REPORT OF THE TECHNICAL WORKING GROUP
FOR THE
RESPONSE TO ASIAN GYPSY MOTH CAPTURES
WASHINGTON-OREGON 2015

OCTOBER 30, 2015

A Technical Working Group (TWG) was convened initially by teleconference on 11 September, 2015, to develop recommendations on responses to the capture of nine Asian gypsy moths (AGM) in Washington State (a tenth moth was submitted subsequently) and two additional captures in Oregon. The panel consisted of Ring Cardé (UC Riverside), Gericke Cook (APHIS-PPQ), Lee Humble (NRCan), Dave Lance (APHIS-PPQ, Chair), Donna Leonard (USDA-FS), Sandy Liebhold (USDA-FS), Vic Mastro (APHIS-PPQ, ret.), Steve Munson (USDA-FS) and Patrick Tobin (U. WA). Also present on the initial call were Paul Chaloux, Christopher Deegan, Mark Hitchcox, Diana Hoffman, Phil Lewis, and Anthony Man-Son-Hing (APHIS-PPQ); Bob Rabaglia (USDA-FS); Jim Marra, Randy Taylor, and Brad White (WSDA); and Helmuth Rogg and Clint Burfitt, (ODA). The call lasted nearly 3 h. During the first 1.5 h, State and PPQ program personnel from the affected states described the situation as well as expressing concerns and opinions about possible responses. After that point, the TWG continued with internal conversations. These continued through weekly teleconferences until October 16 as well as email correspondence. The following observations, discussions, and recommendations were developed.

The situation:

A number of male gypsy moths were captured in both Oregon and Washington during the 2015 flight season, and all were sent to the PPQ's CPHST Otis Laboratory, Buzzards Bay, MA, for molecular characterization. The majority of the males showed more or less typical North American profiles, but ten of the males from Washington and two from Oregon were homozygous for the Asian marker at the FS1 (nuclear DNA) site and N+/B+ ("A2" type) on restriction enzyme digestion of the COI coding region. This combination is almost exclusively restricted to eastern Asia. In addition, 11 of the Asian males had an identical COI ("barcoding") sequence (the 12th hadn't been sequenced as of this writing) that was consistent with eastern Asian populations. Three additional segments of the mitochondrial DNA were sequenced for 6 of the males (the others will be done but were not completed at the time of this report), and variations in two of those three segments indicated that 5 of the 6 were associated with populations on mainland Asia while the other, one of the moths from the Tacoma area, had a pattern more typical of Japanese populations. This suggests, but isn't conclusive regarding, multiple introductions. Further characterization of captured males' DNA will be done including the use of short sequence repeats (SSR's, a.k.a. microsatellite DNA), which hopefully will provide a more complete story regarding potential sources and relatedness of the introduced AGM. In addition, there appears to be an incipient population of North American gypsy moths in the Capitol Hill area of Seattle. Males from that area had an unusually high incidence (for North American populations) of the Asian-type FS1 allele. At present we are attributing that to sampling error or "founder effect" but these will also be analyzed further.

The AGM captures in these two states were scattered across a broad area geographically, including eight captures around the southern half of Puget Sound and three in the Portland, OR/Vancouver, WA area. While there appeared to be some clustering in the areas of Kent, WA (2), Tacoma (4), Nisqually, WA (2) and Portland/Vancouver (3), with the exception of Kent (where both moths were in one trap), the individual captures tended to be some distance (often miles) apart from one another with zero captures in

traps in between, which would not be typical for moths dispersing from the core of an incipient population (similar to North American gypsy moths). The local situations varied quite a bit also, as the finds occurred in port, industrial, residential, and wooded areas. Trap densities also varied widely, from ca. 1 trap per square mile to areas where port and/or Asian defoliator surveys resulted in densities of up to 25 traps per square mile. Kent, WA, in particular is of concern because two AGM males were caught in a single trap despite lower trap densities in that area (approximately 1 trap per square mile). The positive Kent trap was immediately adjacent to a Boeing facility.

Both the State representatives and the TWG felt that the pattern of capture suggested a high likelihood that adult Asian female moths were present in the vicinity sometime during the summer of 2015. That opinion, in part, arose from the potential pathways of introduction. A number of potential pathway scenarios exist:

- AGM eggs were deposited on cargo or cargo containers in Asia. Infested containers/cargo were loaded onto a ship that visited WA or OR, where they were transported to off-port locations once unloaded. Hatching larvae found suitable hosts and a number of the resulting adults ended up in traps, either in the summer following the introduction or a subsequent year. This is a feasible scenario for introduction as Asian gypsy moth egg masses are intercepted annually on ships and cargo in WA and OR ports.
- AGM larvae crawled to containers or cargo for pupation. Shipments of that cargo arrived in North American as adult emergence was occurring and the moths flew from cargo (which may or may not have still been on the ship) and either were caught in traps (males) or if males and females, potentially mated and established a viable population that may have been detected this year or will in subsequent years. This is possible but requires fairly precise timing as the pupal period only lasts a couple of weeks, and adult gypsy moths live only a few days.
- AGM egg masses were deposited on ships and hatched while the ships were in, or arriving in, port. First instar larvae ballooned from the ships and found suitable hosts on shore.

With all three scenarios, the presence of adult female moths in the Pacific Northwest is likely and could lead to, or has led to, the presence of incipient populations of AGM on the U.S. Mainland. The TWG and State representatives discussed the interceptions of AGM egg masses on plates of Russian steel, which came into Vancouver and were then moved to a re-heat facility in North Portland, ca. 0.5 mi from one of the OR captures. The second OR capture is within flight range of a dunnage disposal site. Also, several of the sites are in proximity to automobile offloading facilities. Although these discussions cover possible scenarios, there are no smoking guns *per se*. Consequently, the TWG will not be offering a "most likely" explanation for the scattered distribution of AGM finds in WA and OR. Scattered AGM distributions are not unprecedented, as nine AGM captures were similarly distributed at and beyond Tacoma (up to 17 miles distant) in 1991, resulting in a program area of over 100,000 acres. Similar to 2015, all trap captures were single moths with the exception of one trap that caught two males. The scattered distribution may simply be an artifact of a combination of the pathway and the biology of AGM, neither of which is fully understood.

Recommendations:

Given (1) the likelihood that adult female AGM were in the Puget Sound and Portland/Vancouver areas this summer, (2) uncertainty about the underlying AGM populations in areas where captures occurred, and (3) APHIS and State policies that AGM will not become established in the U.S., the TWG recommends an aggressive combination of delimitation trapping and treatment in the areas where AGM males were captured in 2015.

Delimitation and detection trapping:

The current *USDA-APHIS-PPQ Asian Gypsy Moth Survey and Response Guidelines* (January 2014 revision) suggest, for delimitation, "core" trapping levels of 25 to 49 traps per square mile, depending on the desired degree of precision, within two miles of any trap find. The TWG finds these guidelines generally appropriate, but, given the uncertainty around the distribution of the finds in this case, the TWG recommends at least 36 traps per square mile and extending the core trapping area out to 3 miles from the point of 2015 AGM captures. The recommended minimum of 36 traps per square mile retains detectability function comparable to higher trap densities (Appendix 1) and would reduce program costs over the increased detection radius of 3 miles when compared with 49 traps per square mile.

Beyond the core zones, trapping should be conducted at 25 traps per square mile for the next 3 miles (i.e., extending out to 6 miles from 2015 capture sites). The level of 25 traps per square mile is preferable but may not be realistic given the scope of the project; a fall-off of system sensitivity occurs from 25 to 16 but the latter still provides a highly sensitive detection system compared to most pest detection and delimitation grids (Appendix 1).

Beyond areas of delimitation, normal port, gypsy moth detection, and Asian defoliator trapping should be conducted. The TWG agreed that it is important to conduct at least protocol-level GM detection trapping throughout the states of Oregon and Washington during the upcoming years.

The TWG expressed concern about gaps in trap coverage and recommends that APHIS-PPQ, USDA Forest Service, and affected states work together to ensure recommended levels of trapping are initiated on such areas as tribal and federal lands. For example, in the Nisqually find, AGM moths were captured in an area where the trap grid was not as dense as that around some of the other AGM trap catches (e.g., Tacoma or Portland/Vancouver). Moreover, there was a large forested area to the east of the eastern Nisqually find that apparently was not trapped. That area included portions of the Nisqually Indian Community and Joint Base Lewis-McChord. Participants in the gypsy moth program need to contact the Nisqually Community and the military to ensure that appropriate delimitation or detection trapping can be conducted in those areas. USDA-FS-FHP can be engaged in this effort as they have responsibility for providing entomology/pathology assistance, including coordination of gypsy moth trapping, for all federal land management agencies.

The program should ensure that traps are in place throughout the entire GM flight period, based on degree-day modeling. Buffers of 3-4 weeks should be provided for both adult flight initiation and cessation to allow for inaccuracies of the degree-day model and possible biological differences between AGM and NAGM. Traps within grids should be spaced as evenly as possible to avoid areas with large gaps in coverage. Viable populations may exist within these gaps and, if left undetected due to insufficient trap coverage, they could increase to undesirably larger population levels affecting much greater areas. Trap servicing schedules should allow for moths to be submitted for molecular analysis in a timely manner; i.e., within 2 weeks of capture wherever possible.

The program should make preparations to continue trapping at these levels through 2018 even if no additional AGM are captured during that period to ensure no population establishment has occurred.

In addition to trapping recommendations for areas associated with these AGM finds, the TWG is recommending an increase in trapping efforts further inland in the vicinity of high-volume container yards or other locations housing objects that may have resided outside pre-shipment in areas with significant AGM populations. Of the scenarios listed above regarding the source of the trapped AGM, entry of egg masses on cargo would seem the most probable, especially since large numbers of egg masses were

intercepted on ships and cargo in 2013 and 2014. Infested containers, cargo, or packaging materials could easily have been carried inland and remained outdoors through the hatch period in locations with minimal or no gypsy moth monitoring and a suitable host component.

Treatments:

Background, rationale and overview: Historically, eradication treatments were applied around the sites of all AGM trap captures in the U.S. in the year following the initial detection. This policy was based on (1) the high overall risk to U.S. forests posed by AGM given its broad host range and capability of female flight, (2) the potential of the population to spread if not treated, which could make eradication difficult and expensive if delayed a year or more, (3) compared with NAGM, trap captures of AGM are likely not as accurate in delimiting the geographic area occupied by an incipient population (again due to female flight), and (4) uncertainty at the time of detection regarding the geographic and numerical size of the population. In recent years, an AGM port monitoring protocol was put in place and was followed by Oregon and Washington in 2015. The protocol specifies trapping densities that approximate delimitation grids within high-risk port areas (up to 25 traps per square mile, as in Tacoma and Vancouver/Portland areas) as well as enhanced trapping along waterways to and from those ports. In addition, there were NAGM and other Asian defoliator trapping programs in the affected areas. Thus, with the exception of the Kent captures (where traps were placed at approximately one per square mile), the TWG believes that the program has better year-of-detection information than they may have had in the past regarding the potential size and geographic distribution of any incipient AGM population. Especially in the port environs, it's unlikely that any areas with more than a very few AGM escaped detection (Appendix 1). Given the available information, the TWG is not recommending area-wide treatments throughout the broader program area at this time, and the recommended treatment areas may seem smaller than they would have been under similar situations in the past.

Specifically, TWG members believe that eradication treatments are warranted in the four general areas where multiple moths were caught. Even in areas with 25 traps per square mile, only ca. 10% of males are likely captured, and numbers of adult male and female AGM in those areas are likely comparable. As such, there is a reasonable expectation that breeding populations exist in those areas. Treatments should generally follow recommendations in the *USDA-APHIS-PPQ Asian Gypsy Moth Survey and Response Guidelines* (January 2014 revision) with some modifications (see below). The TWG is not specifically recommending treatment for the single capture in Gig Harbor for reasons noted above. Recent captures of single AGM in port areas of California, South Carolina, and British Columbia were similarly followed by enhanced delimitation rather than treatment, and populations did not subsequently develop[1]. However, given the overall number of AGM captured in the Pacific Northwest in 2015, the TWG also believes that it would not be unreasonable for the program to decide to err on the side of caution and treat a square mile area centered on the Gig Harbor site.

[1] The SC detection was in Charleston in 2014, and a single male AGM was caught again during the delimitation in 2015. Current plans are to continue delimitation. It should be noted that capture of a single male AGM in Oklahoma in 2013 also was not followed with treatment despite being in an area of low trap density. In this case, there was no apparent pathway to the area of the trap, with the most likely source being a military base that was centered ca. 9 miles from the capture. As such, it was felt that treatment around the trap (per AGM Response Guidelines) would likely have missed the actual population. An area-wide delimitation in 2014 again caught one AGM, this time on the base itself. Trapping in 2015 turned up no males.

Treatment areas:

(1) Tacoma. Although the finds in this area are distant from one another and trap density was high, the TWG believes that a single area that encompasses all four captures would be most effective. More specifically, the treatment area would extend outward half a mile from a polygon that connects the locations of the four positive traps. The half-mile distance will likely encompass the vast majority of first instars dispersing from the vicinity of trap captures and has been demonstrated to be an effective strategy in previous AGM eradication programs.

(2) Portland/Vancouver. As in Tacoma, the TWG recommends a single treatment area that encompasses all three captures. In this case, the program may consider expanding the treatment area to the northwest to encompass at least two of the NAGM finds that occurred in the vicinity of the AGM captures. The TWG believes that, to the degree that these captures may indicate the presence of breeding NAGM in the area, they can actually enhance the risk from AGM as well. This could occur (1) if AGM genes were introduced into the NAGM population via male AGM mating with female NAGM (note that in this case, the offspring would not be determined to be AGM using standard genetic screening, which relies heavily on mitochondrial DNA), and (2) through increased risk of AGM establishment by mating with NAGM males in areas where populations are sparse and females potentially might have gone unmated otherwise (i.e., would have failed to reproduce due to mating related Allee effects).

(3) Kent. Treatments should occur over a minimum of a 1 × 1 mile block centered on the trap find. Program staff should consider continuing treatments outward from the edges of that block to the approximate locations of the nearest negative 2015 traps. The Kent captures are concerning given the double capture and low density of traps in the area. Compared with the other areas where AGM were captured, there is a greater chance here that an established population exists within the detection system (Appendix 1).

(4) Nisqually. Treatments should extend over 1x1 mile blocks centered on each of the trap detections. Although only two moths were captured and they were several miles apart, the proximity of the finds and the lower trapping density (compared to port areas) increases the uncertainty of ascertaining the presence or size of a population; therefore the TWG is recommending treatment. The TWG considered combining the two capture sites into a single treatment area but felt that any attempts to define the boundaries of a single area would be arbitrary and biologically unrealistic, hence the smaller 1-square-mile treatment blocks.

Insecticide recommendations. Several options are available for insecticide treatments and the program should select the material and application methods that best suit the needs of the area being treated. Given the size of proposed treatment areas and the presence of susceptible hosts, aerial spraying will be the preferred application method to ensure thorough and cost effective coverage of the aerially treated sites. In areas with few or widely scattered susceptible hosts (i.e. industrial or port areas), ground-based equipment may be an acceptable alternative.

Btk is a preferred option due to its combination of efficacy and minimal non-target and human health effects. The activity of Btk is short-lived in the field, and it is most effective for early instars (I-II). As a result, multiple applications (typically three, spaced at 4-10 d intervals depending on conditions and weather) and accurate timing are critical. Degree-day models should be used to calculate appropriate timing of sprays and can be augmented by monitoring hatch of caged F1-sterile eggs placed in the environment. F1-sterile egg masses are available from the USDA-APHIS-PPQ Otis Laboratory in Buzzards Bay, MA. In addition, phenology of hosts in the area should be monitored to ensure that there is adequate foliage on the target trees at the time of the first application.

Diflubenzuron ("Dimilin") is an alternative material that, compared to Btk, has a longer residual and is more effective, particularly for later instars. Based on efficacy alone, diflubenzuron would be recommended over Btk, but it has the disadvantage of relatively greater environmental impact as it is toxic to a broader range of non-target arthropods. Diflubenzuron is an insect growth regulator (IGR).

Tebufenozide ("Mimic") is labeled for gypsy moth and was added to the Joint Forest Service/APHIS Environmental Impact Statement for gypsy moth in 2012. Tebufenozide is another IGR, but its activity is largely restricted to larval Lepidoptera. In both efficacy and non-target effects, it appears to fall somewhere between Btk and diflubenzuron. There is less programmatic experience spraying for gypsy moth with tebufenozide than with diflubenzuron or Btk. However, substantial data exist for this compound.

Gypchek (EPA registered name for gypsy moth nuclear polyhedrosis virus) can be applied similarly to an insecticide and is highly host specific, but achieving required efficacy for eradication can be problematic and availability is limited. It may be an alternative for small areas with environmental sensitivities, such as sites with threatened or endangered insects.

Semiochemical- and genetically-based control methods. While PPQ and states have been considering, and in some cases have used, mating disruption (MD) as an eradication tool for North American gypsy moth, we are not recommending its use for this proposed eradication program. Exceptions could be, for example, if there are areas where threatened or endangered Lepidoptera species are or may be present and MD appears to be the best option. Two reasons for this recommendation: First, despite the effectiveness of MD treatments for North American gypsy moths, this product has not been field tested as an eradication strategy for AGM. Thus, its proposed use for AGM eradication in this case would be based on the assumption that MD will affect AGM populations in the same manner as North American gypsy moth. Second, MD *severely* reduces the effectiveness of GM traps critically needed to determine the effectiveness of a treatment strategy. It is critical, given the spatial spread of the 2015 captures, that the nature and extent of AGM introductions in OR and WA are monitored through traps by the end of the 2016 season, and (+)-disparlure-baited traps are our most effective strategy for this task.

Mass trapping is another tactic that is often mentioned as a potential eradication strategy. Testing by PPQ and FS scientists has indicated that trapping levels approaching 9 traps per acre (5000-6000 per square mile, evenly distributed) are required to achieve a relatively high likelihood that males will arrive at a trap before intercepting a female (i.e., will avert mating). Mass trapping even at this level has not, in testing, provided a high degree of eradication assurance; therefore, mass trapping is likely not feasible in this case given cost, treatment efficacy and resource limitations. The TWG does not recommend mass trapping as an eradication strategy for 2016.

The sterile male technique, or sterile insect technique (SIT), is not an option as eradication strategy for AGM in this case. Although the SIT has been shown to be highly effective in eradicating small, isolated populations of NAGM, the capacity to produce the required number of sterile insects for any of the proposed treatment areas does not exist at this time. Also, this method has not been specifically tested against AGM.

Regulatory measures:

The TWG does not believe that the level of risk of transporting AGM out of the program area warrants regulatory restrictions at this time.

Other recommendations:

The TWG felt that the North American gypsy moth population in the Capitol Hill area should be treated aggressively to avoid possible intermixing with AGM adults. WSDA staff should consider expanding the proposed treatment area beyond the planned 28 acre core.

Given the current situation and significant costs associated with detection and eradication treatments, ship inspections at this time should include 100% inspection of high-risk ship traffic. PPQ staff should work closely with CBP officials to ensure the proper levels of inspection are completed to avoid any further introductions of AGM.

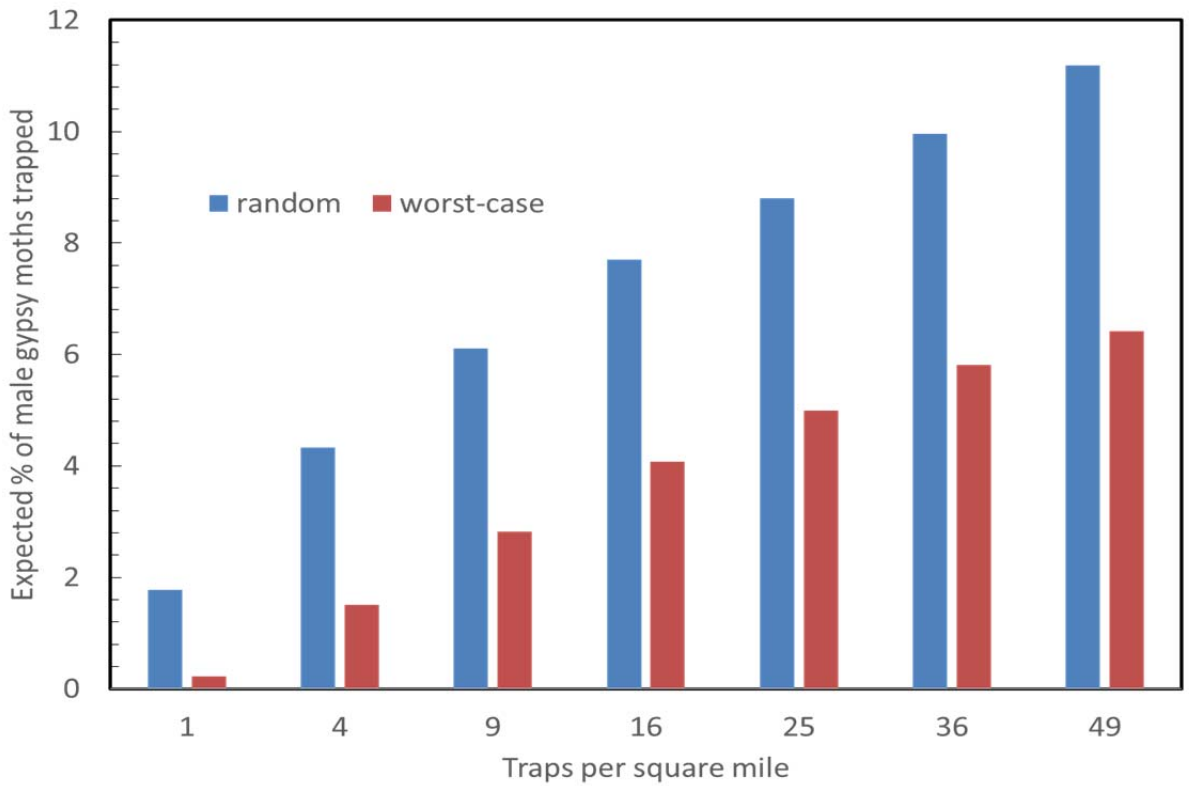
Appendix 1.

Predicted percentages of male gypsy moths captured in (+)-disparlure baited traps deployed at various densities when males are either distributed randomly throughout the habitat or all emerge at maximum insect-to-trap distances within the grid (work conducted on North American gypsy moths).

		Expected % of males captured[a]	
Traps per sq. mi.	Max dist. to trap (m)	Randomly distributed	Worst-case scenario[b]
1	1138	1.78	0.22
4	569	4.33	1.51
9	379	6.11	2.84
16	284	7.71	4.07
25	228	8.80	5.00
36	190	9.95	5.80
49	163	11.12	6.43

[a] Results of Monte Carlo simulations using distance-capture functions derived from multiple release-recapture studies and modeled based on a negative exponential model.

[b] Assumes all insects are initially at the maximum distance from a trap when traps are placed in a "perfect" square grid.



Appendix 2. Estimated total traps, and area trapped, for delimiting AGM populations in Washington and Oregon, 2016 (per recommendations)

TRAPPING TOTALS FROM TWG RECOMMENDATIONS (36 traps per sq. mi. core)

				Washington		Oregon	
Area of Infestation	Trap Group	miles from detection	Traps/sq. mi.	# Traps	Area (Ac.)	# Traps	Area (Ac.)
Tacoma	Core	0-3	36	2157	38,346.43		
Tacoma	Extended	3-6	25	2348	60,108.45		
Portland/Vancouver	Core	0-3	36	524	9,315.50	1355	24,088.74
Portland/Vancouver	Extended	3-6	25	909	23,270.26	1939	49,638.11
Kent	Core	0-3	36	1020	18,133.22		
Kent	Extended	3-6	25	1617	41,394.96		
Nisqually	Core	0-3	36	1758	31,253.14		
Nisqually	Extended	3-6	25	2180	55,807.67		
Gig Harbor	Core	0-3	36	740	13,155.48		
Gig Harbor	Extended	3-6	25	1267	32,435.01		
			TOTAL	14,520	323,220.12	3,294	73,726.85

TRAPPING TOTALS FROM TWG RECOMMENDATIONS (49 traps per sq. mi. core)

				Washington		Oregon	
Area of Infestation	Trap Group	miles from detection	Traps/sq. mi.	# Traps	Area (Ac.)	# Traps	Area (Ac.)
Tacoma	Core	0-3	49	2895	37,812.02		
Tacoma	Extended	3-6	25	2348	60,108.45		
Portland/Vancouver	Core	0-3	49	717	9,364.84	1846	24,110.88
Portland/Vancouver	Extended	3-6	25	909	23,270.26	1939	49,638.11
Kent	Core	0-3	49	1376	17,972.14		
Kent	Extended	3-6	25	1617	41,394.96		
Nisqually	Core	0-3	49	2380	31,085.53		
Nisqually	Extended	3-6	25	2180	55,807.67		
Gig Harbor	Core	0-3	49	1016	13,270.12		
Gig Harbor	Extended	3-6	25	1267	32,435.01		
			TOTAL	16,705	322,521.00	3,785	73,748.99

Estimated number of traps is based on recommendations of buffer extent and trap density. Traps were removed if intersecting with permanent water.

Does not account for other features/inaccessible areas that might preclude trapping.

Appendix 3.

TREATMENT TOTALS FROM TWG RECOMMENDATIONS				
	Washington		Oregon	
Area of Infestation	Area (sq. miles)	Area (acres)	Area (sq. miles)	Area (acres)
Tacoma	10.9055	6,979.52		
Portland/Vancouver	1.261173	807.15072	13.5018	8,641.16
Kent	1.0000	640.00		
Nisqually	2.0000	1,280.00		
	15.17	9,706.67	13.50	8,641.16

Area calculations are based on minimum bounding polygons around 1/2 mile buffers of AGM detection clusters. Areas of polygons overlapping permanent water features were removed. In Portland, a small sliver of treatment area on the opposite side of the river was removed.