used with permission.

## Egg Parasitoids

Two egg parasitoids are established in North America: *Ooencyrtus kuvanae* (Howard) (Hymenoptera: Encyrtidae) and *Anastatus japonicus* Ashmead (Hymenoptera: Eupelmidae). *Ooencyrtus kuvanae* is the most common, often causing 10-40 percent of gypsy moth mortality, with higher mortality rates occurring in dense host populations (see Brown [1984] for a review). *Ooencyrtus kuvanae* has also been reported as a hyperparasite of *Cotesia melanoscela,* though this occurred at low rates (<0.2 percent) (Crossman 1925, Weseloh 1978). *Anastatus japonicus* is the more cold tolerant of these two egg parasitoid species (Leonard 1974) and has the ability to parasitize at least 35 percent of gypsy moth eggs (Crossman 1925). Details on distinguishing characteristics of these egg parasitoids can be found in older publications (Burgess and Crossman 1929, Crossman 1925, Parker 1933, Tigner 1974). For a key to *Anastatus*, including *A. japonicus*, refer to Narendran (2009).

## UNCOMMON PARASITOIDS

### Hymenoptera (Wasps)

#### *Aleiodes indiscretus* (Reardon)

This braconid is native to India where it is a parasitoid of *Lymantria obfuscata*, a relative of the gypsy moth (Shaw et al. 2013). In the late 1960s, it was introduced to the eastern United States for biological control of *L. dispar* (Reardon 1970) and can now be found from Massachusetts south to Maryland (Shaw 2006). *Aleiodes indiscretus* is an oligophagous endoparasitoid of second and early third instar larvae of *Lymantria* and *Dasychira*



Figure 22.—*Aleiodes indiscretus* parasitizing a gypsy moth larva. Photo by Scott Bauer, USDA.

species. As with all species of *Aleiodes*, the dead host becomes dried and preserved (*i.e.,* mummified). The parasitoid larva pupates and overwinters inside the mummified larval host. The adult parasitoid emerges from the penultimate larval instar through a circular postero-dorsal hole (Reardon 1981, Shaw 2006). Adults are honey-yellow (Fig. 22) with a black ocellar triangle and a body length of 6-8 mm (Shaw 2006). Females have fulvous antennae and a slightly larger body than males, which have fuscous antennae (Reardon 1970).

#### *Monodontomerus aereus* Walker

This torymid pupal parasitoid was imported from Europe between 1905 and 1911 (Clausen 1956). It is oligophagous and univoltine, developing in the pupae of gypsy moth, browntail moth, and several native Lepidoptera. *Monodontomerus aereus* is also a secondary parasitoid of Hymenoptera and Diptera associated with gypsy moth and browntail moth (Clausen 1956, Fuester et al. 2014, Muesebeck 1931). While development is internal in a lepidopteran pupa, development in a tachinid puparium or braconid cocoon is external (Muesebeck 1931). This hyperparasitic habit likely causes more harm than good, though it has not been found in sufficient numbers to impact either the primary or secondary host (Clausen 1956, Hoy 1976). The adults overwinter in old cocoons and webs of browntail moths, with only females surviving winters (Muesebeck 1931).

Females break hibernation in mid-April to mid-May and oviposit in June when a host cocoon, puparium, or pupa is found (Muesebeck 1931). A single female may lay up to 350 minutely-spined, grayish-white eggs over a two-month period. Eggs are 0.6-0.65 mm long by 0.2 mm wide at their widest and tapered at both ends. The time from egg deposition to adult emergence ranges between 18 and 24 days. Up to 24 adults can emerge through a single, mostly circular hole in the host's pupal envelope. Mating occurs before hibernation, though it is also possible for this species to reproduce parthenogenetically, in which case all offspring will be males (as is typical of all Hymenoptera). Female length is 1.5-3.5 mm, with males slightly smaller in size (Muesebeck 1931). Adults have a greenish-black head, thorax, and abdomen with brown tibiae and tarsi (Fig. 23). See Muesebeck (1931) for life history and biological information on this parasitoid/hyperparasitoid.

### *Pimpla pedalis* Cresson

This wasp is multivoltine and native to the United States. *Pimpla pedalis* is polyphagous and commonly uses tent caterpillar larvae as hosts, although historically it has been listed as a parasitoid of gypsy moth (Gupta 1983). Even though parasitism of the gypsy moth by *P. pedalis* is rare, this wasp still kills many host prepupae and pupae through stinging alone (Campbell 1963). *Pimpla disparis* and *P. pedalis* (Figs. 12 and 24) are easily distinguished by their middle and hind coxae, which are orange in *P. pedalis* and black in *P. disparis*. Adults are 12-15 mm in length with a fore wing length of 8-12 mm (Gupta 1983).

### *Itoplectis conquisitor* (Say)

Native to the United States, this is a primary parasitoid of lepidopterans. This parasitoid is multivoltine and highly polyphagous, occasionally acting as a hyperparasitoid (Moser et al. 2008). Similar to *Pimpla* and *Theronia*, this endoparasitoid readily stings gypsy moth pupae, though it rarely develops in them (Campbell 1963). Females readily feed on the hemolymph or body fluids of their host, which leads to host mortality (Leius

1961). *Itoplectis conquisitor* prefers an open canopy and is positively associated with highly defoliated areas (Campbell 1963). Adults are black with a banded appearance on the hind leg and abdomen; the legs are mostly reddish-brown while the hind tibia has a broad yellowish-white band (Gupta 1983), and the tarsus is black and white striped (Fig. 25). The size of an adult is typically 9-14 mm, though some specimens are shorter (5-6 mm).



Figure 23.—*Monodontomerus aereus* adult. Photo by Josef Dvořák, via Biolib.cz, used with permission.



Figure 24.—*Pimpla pedalis* adult. Photo by KNWR Entomology Collection (CC0), via Wikimedia Commons.
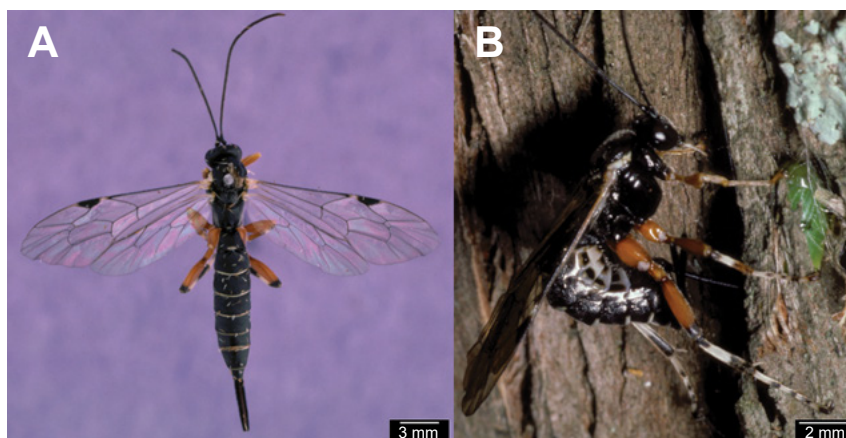


Figure 25.—*Itoplectis conquisitor* adult (A) dorsal view and (B) side view. Photo by Gerald J. Lenhard, Louisiana State University, via Bugwood.org.

### *Perilampus hyalinus* Say

This is a primary parasitoid of some species of sawflies in the genus *Neodiprion* and a hyperparasitoid in association with Lepidoptera and Orthoptera. In gypsy moth and brown-tail moth, *Perilampus hyalinus* is a hyperparasitoid of the tachinids *Compsilura concinnata, Exorista mella,* and *Lespesia frenchii*, and the braconid *Cotesia melanoscela.* In 1912, *P. hyalinus* was reported as being regularly reared from cocoons and puparia of primary parasitoids (Smith 1912), yet today these species are not found throughout the gypsy moth range. These hyperparasitic adults generally emerge from their host puparia 4 to 7 days after the typical emergence of the host parasitoid (Rees 1973). Females can produce up to 367 eggs, ovipositing on foliage at least 4 days following emergence (Tripp 1962). Eggs are elongate with rounded ends and are 0.3 mm long by 0.07 mm wide (Tripp 1962). Planidia (highly mobile first instar larvae) emerge 8-10 days later and are less than 0.3 mm in length (Tripp 1962). Planidia seldom move far from their oviposition site, where they stand erect and wait to make contact with a host. Once they have found a host, they enter the integument in the soft area between the segments in search of a parasitoid on which to complete their development (Smith 1912). *Perilampus hyalinus* completes four instars, and the pupa develops inside the host. Adults emerge from puparia or cocoons through a jagged hole roughly 1.9 mm in diameter. Adults are metallic green and 5 mm in length (Rees 1973) (Fig. 26).

## Diptera

### Sarcophagidae

Referred to as scavengers, some sarcophagids are known to attack gypsy moth pupae already parasitized by ichneumonids (Campbell 1963). *Arachnidomyia aldrichi* Parker exhibits a delayed density-dependent response, increasing their population prior to host population collapse (Brown 1938). In one study, Campbell (1963) found that for 33-46 percent of gypsy moth pupae initially parasitized by ichneumonids, sarcophagids successfully emerged. This native, univoltine fly larviposits in prepupae and pupae of the forest tent caterpillar larva, but may also utilize the gypsy moth, satin moth, and spruce budworm (*Choristoneura fumiferana*) (Dodge 1961). Yellowish-white maggots emerge from the host pupa 8-10 days after parasitism and then drop to the ground (or



Figure 26.—*Perilampus hyalinus* adult. Photo by John Rosenfeld, via BugGuide.net, used with permission.
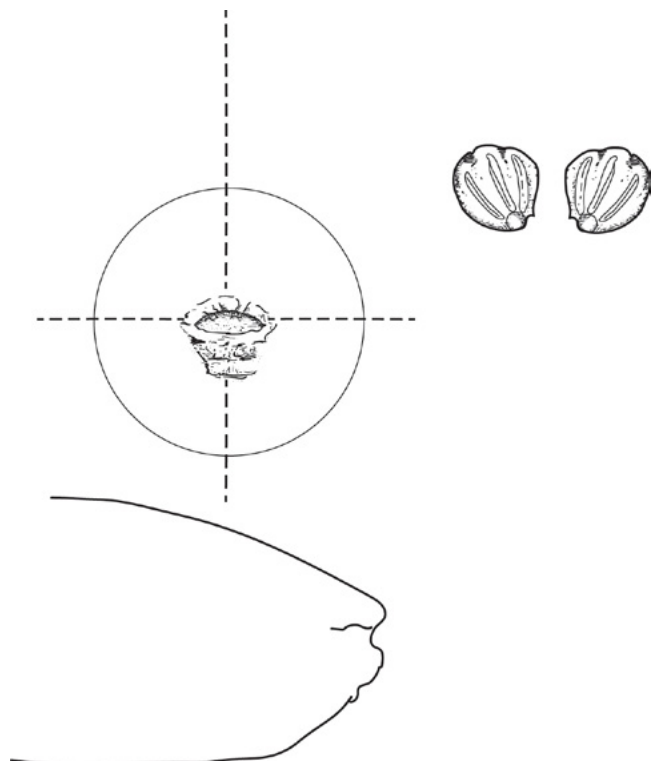


Figure 27.—Line drawing of *Arachnidomyia aldrichi* puparium. Illustration adapted from Figure 1 of Sippell (1961) by Laura Blackburn, USDA Forest Service.

burrow into the diet in the cup) to form a puparium and hibernate. *Arachnidomyia aldrichi* is easily identified in the maggot and puparial stage by the deep posterior depression where the stigmatal slits are located (Fig. 27). The spiracular slits are almost straight and nearly vertical in position (Tigner 1974). Adults measure 8-10 mm in length (Hallock 1940), and, as is characteristic of many Sarcophagidae, there are three prominent longitudinal stripes on the thorax

(Fig. 28). Another sarcophagid species that has been reared from gypsy moth late instar larvae and pupae is *Agria housei* Shewell (Tobin and Hajek 2012), and additional species of sarcophagids likely associate with gypsy moths and attack wounded and moribund individuals.[2]

### *Exorista larvarum* (Linnaeus) and *Exorista mella* (Walker)

*Exorista larvarum* (Fig. 29) is native to the Palearctic region and was released in New England as early as 1906 for biological control of the gypsy moth (Clausen 1956). This species is often confused with the native *E. mella* (Fig. 30). Both species of *Exorista* are multivoltine and polyphagous. Similar to *Parasetigena silvestris*, *Exorista* species lay eggs directly on the surfaces of late-instar host larvae (Sellers 1953). These eggs hatch and the maggots immediately burrow into the host's body. However, *E. larvarum* forms its puparium outside the skin of the host, while *E. mella* forms its puparium inside the skin of the host (Sellers 1953). The puparia of both *Exorista* species have a rounded posterior end, which is somewhat depressed above the apex, with very little elevation of the spiracular plates above the surrounding surface. The spiracular plates, located well above the apex of the puparium, have three slits each on a well-defined ridge (Fig. 31). Adults emerge roughly 15 days after the puparium is formed, nearly 1 month following oviposition. Adult size ranges from 5 to 13 mm, depending on the size of the host (Adam and Watson 1971).

### *Lespesia frenchii* (Williston)

*Lespesia frenchii* is another rarely occurring parasitoid that is native to the United States. This multivoltine and highly polyphagous parasitoid uses both gypsy moth and forest tent caterpillar larvae as hosts. *Lespesia frenchii* exhibits high rates of parasitism in fragmented forests and along the forest edge (Roth et al. 2006). Puparia are 8.5 mm long and have strongly sunken, sinuous spiracular slits with distinct loops (Fig. 32) (Sabrosky and Reardon 1976). Adults are 7-9 mm long (Fig. 33). For more information, see Rees (1973) or Sabrosky (1980).

[2]Pape, T. 2012. Personal communication. Associate Professor and curator, Natural History Museum of Denmark, Universitetsparken 15, 2100 København Ø, 11, Building: 02-4-411 Denmark.

Figure 28.—*Arachnidomyia* species, adult. Photo by Joseph Berger, via Bugwood.org.



Figure 29.—*Exorista larvarum*, adult. Photo by Janet Graham (CC-BY 2.0), via Wikimedia Commons.



Figure 30.—*Exorista mella*, 12 mm adult male. Photo by Ombor Mitra, Agriculture and Agri-Food Canada, via TachImage, used with permission.
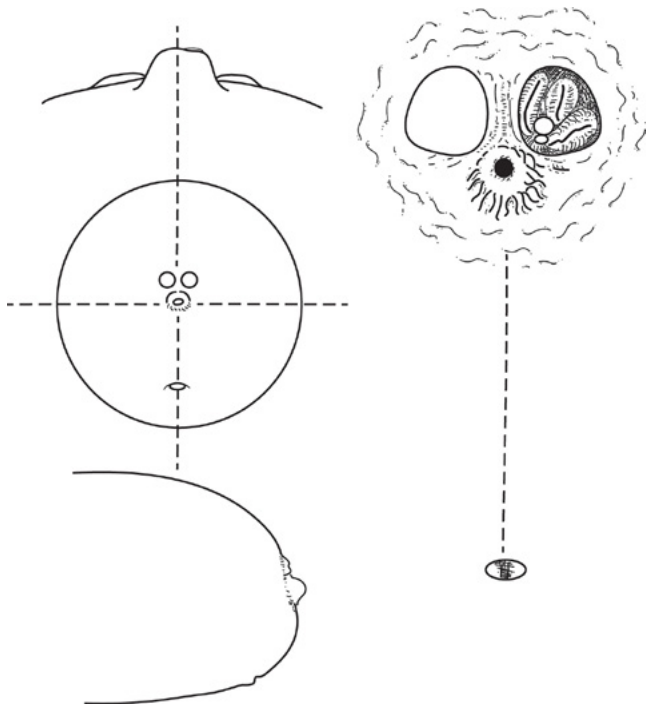
Figure 31.—Line drawing of *Exorista mella* puparium. Illustration adapted from Figure 72 of Greene (1922) and Figure 58 of Sabrosky and Reardon (1976) by Laura Blackburn, USDA Forest Service.
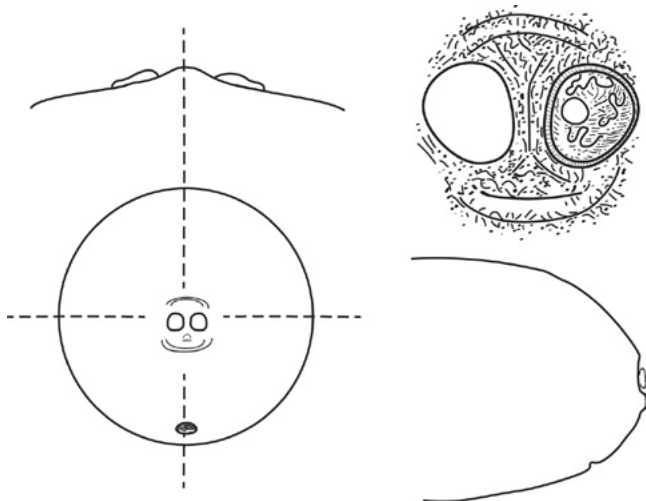


Figure 32.—Line drawing of *Lespesia frenchii* puparium. Illustration adapted from Figure 10 of Sippell (1961), Figure 72 of Sabrosky and Reardon (1976), and Figure 69 of Simons et al. (1979) by Laura Blackburn, USDA Forest Service.



Figure 33.—*Lespesia frenchii*, 8.6 mm adult male. Photo by A.J. Fleming, Agriculture and Agri-Food Canada, via, TachImage, used with permission.

# GYPSY MOTH COLLECTION AND REARING TECHNIQUES

## Field Collections

Larvae can be collected in a number of ways, and two methods are outlined in this guide. The first method is to search for dead larvae. The shape of the cadaver is likely to reveal the underlying cause of death, so be sure to note this while collecting specimens. The second method is to collect live larvae and rear them individually in the laboratory (see section below on rearing larvae). While rearing, check frequently to detect larval death, after which a parasitoid might emerge or the cadaver may be examined microscopically for the presence of pathogens.

When collecting gypsy moth life stages, it is important to take time of year and behavioral characteristics into consideration. Gypsy moth larvae hatch in early spring, roughly coinciding with oak bud break, and their development is strongly influenced by temperature. In the northeastern United States, larvae are generally found from May to early July. Newly hatched larvae may remain near the egg mass from which they hatched if emergence occurs during rainy weather or if temperatures are below 7 °C (Leonard 1981). Larvae then ascend trees, where aerial dispersal, called ballooning, occurs as they release silken threads and are blown on the wind (McManus 1973).

Gypsy moth larvae in the early instar stages are the most specific when it comes to host selection, preferring *Quercus, Populus,* and *Salix* species

(Elkinton and Liebhold 1990). Except in outbreak populations, larvae feed during the day, mainly in early morning and late afternoon (Leonard 1981). During periods of inactivity, early instars can be found along the midrib on the undersides of leaves.

As the larvae grow, their feeding behaviors change. Late instars crawl down tree trunks, where they seek darker daytime resting sites, such as under the base of limbs, in bark crevices, and under the leaf litter or on the underside of objects on the forest floor. Late instars can be found resting on a wide variety of host and nonhost trees, and pupae are typically found in these same locations (Elkinton and Liebhold 1990). For an extensive list of gypsy moth preferred host species, see Liebhold et al. (1995).

Behavior of gypsy moth larvae can be dependent upon the density of the population. Campbell (1967) and Semevsky (1971) suggest that first instars from high-density populations have higher rates of dispersal than larvae from low-density populations. Late larval instars in low-density populations are nocturnal, feeding during the night and spending the day in resting sites on the ground or in protected places on the trunks of trees. In high-density populations, late-larval instars are cathemeral, feeding in the tree canopy during both the day and night, only descending from the canopy when foliage no longer remains (Lance et al. 1987). All of these behaviors should be considered when collecting specimens.

Locating larvae can be a challenge depending on the density of the local population. Look for clues such as a build-up of frass pellets on the ground or damaged leaves on preferred species of shrubs or trees. Focus your search on new leaves, saplings, and preferred plants along a forest edge (Wagner et al. 1997). During the day, early instars will be in the foliage or on the undersides of twigs and branches if it is raining, while later instars will often be on tree trunks. Finding early instar larvae in stands with mature trees can be difficult because of the canopy height, so in this case, search for larvae on preferred understory shrubs.

In areas of high gypsy moth density, "beating" can be an efficient method for collecting early instar larvae from trees and shrubs. Spread a sheet over the ground and use a bat or sturdy stick to rap against a series of branches over the sheet, thus dislodging larvae. Recording the host species can be of value, and

focusing your collection efforts on one tree species at a time will help you to keep more accurate records.

In areas of low to moderate gypsy moth density, it is often efficient to collect late instar larvae and pupae under burlap bands that provide daytime resting sites. Encircle a tree at chest height with a strip of burlap 20 to 25 cm in width; staple along the top edge and cut vertical strips to allow the flaps to be lifted while inspecting for larvae. It should be noted that collecting larvae from bands may provide a biased sample of larval mortality agents because larvae resting beneath burlap bands will not crawl to the leaf litter during the day. Thus, their behavior is changed if burlap bands are used, and larvae then avoid resting under the leaf litter, a major site where infection by fungal pathogens, such as *Entomophaga maimaiga*, can occur.

Photographs may be taken of the insect in its natural setting, and recording notes about the tree species or substrate where the sample is collected from may also be desired. Field samples should be placed in individual containers. If live larvae are collected, place them in a container with an artificial diet (see section below on rearing larvae). If a cadaver or pupa is found attached to foliage or a twig, use a knife or pruners to remove the sample while it is still attached to its natural substrate.

## Rearing Gypsy Moth Larvae

Gypsy moth larval specimens should be placed individually in closed containers and kept at approximately 22 °C at low to moderate moisture levels. Do not rear larvae at constant temperatures over about 25 °C because larvae may not tolerate high temperatures. Also, take care to store containers in the shade because exposure to direct sunlight can cause excessive heating. Live larvae should optimally be placed in 30 ml plastic cups containing an artificial diet. See Bell et al. (1981) for a recipe, or use a ready-to-prepare gypsy moth artificial diet that is available commercially. When rearing larvae on an artificial diet, care should be taken not to touch the diet or introduce contaminants that can lead to fungal or bacterial growth. When larvae pupate, transfer day-old pupae to empty diet cups to avoid excessive moisture from the diet medium. Newly molted pupae are delicate and should be handled with care.

Larvae can also be kept in containers with live foliage. Foliage should be placed in a vial of water packed with cotton to anchor the stem or branch, and leaves

should be replaced regularly (and checked daily) so that acceptable foliage is available. However, providing fresh leaves may not be efficient when rearing numerous samples or when parasitoid emergence requires a long waiting period. It is also possible that foliage may be contaminated with a virus or other pathogens.

For the 10 days after collection, all larvae should be checked for death daily. After larvae die, make sure that the larval container is at high humidity for 3 days to facilitate conidial production by *E. maimaiga.* Create a high humidity environment by closing the container with the artificial diet or by adding a piece of wet paper towel inside of a closed container. Larval death due to fungal infections usually occurs within one week of collection, although this can extend to 10 days (depending on rearing temperature and conditions). After the first 10 days, if a larva has not died, checks should be made weekly to look for host death. Because larval deaths caused by viral infections may occur for up to a month after collection, continue rearing and checking the larva for at least 1 month.

When checking rearings also look for parasitoids, making certain to examine the underside of the cup for any parasitoid larvae that may have emerged and burrowed into the artificial diet. If mold starts to grow on the artificial diet, transfer the specimen to a clean diet cup. It can take several weeks before a parasitoid emerges to form a cocoon (wasp) or a puparium (fly). Adults of some gypsy moth parasitoids emerge from cocoons or puparia in the following year, so pupal characteristics are a useful feature for parasitoid identification. Approximately 1 month after collecting larvae or pupae, all samples should be stored in a refrigerator and dissected to search for dead parasitoid larvae or pupae and microscopically examined for pathogens.

# PATHOGEN IDENTIFICATION

## Laboratory Dissections

To determine the cause of larval death, initially note the external condition of cadavers in the field. Both fungal and viral pathogens are more commonly found in later instar hosts. Bodies of larvae that die from LdMNPV are often loosely attached to surfaces (like tree trunks) by their anterior prolegs only (often creating an inverted V; Fig. 7). The cadavers are internally liquefied, so once the cuticle breaks, the cadaver drips and rapidly

disintegrates. In contrast, late instar larvae killed by the fungus *E. maimaiga* will often hang vertically on the tree trunk with heads downward and prolegs at a 90º angle (Fig. 4), and their bodies will eventually appear dry and stiff (although if recently dead, the internal contents of these cadavers are not solid). However, the vertical orientation of cadavers alone cannot be used to identify the cause of death (Hajek and Roberts 1992). Usually within several days of larval death, cadavers fall to the ground and decompose, releasing spores or occlusion bodies which overwinter in the soil. A cadaver can contain either a single pathogen or both the fungus and the virus. Thus for accurate diagnosis, microscopic examination is necessary.

The size, structure, and odor of pathogens provide identifying characteristics. Spores of *E. maimaiga* can easily be seen since they are larger than viral occlusion bodies. Conidia may be produced by all instars. These ephemeral pear-shaped spores are approximately 20 µm x 25 µm (Hajek and Snyder 1992) and are typically visible microscopically at 100x to 200x. Viral occlusion bodies are comparatively much smaller but readily visible under a compound microscope at 200x to 400x. Viral structures vary between 1 and 10 µm in diameter and are polyhedral in shape (Hajek 1994). Cadavers killed by LdMNPV exude a characteristically unpleasant and unique odor, while larvae killed by *E. maimaiga* are typically odorless (Hajek and Roberts 1992, Koyama 1954).

**Equipment**

- Dissecting microscope for parasitoids (6x-50x such as Wild M5 or M5A)
- Compound microscope with phase contrast
- Gloves
- Lighter
- Alcohol lamp with 95 percent ethanol*
- Small beaker with tap water
- Two dissecting probes
- Bottle with 1M KOH and a pipet*
- Bottle with distilled water and a pipet*
- Glass microscope slides
- 18 mm x 18 mm cover slips
- Paper towels

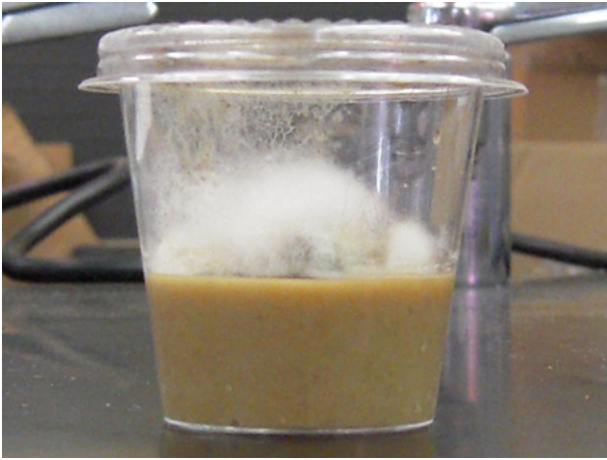* Bottles should be kept closed or covered to prevent evaporation.

Figure 34.—Gypsy moth cadaver covered in mold in a diet cup. Photo by Ruth Plymale, Ouachita Baptist University, used with permission.
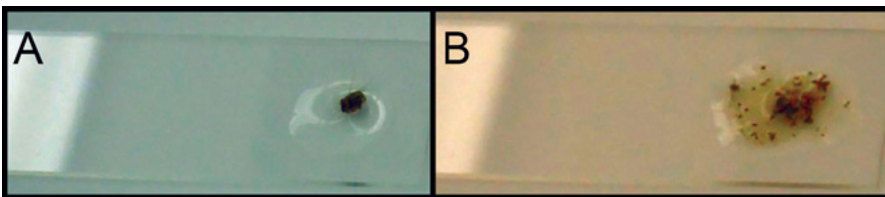


Figure 35.—Lab dissections may be needed to identify cause of death. (A) Add 1-2 drops of water onto microscope slide with a dissected piece of a cadaver. (B) Smash the cadaver piece with probes to form a homogenized mixture (less material is better). Photos by Ruth Plymale, Ouachita Baptist University, used with permission.



Figure 36.—Slide with large fragments of the cadaver removed from the cadaver and water mixture. To observe viral occlusion bodies, the smear between the cover slip and slide must be thin and nearly transparent. Photo by Ruth Plymale, Ouachita Baptist University, used with permission.

## Techniques for Pathogen Identification

1. Put on snug-fitting gloves for sterility.

2. Organize samples for dissection and prepare a notepad or other medium for recording results next to the microscope.

3. Flame two dissection probes by passing them into a flame (e.g., alcohol lamp) for several seconds.

4. Dip dissection probes into water to cool (they should sizzle). Wipe probes on paper towels to clean them.

5. Use both probes to remove a 1 to 2 mm-sized piece of cadaver from the cup and place it on a microscope slide. If saprophytic mold has taken over the sample and artificial diet (Fig. 34), look for hairs or the head capsule to identify the cadaver. If all you can find is the head capsule, transfer it and any surrounding bits of cadaver or hairs to the middle of a microscope slide. This technique works much better with less material rather than more.

6. Add 1-2 drops of distilled water from the water bottle to the cadaver piece on the slide (Fig. 35A) and gently pulverize the piece with the probes until the cadaver piece is homogenized and well mixed with the water (Fig. 35B).

7. Return large fragments from the cadaver back to the bioassay cup, leaving the smear in water on the slide (Fig. 36). Close the cup. Removing these larger fragments makes viewing under the compound microscope easier.
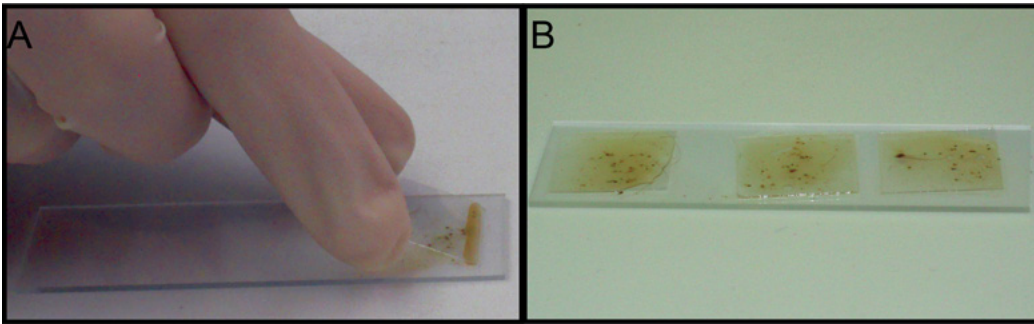
Figure 37.—(A) Place the cover slip over the sample from the side to prevent air bubbles. (B) Three samples can be placed on one slide. Photos by Ruth Plymale, Ouachita Baptist University, used with permission.

8. Add an 18 mm x 18 mm cover slip by laying it on the sample, starting at one side to avoid air bubbles (Fig. 37A). Using the 18 mm x 18 mm cover slips, three samples will fit onto a normal glass microscope slide (Fig. 37B).

9. For typical samples, view under the compound microscope with phase contrast at 200x (10x eyepiece x 20x objective). Make sure the phase ring is on the appropriate phase (20x phase setting if you are using the 20x objective). Scroll from side to side across the cover slip and move the slide backward and forward, making sure to see the entire cover slip. Look for resting spores and then viral occlusion bodies and conidia (but remember that these are very different sizes). If only one resting spore is found, take a second sample from the cadaver to verify this diagnosis. Make sure to clean the dissecting probes and work area between samples so that the contents of one cadaver are not mistaken for the contents of another cadaver.

It can be difficult at high magnification to feel certain whether bright dots within the smear are viral occlusion bodies or not. Test each sample for LdMNPV occlusion bodies by adding 1M KOH and watching for the occlusion bodies to dissolve; but note that this only works with phase contrast, and doing this at 200x magnification is suggested. Add 1 small drop of 1M KOH from a small pipet to one side of the cover slip. As that drop of KOH moves under the cover slip and across the slide, any occlusion bodies that come into contact with the KOH will dissolve (i.e., they will turn from bright to black and often will then disappear). If the KOH does not seem to be moving under the cover slip, use a piece of fine tissue at the opposite edge of the cover slip to take up the liquid, thereby causing the KOH to flow under the cover slip. Watch closely under the microscope as the KOH moves across the slide in order to observe the dissolution of the occlusion bodies. If too much KOH is added, the cover slip gets flooded and the virus is washed away, losing the chance to see it dissolve, so be frugal with KOH usage. KOH is corrosive, so always take care to avoid contact between the KOH and the microscope. If you only observe the dissolving of a small number of occlusion bodies, take a second sample from the cadaver to verify this diagnosis.

## Molecular Methods

Molecular methods can also be used to identify the pathogens/parasitoids in gypsy moth larvae, but there are several issues complicating the molecular analysis. For instance, developing the facilities needed for molecular analysis is expensive and requires high-end instrumentation. Also, molecular analysis is laborious and requires technical skill. With living larvae it is possible to pulverize individual larvae, extract DNA, and then use unique primers for the fungus, virus, and the parasitoids as a group. One would then use DNA fingerprinting to tell the different parasitoid species apart (Bashasab et al. 2006, Chatterjee et al. 2013, Greenstone 2006, Landry et al. 1993, Loxdale and Lushai 1998).

Dead larvae also require three separate analyses to identify fungus, virus, and parasitoids, and several issues can complicate the molecular analysis:

1. If the larva dies from *E. maimaiga* and produces only conidia, these spores decompose within a short time at room temperature, so a sporulating cadaver must be stored in 95 percent ethanol or storage buffer (such as RNAlater™ stabilization solution) during the relatively short time that the fungal cells are alive after host death.

2. If the larva dies from *E. maimaiga* and resting spores are only produced, these must be broken open in order to reach the fungal DNA. See Castrillo et al. (2007) for a method on how to break resting spores to harvest *E. maimaiga* DNA.

3. Cadavers need to be pulverized as for living larvae to detect viruses or parasitoids.

4. In some cases, the parasitoid might have left the cadaver to pupate in the artificial diet, so rearing containers must always be closely searched for parasitoid larvae or pupae which can then be pulverized for DNA extraction and identification with DNA fingerprinting.

Potentially, identification with molecular methods may be more sensitive. However, there is uncertainty about the relative lengths of time for the different methods, and use of molecular methods may be more expensive.

## SUMMARY

The introduction of natural enemies against the gypsy moth in the United States is likely the most intensive biological control effort directed against any individual species in the world. Over the past century there have been many unsuccessful attempts at biological control of gypsy moth. Of the 34 parasitoids introduced to control gypsy moth, only 12 are established in North America (Fuester et al. 2014), with the majority of these natural enemies becoming established prior to 1920 (Burgess and Crossman 1929, Howard and Fiske 1911). Though keys to these parasitoids have been provided in the past (e.g., Sabrosky 1980, Sabrosky and Reardon 1976), they are quite exhaustive and include many species that were released in North America but never became established. This guide helps to identify the most commonly occurring pathogens and parasitoids found today within the North American range of the gypsy moth.

## ACKNOWLEDGMENTS

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# GLOSSARY

**apex**—the top or highest point of something.

**cathemeral**—irregularly active through the day and night, not falling into the strict definitions of diurnal or nocturnal or crepuscular.

**cocoon**—a papery or silken structure enclosing the pupa.

**conidiophore**—a specialized hypha upon which conidia develop.

**conidium** (sing.); **conidia** (pl.)—an asexual reproductive propagule formed in any manner that does not involve cytoplasmic cleavage. Conidia function as organs of reproduction and dissemination. Here, use of this term refers to fungi.

**coxa** (sing.); **coxae** (pl.)—the basal segment of a leg on insects.

**diurnal**—active mainly during the daylight hours.

**endoparasitoid**—a parasitoid that lives inside another insect and ultimately kills it.

**epizootic**—an unusually large number of cases of a disease within an animal population that is not human.

**femur** (sing.); **femora** (pl.)—the segment of an insect's leg that is third from the body.

**frass**—excrement or debris produced by insects.

**fulvous**—reddish or brownish yellow; tawny.

**fuscous**—dark gray or grayish brown in color; dusky.

**hemolymph**—the circulatory fluid of various invertebrate animals that is functionally comparable to the blood and lymph of vertebrates.

**hyperparasitoid**—An insect that is a parasitoid of a parasitoid.

**larva** (sing.); **larvae** (pl.)—the active immature form of an insect, especially one that differs greatly from the adult. This is the stage between egg and pupa (e.g., a caterpillar, maggot, or grub).

**larviposit**—to bear and deposit living larvae instead of eggs.

**maggot**—a soft-bodied legless stage that is the larva of a dipterous insect (e.g., the housefly).

**mesic**—a type of habitat with a moderate or well-balanced supply of moisture.

**multivoltine**—having more than one generation per year.

**nocturnal**—active mainly during the night.

**ocellar triangle**—a three sided space, sharply defined in many insects, on which the ocelli (or simple eyes) are located.

**oligophagous**—feeding on a limited number of foods, usually within one taxonomic family of plants or animals.

**oviposit** (verb); **oviposition** (noun)—to lay eggs, especially by means of an ovipositor (a tubular structure, usually concealed but sometimes extending outside the abdomen, with which many female insects deposit eggs).

**parasitoid**—an insect, and especially a wasp or fly, that completes its larval development feeding on the body of another insect, eventually killing it, and is free-living as an adult.

**parthenogenetic** (adj.); **parthenogenesis** (noun)—a form of reproduction in which an unfertilized egg develops into a new individual; commonly occurs among insects and certain other arthropods.

**pathogenic** (adj.)—causing or capable of causing disease.

**penultimate**—occurring immediately before the last one; next to the last.

**phagocytosis**—the engulfing and usually the destruction of non-self matter by individual immune system cells that perform this service; this term often refers to engulfing and killing microbes that have invaded a host.

**planidium** (sing.); **planidia** (pl.)—a first-stage larva of various parasitic hymenopteran and dipteran insects, known as a dispersal stage.

**polyphagous**—feeding on a broad array of plant or animal species.

**postero-dorsal**—relating to, or situated at, the back of the upper surface of an organism or object.

**prepupa** (sing.); **prepupae** (pl.)—a stage in the development of many insects immediately preceding the change to a pupa and usually marked by cessation of feeding and reduction of activity.

**protuberance**—usually a rounded part that sticks out from a surface.

**pubescence**—a covering of fine soft short hairs.

**pupa** (sing.); **pupae** (pl.)—the inactive stage in some insects, such as true flies and wasps, that falls between the larval and adult stages, during which the insect typically undergoes complete transformation within a protective cocoon or hardened case.

**puparium** (sing.); **puparia** (pl.)—a rigid outer shell formed from the larval skin that covers some pupae (as in dipteran parasitoids discussed above).

**rachis** (sing.); **rachises** (pl.)—a bent or sometimes zig-zag extension at the tip of a conidiogenous cell produced by lateral branching of the elongating extension beneath each successive conidium that is formed.

**resting spores**—a persistent spore created by some fungi which has a thick wall in order to survive through stressful times, such as drought or periods when hosts are not active. It protects the spore from biotic (microbial, fungal, viral), as well as abiotic (wind, heat, xeric conditions) factors.

**spiracular plates**—In Diptera, the flattened tip of each tube that bears the posterior spiracles (or breathing holes) for puparia.

**stigmatal slits**—narrow breathing pores in fly larvae or puparia.

**tarsus** (sing.); **tarsi** (pl.)—the distal part(s) of the limb(s) of an insect.

**tibia**—the fourth segment of the leg of an insect, between the femur and tarsi.

**univoltine**—having one generation per year.

**viral occlusion bodies**—a crystalline protein matrix surrounding the virions of some insect viruses. For nucleopolyhedroviruses, occlusion bodies are produced within the nuclei of host cells while the insect is still alive, and the occlusion body protects the infectious virions after death of the host.

**virion**—a complete virus particle that consists of an RNA or DNA core with a protein coat and sometimes with an external envelope; this is the extracellular infective form of a virus.

**viviparous**—giving birth to live young (not eggs).

**xeric**—of, relating to, or adapted to a dry environment.

Since the early 1900s, a number of parasitoids have been released for classical biological control of the introduced destructive forest insect, *Lymantria dispar* (gypsy moth), in North America.  During this time, two pathogens were accidentally introduced. These pathogens and several of the parasitoid species are now commonly found in North American gypsy moth populations. The aim in creating this guide was to provide laboratory techniques for distinguishing between two common pathogens, the fungus *Entomophaga maimaiga* and the gypsy moth multiple nucleopolyhedrovirus, and provide illustrations and images for adults, puparia, and cocoons of established gypsy moth parasitoids commonly found in the larval or pupal stages of gypsy moth in North America. Gypsy moth collection and rearing techniques are also reviewed, and a technical glossary and summary table highlighting the affected life stage by gypsy moth parasitoids in North America are included in this guide.

KEY WORDS: *Lymantria dispar*, pathogens, parasitoids, hyperparasitoids, life table studies, cause of death, *Entomophaga*, Tachinidae, Ichneumonidae