



USDA-APHIS-PPQ Asian Gypsy Moth Survey and Response Guidelines



Photo Credit: John H. Ghent, USDA Forest Service, Bugwood.org

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Dedicated to Roeland Elliston, PPQ Western Region Gypsy Moth Program Manager

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Purpose

The information contained in these response guidelines is intended for use when surveying and controlling introductions of Asian gypsy moth (AGM, *L. dispar japonica*, *L. dispar asiatica*, *L. umbrosa*, *L. albescens* and *L. postalba*). These guidelines provide technical and general guidelines for detection, delimiting, eradication treatment, and follow-up delimiting survey activities for occurrences of AGM beyond Department of Homeland Security Customs and Border Protection finds during port of entry inspections.

To avoid the establishment of AGM, the nationwide PPQ response policy for any AGM detection is eradication as outlined in the 1995 USDA Asian Gypsy Moth Policy (Appendix A). These guidelines support this policy with the addition of a decision table to determine the appropriate response method.

Authority and Statutes

The Plant Protection Act, Public Law 106-224, June 2000, provides authority to the United States Department of Agriculture to order treatment or quarantine of an area when the Secretary considers such action necessary to prevent the dissemination of a plant pest.

The choice of treatment material for the Asian Gypsy Moth is governed by the Supplemental Environmental Impact Statement (SEIS) that was filed with the United States Environmental Protection Agency on October 12, 2012.

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I. Pest Information

A. Description and classification

Adult Asian gypsy moth (AGM) males have grayish-brown wings with black markings and a 1½ to 2 inch (3.8-5 cm) wingspan. Adult female AGM are heavy-bodied and can have a wingspan of 3½ inches (8.9 cm) or more. Female wings are white with black markings (Figure 1); however, they rarely exhibit color morphs and appear almost black (PPQ, 2003). Early-instar AGM larvae appear black and have long hair-like setae. Older instars (IV through VI) have distinctive markings on the head capsule and are gray with five pairs of raised blue to blackish spots and six pairs of raised red to brownish-red spots along their back (CAPS, 2008). Occasionally, the instars express color morphs. There are several different types, with the most common being a black or nearly black stripe along the dorsal surface (Figure 2) (Mastro, 2009).



Figure 1. Male (left) and female (right) adult Asian gypsy moths (PPQ).



Figure 2. Late instar Asian gypsy moth larvae exhibiting typical coloration (top) and color morphs (bottom) (PPQ).

Lymantria dispar is a moth in the family Lymantriidae (some recent classifications consider Lymantriinae to be a subfamily of Noctuidae; see Pogue and Schaefer, 2007). In a recent review of the genus

Lymantria, Pogue and Schaefer (2007) recognized three subspecies of *Lymantria dispar*: *L. d. dispar* (L.) (European gypsy moth), *L. d. asiatica* Vnukovskij, and *L. d. japonica* (Motschulsky). For regulatory purposes, the latter two subspecies are both considered Asian gypsy moths (AGM). *L. d. asiatica* occurs in temperate Asia from the Ural Mountains east to China, Korea and the Russian Far East (north of the Himalayans). *L. d. japonica* is found on several major Japanese islands including Honshu, Shikoku, Kyushu, and parts of Hokkaido.

Pogue and Schaefer (2007) also described three new or revised/re-described species that had previously been considered subspecies of *L. dispar*. These are *L. umbrosa* (*Lymantria dispar hokkaidoensis/umbrosa/nesiobia*), *L. albescens* (*L. dispar albescens*), and *L. postalba* (*L. d. postalba/tsushimensis*, *L. albescens tsushimensis*). All three are native to Japan (though their distributions are generally more limited than that of *L. d. japonica*). All three species, like *L. dispar*, use disparlure as the major, if not sole, component of their sex-attractant pheromone and thus may be caught in gypsy moth monitoring traps.

Pogue and Schaefer indicate that regardless of the changes in nomenclature, “Asian Gypsy Moths” are those that have females capable of flight. Therefore, for regulatory purposes, USDA considers all three newly classified species to be AGM in addition to *L. d. asiatica* Vnukovskij, and *L. d. japonica* (Motschulsky) (Table 1). DNA analysis is used to determine what type of moth is trapped; Section XX covers the submission process of samples.

Table 1. Asian Gypsy Moth. Winged and flight-capable females.

Common Name(s)	<i>Lymantria</i> Species or Subspecies (Subgenus: <i>Porthetria</i>)	Latest authoritative reference for name	Brief Distribution	Field ID Characteristics
Asian Gypsy Moth (AGM)	<i>dispar asiatica</i>	Present Study	Asia mostly east of Urals, China Korea	Females fly to lights, often en masse
Japanese Gypsy Moth (JGM)	<i>dispar japonica</i>	Schintmeister, 2004; Present Study	All main islands in Japan; limited in Hokkaido	Large males, very dark brown color
Okinawan Gypsy Moth (OGM)	<i>albescens</i>	Schintmeister, 2004; Present Study	Okinawa, southern Ryukyu Islands	Males with white hind wings, more southerly
Hokkaido Gypsy Moth (HGM)	<i>umbrosa</i>	Present Study	Hokkaido, esp. eastern part	Smaller males, light in color
Tsushima Gypsy Moth (TGM)	<i>postalba</i>	Present Study	S. Kyushu & N. Ryukyu Islands	Male with white hind wing, more northerly

B. History and risk of AGM in North America

AGM was first discovered in North America in 1991 in British Columbia, having arrived as egg masses on cargo ships from Russia (Savotikov, 1995). Immediately after this discovery, traps were set around

ports in Washington and Oregon where additional AGM were caught. In 1993, AGM were found on the east coast when females started flying off of a military cargo vessel that had arrived from Germany and docked in Sunny Point, North Carolina. Eradication protocols were immediately put into effect in these areas and the populations were successfully eradicated (PPQ, 2003). Recent studies have revealed that AGM as well as European gypsy moth (EGM) are capable of surviving in all temperate regions of the world with the exception of sub-alpine and desert regions (Peterson, 2007). As of 2008, AGM has been detected and subsequently eradicated in Washington, Oregon, California, Idaho, Texas, and North Carolina. AGM is not known to be established in any areas of the United States.

C. Life cycle

AGM has four life stages: egg, larva (caterpillar), pupa and adult (moth). Females lay one batch of eggs in a single mass on a host tree or inanimate object. The size of the egg mass varies depending on what the female fed on while in the larval stage (among other factors), but can range up to 1500 eggs. AGM are attracted to light; therefore, eggs are frequently laid near light sources (Figure 3) (Savotikov, 1995). Egg masses are covered with yellowish to tan hairs (appearing fuzzy) but can bleach out to near white after prolonged exposure to direct sunlight. On average they are 1½ inches (3.8 cm) long and ¾ inch (1.9 cm) wide (Figure 4) (PPQ, 2003). In 4-6 weeks, embryos develop into larvae but remain unhatched, in the egg, until spring. Time of hatch in the spring is temperature-dependent, but typically larvae emerge from eggs approximately when buds on host trees have just broken. AGM larvae feed on leaves and are the only stage that causes damage (Doane et al. 1981, Wallner et al. 1989). After five or six molts as larvae, AGM enter the pupal stage which typically lasts 10-14 days (Figure 5). When the moths emerge they do not feed. Females release a sex pheromone to attract males and, after mating, search for a place to lay their eggs (PPQ, 2003). The moth stage is very short, lasting only 2-3 days (Mastro, 2009).



Figure 3. Asian gypsy moth egg masses laid on a light aboard a shipping vessel (PPQ).



Figure 4. Asian gypsy moth egg mass (Canadian Food Inspection Agency).



Figure 5. Asian gypsy moth female (left) and male pupae (PPQ).

D. Biology

Female gypsy moths of the European subspecies do not fly; however, the Asian variety can fly distances up to 25 miles (40 km) (Savotikov, 1995). AGM also require a shorter number of days of exposure to low temperatures to complete diapause (Reineke, 1998). AGM hatch can be induced when ships from infested areas with cold climates reach our much warmer southern ports, even during winter months (Mastro, 2009).

E. Host range

AGM larvae have been known to feed on more than 600 plant species, covering more than 100 botanical families. AGM prefer deciduous trees but can develop on conifers. As a result, they have a broader host range than the European subspecies. Large infestations of AGM can completely defoliate trees, which weaken the trees and expose them to attack by secondary organisms. If defoliation is repeated for two or

more years, it can lead to the death of large sections of forest as well as orchards and plantations (Savotikov, 1995).

F. Spread of infestation

Because females have the ability to fly long distances, they spread rapidly into and through uninfested areas. In addition, newly hatched AGM larvae disperse by “ballooning,” which consists of climbing a tree or other object, dropping on a silken thread, and becoming wind-borne. Humans are also a potential source of long-range spread. Eggs can be laid on ships, shipping containers, outdoor furniture, firewood, timber, rail cars, automobiles and other inanimate objects. While in the egg stage, gypsy moth is in diapause and can remain quiescent and viable for long periods of time. This provides a long opportunity for these contaminated vehicles and materials to be transported to new areas. Also, AGM egg masses are extremely hardy and their tolerance of temperature and moisture extremes enhances the risk of spread (APHIS, 1992). Once hatched in a new location, the broad host range and the ability of the larvae to disperse enhances their ability to establish.

II. Identification

A. Handling and submission of suspect AGM specimens for identification

Specimens that are suspected of being AGM should be submitted to the Center for Plant Health Science and Technology (CPHST) Otis Laboratory for testing (see Appendix B). All specimens collected outside of the EGM quarantine areas will be analyzed. Specimens collected within generally-infested areas will be analyzed based on sub-samples of total catch because of the large number of insects which can be caught in some areas. **It is critical that samples be collected regularly, stored properly, and submitted to the Otis Lab as soon as possible to maintain the integrity of the DNA.** If traps cannot be checked regularly, it may be considered to trap when flight is expected rather than spreading resources out across the whole season. As a general rule, traps should be checked and samples removed every two weeks in order to reduce the degradation of the specimen’s DNA. High temperatures and high humidity speed degradation of specimens and trapping schedules should be adjusted accordingly. If stored unfrozen the specimens should be in containers (paper bags or boxes) which will promote drying. Plastic containers retain moisture that favors the growth of bacteria and fungi, which will quickly degrade the DNA. Specimens should be stored in a freezer if possible (if not, in a cool dry area) and shipped to Otis as soon as practical. Specimens should not be stored unfrozen for extended periods. A PPQ Form 305 (Appendix C) should be sent with each trap, stating the trap number, collection site, number of specimens

(estimates okay), life stage, collection date, and date of last (previous) trap check (to determine maximum time that the moth may have been in the trap prior to the check). Specimens should be shipped via next day delivery for Tuesday through Friday arrival (PPQ, 2008) to:

Molecular Diagnostics Unit-Otis Laboratory
USDA, APHIS, PPQ
1398 West Truck Road
Buzzards Bay, MA 02542-1329

Questions can be directed to John Molongoski (phone: 508-563-0929; email: john.j.molongoski@aphis.usda.gov; or fax: 508-563-0903).

The CPHST Otis Laboratory will communicate negative results directly back to the submitter. For any positive AGM confirmations, they will complete a PPQ form 391 and send an e-mail narrative about the detection and PDF of the completed PPQ form 391 to ppq.nis.urgents@aphis.usda.gov. The data will be entered into the Pest ID system and confirmation communicated by the National Identification Service's Domestic Diagnostics Coordinator to the National Survey Coordinator with Emergency and Domestic Programs staff at APHIS headquarters. According to the agreed upon communication protocol, the National Survey Coordinator will forward the confirmation to the list of contacts including the State Plant Health Director and State Plant Regulatory Officer of the state of origin, and the AGM national and regional program managers.

Milk Carton Trap

- Layer moths loosely between wadded paper towels or tissue paper in a paper bag (brown lunch bag size) to prevent motion and specimen damage during shipment (one bag per trap; if more than one bag is required per trap, label appropriately). Label paper bag clearly with trap numbers matching paperwork.
- Staple or tape paper bag closed.
- Do not attach paperwork to individual bags.
- Do not use plastic bags or paper envelopes as these do not allow moisture release and thus promote fungal growth and decomposition of the moths.
- Do not send traps or paperwork for traps which contain no specimens.

Delta Traps

- Label each trap clearly with trap numbers matching paperwork.
- Package traps to avoid crushing during shipment.
- Do not attach paperwork to individual traps.
- Do not use Styrofoam peanuts or other small packaging materials that could potentially enter the traps.
- Do not disassemble the traps or remove moths from the trap.
- Do not ship traps with sharp staples exposed.

Egg Masses

To ensure that useful molecular information can be extracted, DNA must be able to be recovered. In order to achieve this, please adhere to the following protocols.

- Egg masses that are being shipped to Otis should **not** be treated with oil.
- If egg masses are wet, air dry before shipping.
- Ship individual egg masses in separate Ziploc bags; do NOT mix egg masses.
- Include a shipping permit with any shipment of viable eggs (permit obtained from Otis).
- If practical, notify Otis Lab via email or phone (508-563-9303) that egg masses are being shipped.

B. Morphological description of AGM

1. Distinguish from non-Lymantriids

L. dispar can be most easily distinguished from other non-Lymantriids by their medium sized, non-translucent, intricately patterned, wings (USDA, 1981). Their eggs are large (>1 mm diameter), covered in buff-colored hair, and laid in masses that may contain as many as 1500 eggs. Masses are laid on surfaces such as tree trunks, rocks, cars, and shipping containers (Mastro, 2009).

2. Distinguish from other Lymantriids

L. dispar can be easily confused with *L. monacha*, the nun moth, as well as other Lymantriids. The nun moth is also a defoliator and native to Eurasia. It can be distinguished from *L. dispar* by its wide range in color variation, from chalk white to dark brown, covered with intricate patterns. The exception is the dark phase males which are especially difficult to separate. Also, the female nun moths have a narrower abdomen and long ovipositor which *L. dispar* lacks. Nun moth eggs are deposited in clusters, not in a

single mass and without a covering of hair (USDA, 1981). Because of the similarities between AGM and other Lymantriids, it is best to send all suspect samples to an identifier.



Figure 6. *L. dispar asiatica* (AGM) male (John H. Ghent, USDA Forest Service, Bugwood.org)



Figure 7. *L. monacha* (Nun moth) male (Peter Lillywhite).



Figure 8. *L. monacha* (Nun moth) female and male. (Melody Keena, USDA Forest Service, Bugwood.org)



Figure 9. *L. mathura* (Rosy moth female) (PPQ)

3. Distinguish AGM from European gypsy moth

AGM adults are similar enough to EGM that the two cannot be reliably distinguished by visual examination alone. AGM tend to be larger than EGM, and AGM females, unlike female EGM, usually have wings that are large enough to completely cover the abdomen when closed, but these differences do not provide definitive identification. In addition, the subspecies can hybridize to produce moths that intermediate in a number of traits including flight (Keena et al., 2001). As a result, DNA analysis is routinely used to distinguish between the subspecies.



Figure 10. European gypsy moth (*L. dispar dispar*) (CAPS).



Figure 11. Asian gypsy moth (*L. dispar japonica*) (CAPS).

C. Molecular methods and DNA markers

Two genetic markers are routinely used to assess the genotype of submitted gypsy moth specimens: the nuclear marker FS1 (Garner and Slavicek, 1996) and a mitochondrial marker (Bogdanowicz et al., 1993). Two alleles or variations occur at the FS1 loci, designated as North American (NA) or Asian (A) respectively. As gypsy moths are diploid organisms, each moth contains two non-identical copies of each autosomal (non-sex) chromosome. The two copies or alleles of FS1 can be identical (homozygous) or different (heterozygous) in a given specimen. Thus, three combinations are possible: a moth can be homozygous North American (possessing two copies of the North American allele), homozygous Asian, or heterozygous (containing one copy each of the North American and Asian allele). As the designation implies, the North American FS1 allele is the allele most frequently found in gypsy moths from North America while the Asian allele is the only FS1 allele present in moths from eastern Asia. The Asian allele is, however, also detected in the US population at a low percentage (approximately 3 to 6% depending upon geographic location in the continental US). Both of the FS1 alleles, on the other hand, are present in high abundance in gypsy moths from Europe and Western Russia.

Unlike nuclear DNA, mitochondrial DNA is always maternally inherited. Thus, the mitochondrial DNA analysis of any given gypsy moth reflects the mitochondrial DNA of its mother. The mitochondrial marker is characterized by four possible haplotypes or variations defined by restriction site polymorphisms. After completion of PCR (DNA amplification), the amplified DNA fragment is incubated in the presence of the restriction enzymes Nla III and Bam H I respectively. For the North American haplotype (NA), neither of the enzymes cut nor digest the amplified DNA (Nla and Bam negative). For the A1 haplotype (commonly found in moths originating in Europe and Western to Central Russia), Nla III cuts the DNA fragment into two pieces (Nla positive), but Bam H I does not cut the DNA fragment (Bam negative). For the A2 haplotype (common to Far East Russia and the Orient), both

enzymes cut the DNA fragment (Nla and Bam positive). The fourth possible haplotype (Nla negative and Bam positive) has only been detected to date in a few moths from China and is rare. This haplotype is designated A3. Similar to the FS1 A allele, the A1 mitochondrial haplotype is also found in the US population at a low percentage. In both cases, the exact percentage varies depending upon location within the United States. It is important to keep in mind that these designations for the nuclear FS1 marker and the mitochondrial marker are not meant to indicate the evolutionary origin of the moths tested, but rather implies the geographic region where they are found in the highest abundance (Table 2).

Table 2. Gypsy Moth Genotype Determination

Band Size (bp) produced in FS1 PCR	FS1 Designation	Band Size (bp) produced in Mitochondrial RFLP	Mitochondrial DNA RFLP Data	Mitochondrial DNA Haplotype	Distribution	Determination	Additional Analysis Advised
207	NA	500	Nla-Bam-	NA	Prevalent genotype in North America	North American	None
207	NA	350/150	Nla+Bam-	A1	Europe and North America (E>NA); % varies with location in North America)	Subject to further analysis	Microsatellite Analysis
207	NA	300/150/50	Nla+Bam+	A2	Rare; 2 in Austria, 1 in Slovak Republic, 15 in Tunisia all from 1990's	Subject to further analysis	Microsatellite Analysis
207	NA	450/50	Nla-Bam+	A3	Very rare: 1 moth found in North America (Delaware, 2009)	Subject to further analysis	Microsatellite Analysis
207/312	H	500	Nla-Bam-	NA	North America (% varies with location)	Subject to further analysis	Microsatellite Analysis
207/312	H	350/150	Nla+Bam-	A1	Europe and North America (E>NA); varies with location in North America	Subject to further analysis	Microsatellite Analysis
207/312	H	300/150/50	Nla+Bam+	A2	Rare; 2 in Austria, 1 each in China, Korea, & Tunisia	Subject to further analysis	Microsatellite Analysis
207/312	H	450/50	Nla-Bam+	A3	Not found to date	None	None
312	A	500	Nla-Bam-	NA	North America (% varies with location)	Subject to further analysis	Microsatellite Analysis
312	A	350/150	Nla+Bam-	A1	Europe and Asia (E>A)	Asian	Microsatellite Analysis
312	A	300/150/50	Nla+Bam+	A2	Prevalent genotype in Asia	Asian	Microsatellite Analysis
312	A	450/50	Nla-Bam+	A3	Rare; 8 found in China; 1 in Japan	Asian	Microsatellite Analysis

Genetic markers termed “microsatellites” are available to provide more precise information on the possible origins of captured or intercepted gypsy moths. Microsatellites are repeated sequences of typically 2 to 4 base pairs in length (for example, [GTT]*n* or [AT]*n* or similar repeats). They are found in nuclear DNA but are not in portions of the DNA that actually code for proteins. Consequently, mutations in microsatellites are typically not “weeded out” by selection, and thus numerous alleles of a given microsatellite can accumulate in a species’ genome. This makes microsatellites potentially useful for answering within-species questions relating to such things as geographic or population-level origins of an individual. In humans, they are used for determining paternity. Microsatellites are inherited in

Mendelian fashion; i.e., an individual has two copies of each microsatellite – one from each parent. For analysis, they are amplified (many copies are made) using PCR primers that adhere to the flanking regions of each microsatellite. To determine which alleles the individual has, the lengths of the microsatellite DNA are then measured, as the various alleles differ only in the number of times the basic 2- to 4-base sequence is repeated. Data generated using multiple microsatellite markers (6-12 markers minimum) are subject to analysis using population assignment software such as STRUCTURE (Pritchard et. al., 2000). STRUCTURE is a Bayesian-based clustering method for inferring population structure; the program uses allele data from multiple microsatellites to identify distinct genetic populations (termed clusters) and assigns individual specimens into those populations. The use of microsatellites may potentially provide information relating to the country of origin of AGM intercepts, the possible relatedness of multiple AGM interceptions, and the possible infiltration of AGM genes into the established North American gypsy moth population.

D. Rearing

Immature insects can be difficult to identify taxonomically, and for this and other reasons it may occasionally be advantageous to rear out suspect AGM that were intercepted as eggs or early-instar larvae. EGM have been reared routinely in large numbers for such purposes as virus production and pilot sterile insect release programs (Bell et al. 1981), and these methods are applicable to AGM. Within the U.S., however, suspect AGM must be reared under permit and within a PPQ-approved quarantine facility. Contact the CPHST Otis laboratory for shipping instructions for live egg masses and other viable life forms, and to obtain a copy of the permit, which must accompany the shipment.

III. Survey

A. Traps and lures

Pheromone baited traps are used for detecting and delimiting AGM populations in North America. With rare exception, these traps capture only males. Black-light traps can capture AGM of both sexes and are used to monitor AGM in port environs in Asia, but they are not considered sensitive or specific enough for detection and delimitation survey purposes.

Gypsy moth pheromone lures contain 0.5 milligrams of disparlure in either a PVC-coated string or laminated plastic strip. These dispensers provide slow release of the attractant into the air over a period of several months. The lures are used in delta or milk-carton traps. Delta traps are used outside of areas that are generally infested with gypsy moth, where catch is expected to be less than 10 moths per trap.

They are prism-shaped traps made of plastic-coated cardboard; moths enter through openings on the triangular ends and are captured in a sticky substance that coats two of the three inner surfaces. Milk carton traps resemble basic 0.5 gallon (2 quart) paper milk cartons, but contain small entry ports on each side as well as a trap hood around the carton which serves as a behavioral stop for males so they will enter the ports. The lure hangs inside the carton at approximately the same height as the entry ports. Moths that enter the milk carton trap are killed by an insecticide (DDVP) that is released from a laminated plastic strip which is also hung inside the carton. Milk carton traps can hold as many as 1000 moths.

B. AGM detection survey

Detecting insect populations with traps is probabilistic in nature. All other things equal, the closer a moth is to a trap, the more likely it is to be captured. Because of that, the sensitivity of a detection trapping grid, that is, the probability of detecting a relatively small population, improves with increases in the number of traps per unit area (Figure 12).

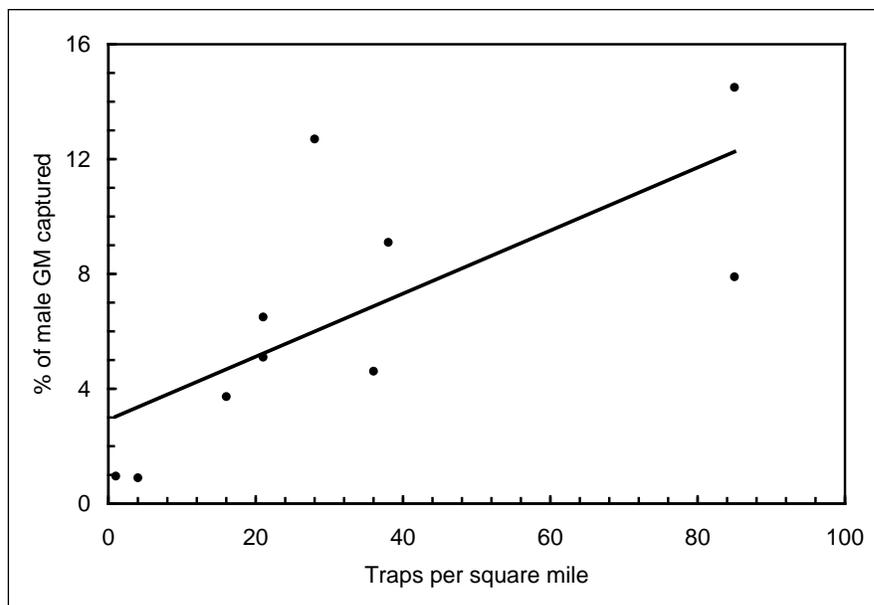


Figure 12. Percentages of marked male European gypsy moths captured in grids of disparlure-baited traps at various densities in testing conducted from the mid-1970s through 2002 (Lance, *et al.* 2003).

Ideally, moths will be detected in the same year they are introduced. Trap density will vary depending on the local risk, which is based on pathways of introduction and spread, host availability, and other factors as determined by local managers. General guidelines are provided below.

Areas at risk should be surveyed using pheromone traps. Ports and other high risk areas should set up to 25-36 traps per square mile (2.6 square km) within a two-mile (3.2 km) radius of docking areas and other high risk sites. An additional 16 traps per square mile (2.6 square km) should be set out to a distance of 5 miles (8 km), where there is host vegetation (Figure 13 below). Areas within 1 mile (1.6 km) from the banks of high-risk waterways should be set with up to 25-36 traps per square mile (2.6 square km).

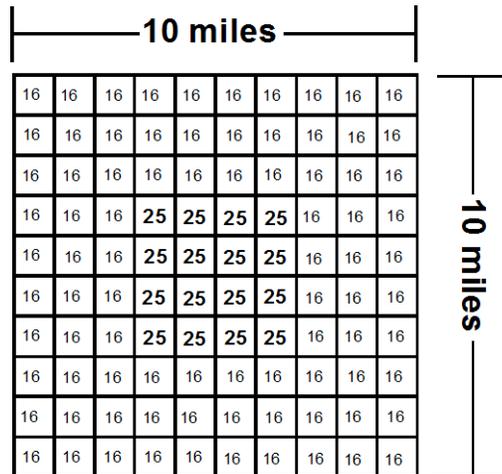


Figure 13. Sample trap density grid for high risk areas

In other risk areas, traps should be set at 9 traps per square mile (2.6 square km) out to a 1 mile (1.6 km) radius from docking areas and other sites at risk. An additional 4 traps per square mile (2.6 square km) should be set out to a distance of 3 miles (4.8 km), where there is host vegetation (Figure 14 below). Waterways should be set with 9 traps per square mile (2.6 square km) along the water out to 1 mile (1.6 km) inland on each side of the waterway (APHIS, 1992).

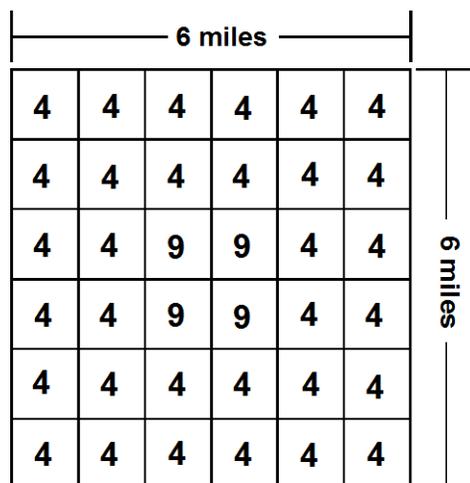


Figure 14. Sample trap density grid for risk areas

C. Egg masses

1. Egg masses to determine hatch dates

If male gypsy moths are treated with the proper substerilizing dose of radiation and mated to an unirradiated female, a portion of the resulting eggs will hatch, but the larvae will develop into fully sterile adults. These egg masses can be placed in areas with newly detected gypsy moth populations to help estimate timing of hatch without risk of adding to the incipient population. The egg masses are caged as an added security measure, to protect the egg mass, and to avoid possible confusion over the origin of any males that are subsequently captured. The Otis laboratory provides sterile EGM egg masses and can also provide cages or guidance on cage construction.

Egg masses should be placed outdoors in the area where suspect AGM were captured. This should be done as early as possible, preferably during the fall following the summer when the AGM were trapped. Cages should be placed in a variety of locations near the ground, but in places where they will not be disturbed by the public. Masses should be checked for hatch three times weekly starting about 3 weeks before phenological models or other indices indicate that hatch is expected. In areas with warm winters (e.g., coastal California or the Gulf Coast), masses should also be checked weekly through the winter.

An alternative, if available, is to monitor hatch of wild egg masses that are found in the area. This reduces possible environmental differences that could affect timing of hatch and would also eliminate possible differences in timing of hatch between the laboratory (EGM) insects and the target (AGM) population. However, a high degree of security would be required to ensure that no larvae escape. Eggs should be carefully scraped from their substrate, keeping the mass intact as much as possible and placed in a cage (as above) in a secure area.

2. Egg mass surveys

Various types of egg mass surveys, all based on visual search, have been designed for, and used in, managing gypsy moth, but they are typically used in dealing with outbreak-level populations (Kolodny-Hirsch 1986, Buss et al. 1999). Female European GM doesn't fly and typically deposits egg masses near their pupation sites, which are often in relatively hidden locations such as under bark flaps, in the interior of wood piles, or under decks. AGM females may also do this but instead often fly some distance prior to oviposition. Searching for egg masses in indiscriminate and often cryptic locations is much less efficient than pheromone trapping for finding and characterizing Asian gypsy moth populations, especially at the low population levels typical of incipient populations. As a result, AGM egg mass surveys are not

recommended for detecting or delimiting a newly introduced population. In addition, eradication protocols are based on data from trapping grids.

Egg mass searches, however, may be desirable if resources are available and masses would be considered useful for such tasks as confirming the presence of a locally reproducing population, molecular characterization of a population, or timing control measures. Tree trunks, as well as the cryptic types of locations noted above, should be systematically searched in the vicinity of trap captures. In addition, AGM females in many areas tend to fly to lights at night, and searches of well-lit light poles and architectural surfaces can prove successful.

D. Trap deployment

1. Timing for start and end in different geographic areas

Traps should be set out before male gypsy moths emerge; however, times conducive to adult flight at various U.S. port areas and months when male moths from various sources could potentially be present vary. The highest risk would come from any moths that had left ships or cargo in previous seasons and were reproducing locally. The second highest risk would come from moths that dispersed away from ships or containers, either as adults that had pupated prior to transit or, earlier in the season, as newly hatch larvae. Note that this risk would also be present throughout the period when flight of local populations could be occurring.

A lesser but non-zero risk is present through other portions of the year when temperatures at a port allow for flight. This occurs because ships coming from foreign ports in the northern hemisphere sometimes transit southern hemisphere ports before coming to the U.S. In those cases, the reversal of season could affect temperature conditioning of egg masses on the ships, causing eggs to hatch at unusual times of year. Under this scenario, larvae in sub-tropical ports could potentially disperse from ships, find host material, and develop into adults at most any time (an alternative possibility is transport of AGM from breeding populations in the southern-hemisphere, though none are known at this time). While the risk of such an occurrence is low, managers at southern ports can consider keeping traps in place, if resources are available to do so.

Trap lures can draw in moths for up to one year in the field, so, in general, they will last throughout a year's trapping period, and setting them out early will not decrease their effectiveness (APHIS, 1992).

However, if trapping is conducted year-around, lures should be changed every six months to ensure optimum effectiveness.

2. Trap placement

Trap locations should focus on high risk ports and waterways, first and foremost, as determined by the SPHD and SPRO. Determinations should be based on past experience of interceptions, volume of ships arriving from Asia, and other factors. Other risk areas should be trapped as resources allow.

Milk carton traps should be hung using a string, tied to a branch of a host tree (Figure 15). Delta traps are most effective when attached directly to the bole of a host tree. The most efficient way of doing this is by stapling a binder clip to the bole of a host tree then clipping the trap in place. However, if a homeowner objects to this method, it can also be hung using a paper clip (Figure 16) or by tying a string around the tree and hanging the clip on the string. The traps should be hung at breast height unless vandalism is a problem in which case they should be hung higher (Lance, 2009). If a trap cannot be hung on a host tree, another vertical surface, such as a telephone pole, can be used to hang the trap, preferably within 100 meters of a host. Never hang the traps on branch tips.



**Figure 15. Milk carton trap
(William A. Carothers).**



Figure 16. Delta trap (PPQ).

3. Trap monitoring, frequency, and timing

Generally, traps should be monitored as often as possible. Past experience has shown that vandalism usually occurs soon after a trap is set; therefore, it is desirable to check traps at least once before male moths would be expected to emerge. Pheromone traps should be checked every two weeks if possible. Collected specimens should be sent to the Otis Diagnostic Laboratory in Massachusetts (section II.A.).

4. Detection of suspected AGM in trapping season

If a suspect AGM is identified during the trapping season, it is recommended that the number of traps be increased within the detection grid to help delimit the population. In addition, staff can visually survey light structures in the area around the trap and/or ports for female moths or egg masses.

IV. Eradication Actions

A. Determining response

A Technical Working Group (TWG) will be convened or consulted as soon as a diagnostic test confirms AGM are trapped. The TWG will consider each situation prior to rendering a response plan. This includes reviewing prior trapping densities and grids, vegetation, obvious introduction points, etcetera. The TWG will assist in the determination if the population is at a sufficient level to require eradication and the scope of such eradication. If eradication is recommended, the TWG will work with the local federal regulatory managers to determine the best eradication and trapping methodologies. Local federal officials will communicate with their respective state counterparts and other federal and state agencies to determine the type of response.

Consult Tables 4 and 5, which represent an array of time (single season (Table 4), multiple seasons (Table 5)) and situations (single moth catch in a port grid, multiple moth catch in a port grid, etc.) that shows the various levels of response available within an eradication framework. “Level two” listings for land-based detections are shown as either proven eradication treatments or “precision delimiting”. Precision delimiting (see part D of this section) is not a treatment itself, but, as with any form of delimiting trapping, is part of the eradication process. Based on input from the TWG and the possible responses outlined in Tables 4 and 5, apply the appropriate levels of response for the situation. In addition, quarantines may be considered beyond what is listed below if needed to control the spread of AGM.

Table 4. Single season situations and response steps for AGM detections beyond standard detection surveys

Scale	Scenario type	Situation	Type of response	First step of response	Second step of response	Third step of response	Fourth step of response
Foreign	Single season scenarios	AGM populations high in Asia	Detection	Maximum inspection intensity by CBP	Increase detection trapping intensity	Outreach & Public Information	
Domestic (national)		AGM ship-interceptions “low”	Eradication	CBP eradicates life stages found onboard vessel	Maintain normal high-risk trapping grids		
		AGM ship-interceptions “high”	Eradication or Detection	CBP eradicates life stages found onboard vessel	Increase detection trapping intensity	Outreach & Public Information	
	Single season scenarios	One moth <u>in</u> a Port/Waterway (P&W) trap grid	Eradication or Delimiting	Outreach & Public Information	Eradication treatment or Precision Delimiting	Delimiting trapping for 3 years post-treatment	
		One moth <u>outside</u> a Port/Waterway trap grid	Eradication or Delimiting	Outreach & Public Information	Eradication treatment or Precision Delimiting	Delimiting trapping for 3-4 years post-treatment	
		Multiple moths in the same trap grid or finite locality	Eradication or Delimiting	Outreach & Public Information	Eradication treatment or Precision Delimiting	Delimiting trapping for 3 years post-treatment	If/when possible, determine “relatedness”
		One moth and other life stage(s) in the same trap grid	Eradication	Outreach & Public Information	Eradication treatment	Quarantine	Delimiting trapping for 3 years post-treatment

Table 5. Multiple season situations and response steps for AGM detections beyond standard detection surveys

Scale	Scenario type	Situation	Type of response	First step of response	Second step of response	Third step of response	Fourth step of response
	Multiple season scenarios	One moth in season 1 plus one moth in season 2 – <u>in the same</u> Port/Waterway (P&W) trapping grid	Eradication or Delimiting (continues across both seasons)	Outreach & Public Information (increased)	Season 1 – Eradication treatment or Precision Delimiting	Delimiting trapping for 3 years post-treatment	If/when possible, determine “relatedness” of season 1 moth and season 2 moth
					Season 2 – Eradication treatment or Precision Delimiting <u>continues</u>	Delimiting trapping for 3 years post-treatment; [the 3-year clock resets to zero with season 2]	
		One moth in season 1 plus one moth in season 2 – <u>outside a P&W</u> grid	Eradication or Delimiting (continues across both seasons)	Outreach & Public Information (increased)	Season 1 – Eradication treatment or Precision Delimiting	Delimiting trapping for 3 years post-treatment	If/when possible, determine “relatedness” of season 1 moth and season 2 moth
					Season 2 – Eradication treatment or Precision Delimiting <u>continues</u>	Delimiting trapping for 3 years post-treatment	
		Multiple moths in the trap grid (or finite locality)	Eradication	Outreach & Public Information (maximum)	Treatments – i.e. multiple tactics	Quarantine	Delimiting trapping for 3 years post-treatment

B. Size of area to be treated

A minimum of a half mile (0.8 km) radius around the capture site should be treated with a proven eradication treatment (AGM SAP, 2007). If adjacent traps with positive captures are 1 mile (1.6 km) or less apart, the area between the traps should be treated with a proven eradication treatment as well as the half mile (0.8 km) radius. If adjacent traps further than 1 mile (1.6 km) apart both have positive captures, site parameters such as host type, host density, and trap placement would need to be evaluated by the program manager.

C. Proven eradication treatment options

1. Btk

Btk (*Bacillus thuringiensis kurstaki*) has repeatedly been proven efficacious against gypsy moth. It is short-lived in the field, so multiple applications (typically 3) should be made either by air or by ground at intervals of 7 to 10 days (AGM SAP, 2007). The spray zone should extend at least a half-mile (0.8 km) radius from all capture sites. Btk is effective only against young caterpillars (instars I and II and, to a lesser degree, III), so it is critical to apply the material when these stages are present in the field. Btk is more specific than other insecticides; it will kill some non-target caterpillars (Lepidoptera) if they ingest sufficient materials but has little toxicity on other organisms. The bacteria occur naturally and can be found in soil (AGM SAP, 2004). Btk works by disrupting the caterpillar's digestive system, which leads to death in 7-10 days (PPQ, 2003).

2. Diflubenzuron

Diflubenzuron (trade name of Dimilin) is an insect growth regulator that interferes with development by inhibiting synthesis of chitin, a major component of the insect exoskeleton. Dimilin is highly effective against gypsy moth larvae. In comparison with Btk, it has a longer field life (requires fewer applications) and is more effective against older larvae (timing is less critical), but kills a much wider range of insects and other arthropods (APHIS, 2006, TX).

Other eradication treatment options are available in the 2012 Supplemental Final Environmental Impact Statement, which is entitled "Gypsy Moth Management in the United States – a cooperative approach."

D. Precision delimiting

Delimiting for AGM, when applied post-treatment, normally involves a grid with a 5-mile radius and the following trap densities: 25-36 traps per square mile for the first two miles (from center) and 16 traps per square mile for the last three miles (Figure 13). However, precision delimiting may be applied in certain circumstances, such as in an area with limited host material or low trap catches often involved in a port or waterway detection (Tables 4 and 5), to determine the necessity of or prepare for an eradication treatment at the recommendation of the Technical Working Group. The trapping grid for precision delimitation would take on the following enhanced densities—49 traps per square mile for the first two miles (from center) and 25 traps per square mile for the last three miles (Figure 17). No post-treatment delimiting survey is required since no treatment is being applied.

25	25	25	25	25	25	25	25	25	25
25	25	25	25	25	25	25	25	25	25
25	25	25	25	25	25	25	25	25	25
25	25	25	49	49	49	49	25	25	25
25	25	25	49	49	49	49	25	25	25
25	25	25	49	49	49	49	25	25	25
25	25	25	49	49	49	49	25	25	25
25	25	25	25	25	25	25	25	25	25
25	25	25	25	25	25	25	25	25	25
25	25	25	25	25	25	25	25	25	25

Figure 17. Precision delimiting grid showing trap densities per sq. mile

E. Treatment considerations

When treating, special consideration must be given to people living and working in the area. Notices, with information about when an area will be sprayed, what will be sprayed, and any possible side effects, should be sent to residents and businesses and posted in public areas. People should be encouraged to stay indoors at the time the spraying should occur. Open houses and a well-staffed toll free hot line should also be used to help educate the public.

1. Aerial insecticide treatment

Aerial applications of diflubenzuron or Btk are the best-tested and most effective tools available for eradicating isolated gypsy moth populations. Aerial application provides more thorough coverage than ground-based sprayers and is the only viable option for applying insecticides to forested areas and/or areas with very tall vegetation that is beyond the reach of ground-based sprayers. Of the two insecticides listed, diflubenzuron is generally more effective against gypsy moth and requires fewer applications and less precise timing compared to Btk. Btk, however, has fewer non-target effects and is used more often against gypsy moth.

2. Ground-based insecticide treatment

If aerial application of pesticides is not feasible, treatment using ground-based equipment such as hydraulic sprayers may be an alternative. Ground-based equipment allows targeting of host trees while avoiding non-hosts and objects such as houses, cars, and buildings. Along with coverage issues, ground spraying is labor and time intensive and treatments typically have to be made during daylight hours. This can create more exposure to humans than aerial spraying which can be scheduled early mornings. Ground spraying can also be problematic because spray trucks and/or operators often require access to private land (BCMFR, 2007).

F. Degree-day modeling

1. Use of degree-day projections

Degree-day models are phenological simulation models that use temperature data to predict when biological events will occur. Gypsy moth degree-day models are available that use daily minimum and maximum temperatures to estimate the timing of events such as egg hatch or adult flight. In programs to eradicate isolated GM populations, wild egg masses and other immature stages are often difficult to impossible to find, and these models are valuable aids in determining the proper time to apply control measures such as Btk sprays (which are only effective against early instars) or mating disruption applications. Predictions of dates when adult flight will occur can also be used to time placement and removal of disparlure-baited traps. Note that for detection programs, however, trapping periods should generally be much broader than the flight periods predicted by models. Moths that arrived in an area recently may have been exposed, during much of their lives, to temperatures very different from local conditions. As a result, the models would be poor predictors of dates when their flight would occur.

The most commonly used gypsy moth phenology model is GMPHEN (Sheehan 1992). The model was based on data from EGM and is thought to be generally applicable to AGM, although temperature-related differences in development among strains have been demonstrated (Keena 1996). Studies are currently underway to develop better data on potential differences among Asian, Japanese, and European strains, especially with regard to predicting hatch dates. However, the movement of AGM egg masses around the world, from one climate to another complicates the application of a model.

Weather data for GMPHEN typically consist of daily minimum and maximum temperature readings throughout one calendar year (January 1 to December 31), or at least from the first of the year until late in the expected flight season (GMPHEN allows for other options). Daily min/max temperature data, and a variety of related information, are available for many weather stations throughout the United States. The data can be obtained at no charge in comma-delimited files from sites such as www.wunderground.com. Typically, these files are pasted into a spreadsheet such as MS-Excel, placed into the basic format for a GMPHEN data file, and then exported to Notepad or a similar editor for final formatting. For initial planning of program activities, data from one or more locations near the program area can be downloaded, averaged across multiple years if desired, and run in GMPHEN. For finer estimates of gypsy moth phenology at the program site, temperatures can be monitored on-site and used to replace the historical data as the season progresses. GMPHEN can be obtained online from the Forest Service (<http://www.fs.fed.us/ne/morgantown/4557/gypsymth/download.html>) or by contacting the CPHST Otis Laboratory.

2. Predicting gypsy moth hatch

For AGM programs, one shortcoming of using sterile egg masses is that hatch patterns of AGM may differ somewhat from those of European-type GM, and especially those of EGM from a lab-adapted strain that has been irradiated. If wild-type egg masses are found in the program area and the program deems it worth the risk, they can similarly be sealed in escape-proof cages and followed to monitor hatch.

3. Historic hatch data

In some instances, AGM populations will be detected in areas where gypsy moths (AGM or EGM) had been detected in earlier years, and data on timing of hatch of wild eggs had been collected. These data can be used in conjunction with the above methods to improve confidence in date-of-hatch estimates.

4. Phenological indicators

Timing of hatch of gypsy moth eggs tends to coincide in various locales with botanical events. For example, in New England, hatch is said to occur when shadbush (or serviceberry, *Amelanchier* sp.) bloom. More critical to the gypsy moths' ecology, hatch of EGM typically occurs at approximately the time of bud break in members of the red oak subgroup (*Quercus* sp.).

V. Post Treatment Delimiting Surveying to Verify Treatment Effectiveness

A. Area and density to be surveyed

Area within a five mile (8 km) radius of any traps that captured AGM should be trapped at 16-36 traps per square mile (2.6 sq. km) the following year or, if possible, at the end of the season when initial captures occurred (AGM SAP, 2007). These traps should be in place for at least 3 months (and longer in warmer areas) starting several weeks before adult flight is expected to start (AGM SAP, 2004). See Section III.D. for guidance on trapping dates.

B. Duration of surveying necessary to declare successful eradication

Trapping in the five mile (8 km) radius area should be continued for three years, including the year of treatment, to provide assurance that no population remains. If a positive capture takes place during these 3 years, reset delimiting survey.

VI. Environmental Assessment

Though treatment options are included in the 2012 Supplemental Final Environmental Impact Statement, a site-specific Environmental Assessment is required to analyze the specific conditions of the proposed treatment area and what effects an eradication treatment might have on the area.

VII. Collaboration with State Government Entities

Each State in which a suspect AGM is captured has in common with PPQ an interest in detection and control of the pest. The partnerships between PPQ and State Departments of Agriculture are essential. PPQ understands each State's interest in protecting resources that may be threatened by AGM.

VIII. Glossary

Asian gypsy moth (AGM)—For regulatory purposes, Asian gypsy moths include the following species: *Lymantria albescens*, *Lymantria dispar asiatica*, *Lymantria dispar japonica*, *Lymantria postalba*, and *Lymantria umbrosa*.

Btk—The abbreviation used for the biological insecticide *Bacillus thuringiensis* var. *kurstaki*.

Delimiting survey— A survey to determine the limits of an infestation and the approximate size of the infestation.

Delta trap—A triangular shaped trap made of plastic coated cardboard which uses a sticky surface to capture insects. For gypsy moth, disparlure is used to attract male moths to the trap.

Detection survey—A survey to determine if a species is present in an area where it is not known to exist. For gypsy moth, detection surveys most often consist of a systematic deployment of pheromone-baited traps.

Disparlure— The chemical *cis-7,8-epoxy-2-methyloctadecane*, which is the only known active component of the female gypsy moth's sex attractant pheromone.

Eradication—Action taken to eliminate all members of a population or to drive a population to extinction.

GMPHEN—Phenology model used to determine potential for capturing male moths in different areas of the country throughout the year. Based on data from European gypsy moth, and thought to be generally applicable to AGM, although temperature-related differences in development among strains have been demonstrated.

High risk areas—Areas where AGM is most likely to be introduced. These may include, but are not limited to, ports, international or state border crossings, inland waterways, rivers used for transportation of goods, warehouse districts, inspection stations, major highways and railways, rest areas and weigh stations, parks, and inland container storage and intermodal sites.

Isolated infestation—A population that is isolated geographically from other populations of the same species

Lymantria albescens—Found on Okinawa and the southern Ryukyu Islands of Japan. Females are capable of flight. Considered AGM for regulatory purposes. Formerly known as *Lymantria dispar albescens*.

Lymantria dispar asiatica— A subspecies of *L. dispar* found in Asia, mostly east of the Ural Mountains, China, and Korea. Females are capable of flight. Considered AGM for regulatory purposes.

Lymantria dispar dispar—The scientific name for the European gypsy moth, which is established in areas of North America. The females have wings but are flightless.

Lymantria dispar japonica—A subspecies of *L. dispar* found on all of the main islands of Japan. Females are capable of flight. Considered AGM for regulatory purposes.

Lymantria postalba— Found on southern Kyushu and northern Ryukyu Islands of Japan. Females are capable of flight. Considered AGM for regulatory purposes. Formerly known as *Lymantria dispar postalba*, *L. d. tsushimensis*, and *L. albescens tsushimensis*.

Lymantria umbrosa— Found in Hokkaido, Japan. Females are capable of flight. Considered AGM for regulatory purposes. Formerly known as *Lymantria dispar hokkaidoensis*, *L. d. umbrosa*, and *L. d. nesiobia*.

Maritime Waterway—An inland waterway, such as Puget Sound, San Francisco Bay, or the Columbia River, that is transited by trans-oceanic vessels.

Milk carton trap—A milk carton shaped trap made of plastic coated cardboard that uses insecticide to kill insects and has a larger capacity than a delta trap. For gypsy moth trapping, milk carton traps are placed where you can expect to trap 20 or more moths.

Post treatment delimiting survey—A delimiting survey conducted after an eradication program to determine if eradication was successful.

Precision delimiting survey—A more intensive delimiting survey than is usually applied due to circumstances of the detection such as finding one moth or the environment in which the moth was found. May be used to determine the necessity of or prepare for an eradication treatment at the recommendation of the Technical Working Group.

Ship interceptions “high”—When interceptions of the number of ships with egg masses are higher than the average for a location.

Treatment—Any action taken that is intended to control a pest

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Appendix A - USDA ASIAN GYPSY MOTH POLICY

It is well documented that gypsy moths (*Lymantria dispar*) display considerable variation in behavior throughout their range. Most Asian strains of gypsy moth are characterized by females capable of strong directed flight and host ranges broader than that of the gypsy moth strain currently established in North America (narrow genetic range based on isolation of population originally introduced from Europe in 1869; characterized by non-flying female moths). In recognition of these significant behavioral differences, it has been determined that Asian Gypsy Moth (AGM) warrants status as a significant, exotic pest of economic importance. Contrary to USDA's North American gypsy moth (NAGM) policy of not conducting eradication activities within the generally infested area, action will be taken against confirmed AGM infestations in the generally infested area when the source of the introduction is known. Knowledge of the time, location and extent of an Asian introduction will be required to trigger treatment activities. In cases where deductive, circumstantial or investigative information can be developed about an introduction of uncertain origin, appropriate action may also be recommended and taken. The goal of such treatments will be to eliminate all of the gypsy moths that exhibit traits characteristic of AGM.

USDA's current policy of excluding the introduction and preventing the establishments of exotic economic pests will be applied to AGM, regardless of whether an introduction occurs within or outside of the area generally infested with NAGM.

The consequences of an AGM introduction into the United States will be determined by several factors, the most important of which are: 1) source of the introduced AGM, 2) site of introduction and 3) size of introduction relative to any resident North American population. Operational responses to mitigate these consequences will be based upon the specific circumstances of each occurrence to maximize the effectiveness of treatment strategies.

Recent studies indicate that several Asian strains are sexually compatible with NAGM, resulting in hybrid progeny that possess a mixture of behavioral characteristics and demonstrate observed hybrid vigor. While the exact effects of such hybridization are not well defined, the presence of NAGM in superior numbers is believed to dilute the expression of noxious Asian behaviors (i.e. female flight and a broader host range) in mixed populations. However, the possible retention of these traits at some low level requires mitigation measures where feasible to prevent or reduce the likelihood of introducing Asian genetic material into NAGM populations.

In recognition of the behavioral differences between AGM and NAGM, standard programmatic operations used outside of the generally infested area will be modified. Pretreatment delimiting surveys will not be conducted for AGM due to the potential increase in size and scope an AGM population can achieve in a single year. Control measures will commence as soon as possible after confirmation of an Asian introduction based upon the best information available, followed by extensive post-treatment delimiting surveys.

In order to reduce the likelihood of future introductions, USDA will conduct multifaceted exclusionary activities, supported by effective detection surveys at high risk introduction sites. These sites will include ports of entry, selected military bases, and other locations as needed.

Future policy changes will be determined by scientific advances that provide new information required for informed decision making and improved operational support.

Appendix B – AGM Trapping Submission Guidelines

Asian Gypsy Moth Trapping Submission Guidelines

Specimens trapped in the field can be analyzed for the presence of Asian genetic markers by submitting the specimens to the CPHST Otis Laboratory. All specimens submitted from outside the generally-infested area will be analyzed. Because of the quantity of specimens submitted from within the generally-infested area, only a small fraction can be analyzed. **Collect captured moths a minimum of every two weeks to minimize DNA degradation of the specimens, more frequently in warm climates.**

Store specimens in a cool, dry location (frozen if possible).

Ship ASAP after collection

MILK CARTON TRAPS

DO layer loose moths between wadded paper towels or tissue paper in paper bag (brown lunch bag size) to prevent motion and specimen damage during shipment.

DO label paper bag clearly with trap numbers matching paperwork.

DO staple or tape paper bag closed.

DO NOT attach paperwork to bags.

DO NOT use plastic bags or paper envelopes as these promote fungal growth and do not allow moisture release.

DO NOT send traps or paperwork for traps which contain no specimens.

DELTA TRAPS

DO label each trap clearly with trap numbers matching paperwork.

DO package traps to avoid crushing during shipment.

DO NOT attach paperwork to traps.

DO NOT use Styrofoam peanuts for packaging.

DO NOT disassemble the traps or remove moths from the trap.

SHIPPING

DO send a PPQ Form 305 for each trap sent.

Include: • Trap number • Collection Date
 • Collection Site • Life Stage
 • No. of specimens (estimates OK)

DO package moths / traps to prevent crushing or motion during shipping. Moths must be received whole with antennae and legs attached to body.

DO ship via next day delivery for Tuesday through Friday arrival.

DO ship ASAP after each collection.

DO keep moths frozen until shipment.

DO keep specimens dry.

DO NOT attach paperwork to traps or bags.

DO NOT use Styrofoam peanuts with delta traps.

DO NOT send traps or paperwork for traps with no specimens.

SHIP TO:

John Molongoski
USDA, APHIS, PPQ
CPHST Otis Laboratory
1398 West Truck Road
Buzzards Bay, MA 02542-1329
• Voice: (508) 563-9303 ext 218
• Fax: (508) 564-4398
• Email: john.j.molongoski@aphis.usda.gov

PPQ Form 305 can be obtained from the Otis Lab via phone or email requests. Please do not hesitate to contact us if you have any questions.

Appendix C – PPQ Form 305

Public reporting burden for this collection of information is estimated to average .17 hours per response, including the time for reviewing instructions, searching existing data sources, gathering & maintaining the data needed, & completing and review the collection of information, including suggestions for reducing this burden, to Department of Agriculture, Clearance Officer, OIRM, AG Box 7630, Washington, D.C. 20250, & to the Office of Information and Regulatory Affairs, Office of Management and Budget, Washington, D.C. 205

U.S. Department of Agriculture Animal and Plant Health Inspection Service Plant Protection and Quarantine		1. Insect Name:	
Insect Collection Worksheet for Genotype Analysis			
<small>Complete for each trap containing specimens</small>			
2. Submitter's Name:		3. Submitter's Address:	
4. Email Address:		Phone: _____ Fax: _____	
5. Submitting Agency:			
TRAP DATA			
6. Date Collected:	7. Date of Last Trap Check:	8. Trap Number:	9. Nearest Port of Entry
10. Trap Type:			
11. Trap Location:		12. Number of Specimens in trap:	
Address		13. Other Life Stages Collected: Number of Specimens <input type="checkbox"/> Eggs <input type="checkbox"/> Larvae <input type="checkbox"/> Pupae <input type="checkbox"/> Female	
City			
State			
County			
Longitude			
Latitude			
Other Coordinates			
14. Special Treatments of Specimens: (e.g. freezing conditions, use of alcohol, prolonged storage conditions, host if no trap used, etc.)			
15. Send to:		16. Date Sent:	
Molecular Diagnostics USDA, APHIS, PPQ CPHST Otis Lab 1398 West Truck Road Buzzards Bay, MA 02542		Tel: 508-563-0929 Fax: 508-563-0903	
FOR LABORATORY USE ONLY			
Date Received:		Otis MSC ID Number:	

PPQ FORM 305