FINAL PROJECT REPORT

Project Title: Use of methyl bromide to eliminate SWD

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Other funding sources: None

Total Project Funding:

Budget History:

Item	Year 1:	Year 2:	Year 3:
Salaries	10,920		
Benefits			
Wages			
Benefits			
Equipment			
Supplies	2,600		
Travel			
Miscellaneous			
Total	$13,520		

Objectives. The purpose of this study was to demonstrate the insecticidal efficacy of postharvest methyl bromide fumigation toward spotted wing drosophila in sweet cherries, including the exports to Australia, which are treated for 2-h with a 64 mg/L dose at 43 < T < 54°F, a 48 mg/L dose at 54 < T < 63°F, and a 40 mg/L dose at 63 < T < 72°F.

Significant Findings. At 54 ± 0.5 ºF, "CT" concentration – time cross products > 88 mg h/L have resulted in complete mortality of ~55,000 internal feeding large larvae (ca. 96-120 h old at fumigation), the most tolerant life stage of SWD. Between 43-51 ºF, the individual and interactive effect(s) of temperature, time, and methyl bromide (MB) dose on the survivability of the most tolerant SWD life stage was quantitatively delineated; a multifactorial experiment was generated and the results were analyzed using Design Expert 7.0 (Stat-Ease, Inc.). The mathematical model generated in this study can be used as a predictive tool for ensuring that targeted mortality is achieved during individual fumigation events, such as when "Probit 9-level" control of insects (i.e., ≥ 99.9986% mortality) is required by trading partners.

Results & Discussion.

Most MB-tolerant life stage. Direct and indirect analytical methods were used to identify 48-96 h old larvae (60-108 at fumigation) as the life stage of SWD that is most tolerant toward MB. Data support a conclusion that this is a result of larvae burrowing into commodity to feed internally, where fumigant concentrations are relatively lower than on the surface of the fruit. We often observe this larger and older larval life stage completely submerged, including spiracles. On the other hand, eggs, pupae, and younger larvae are exclusively associated with the fruit periphery and an external "feeding" behavior where they have a relatively uniform expose to fumigant concentrations measured in chamber headspace (i.e. external scenario).

Strawberries (▲), cherries (♦), and grapes (■) were infested with SWD over a 48 h period, removed for 48 h, tempered for 12 h, and held as a fumigation control. Adult emergence from fruit obeyed Gaussian distribution over ~ 50h, which was centered 14 day after the initial infestation when incubated at 27 °C and 80% RH. Derivatives of emergence provide a useful illustration, with the slope of the intersects being directly related to uniformity for a particular fruit. One strawberry, two cherries, and three grapes were in each cage; total emerged adults were respectively 1436, 1189, and 2034.

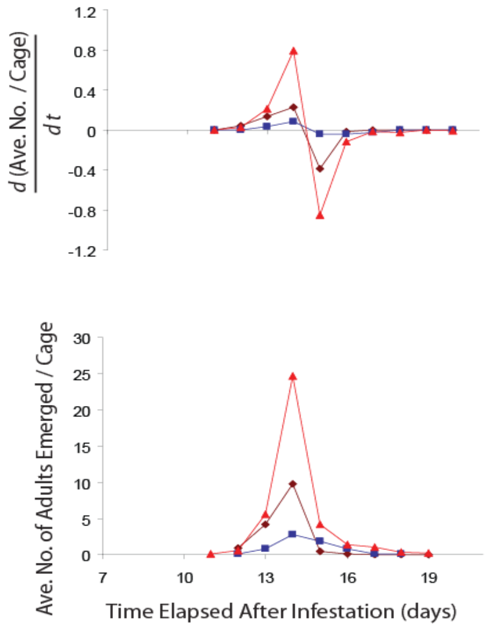

Figure 1. Emergence of adult SWD from fruit can be accurately predicted and occurs with uniform synchronization.

To indirectly diagnose "large larvae" as the most tolerant life stage, strawberries (▲), cherries (♦), and grapes (■) were infested with SWD over a 96 h period, tempered for 12 h, and fumigated with MB exposures of 10.7 mg-h/L at 60°F. The emergence of adults after incubation is at a maximum 14 d after infestation, indicating that "large" larvae (~ 60-108 h-old at time of fumigation) are the most MB-tolerant SWD larval life stage. Numbers of specimens treated are estimated by control emergence of 4,321 in nonfumigated controls.

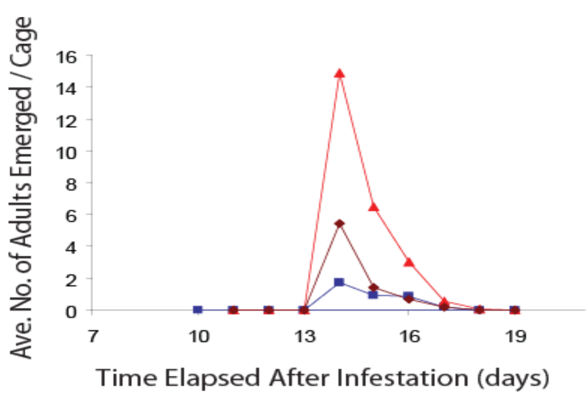

Figure 2. The uniform synchronization of emergence allowed the most tolerant life stage to be determined indirectly.

Dose-mortality data on segregated developmental life stages was used to directly diagnose “large” larvae as the most MB-tolerant SWD life stage. Larvae and eggs specimens were obtained from “natural infestation”, while pupae and adults, which are only encountered on the periphery of the fruit unlike larvae, were treated in cages (Table2).

Table 2. Survivability of SWD dvelopmental stages exposed to MB for 2h at 60F/15.6C

(Stages in order from most (left) to least (right) tolerant)

MB Dose	Lg. larvae (48-96h)		Sm. larvae (0-48h)		egg (0-24h)		Pupae		Adult	
(mg/L)	% Survival	% Mortality	% Survival	% Mortality	% Survival	% Mortality	% Survival	% Mortality (corrected) *	% Survival	% Mortality (corrected) *
0 (CG)	-	-	-	-	-	-	90.8	-	93.6	-
16	72.8	29.2	43.6	56.4	26.4	73.6	10.2	88.8	0.0	100.0
32	13.6	86.4	1.9	98.1	2.7	97.3	0.0	100.0	0.0	100.0
48	0.4	99.5	0.0	100.0	0.1	99.9	0.0	100.0	0.0	100.0
64	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0
n **	4510		4120		3840		3238		1854	

* Mortality correction = ((control % survival - treated % survival)/control % survival) * 100

** n = total number observed in treated groups for each stage; estimated from the number surviving to adult from control groups (CG) for each stage

A paired t-test was used to further differentiate the target stage as being “older and large larvae” and not “younger and smaller” larvae when feeding on fruit; means were converted to probabilities of emergence for graphics. Statistical values for 0-48 h-old larvae vs. 48-96 h-old larvae with MB exposures of 10.2 mg-h / L: t= 3.2, 10df, P = 0.01; 0-48 h-old larvae vs. 48-96 h-old larvae with MB exposures of 22.9 mg-h /L: t = 2.6, 22df, P = 0.018. The tolerance to MB of the “older and larger” SWD larval life stage, ~60-108 h- old at the time of fumigation (12 h temper period prior to fumigation), is likely due to a more pronounced ability to feed completely “submerged” and relocate more quickly within the fruit pulp relative to “younger and smaller” larvae. This behavioral distinction increases the potential for large larvae to be physically separated from MB, which increases in concentration toward the fruit periphery. SWD eggs, pupae, and adults are associated with the external surface of the fruit and are unable to avoid exposure to MB. Percentages of specimens treated are estimated by emergence from untreated controls of 1,499 and 1,572 for 0-48 h-old and 48-96 h-old larvae, respectively.

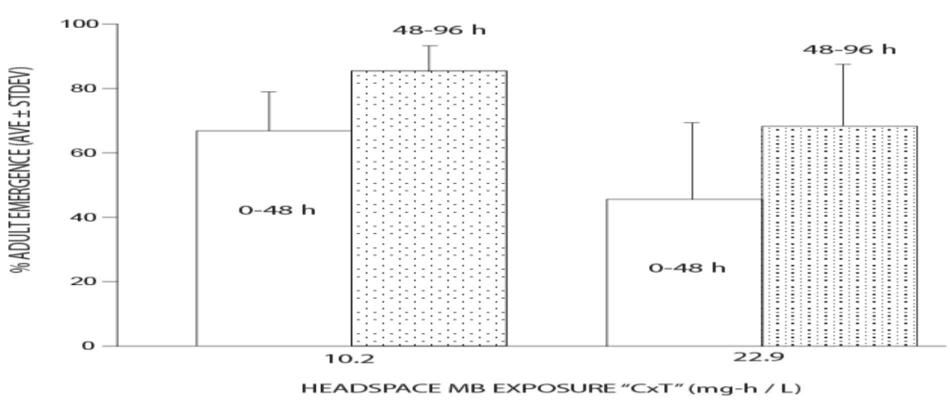

Figure 3. Older and larger larvae are more MB-tolerant than younger and smaller larvae in comparative fumigations of infested grapes at 60 ºF.

Probit Analyses. Dose-mortality regressions were generated using Probit 2007 software; Probit 9 (P9) doses project 99.9968% mortalities. Number of insects specimen treated (n) and regression heterogeneity (H) are noted in Figure 4.

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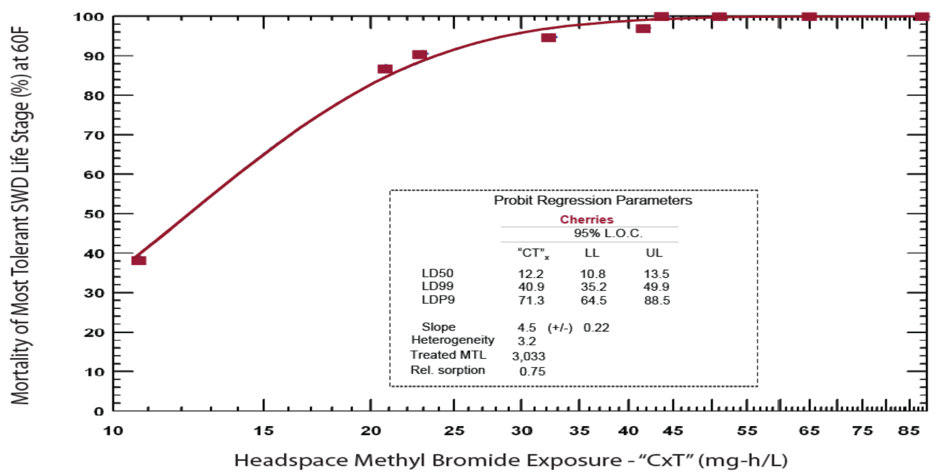

Figure 4. The survivability of the most tolerant SWD life stage(MTL), 60-108 h- old larvae, to MB is directly related to the sorption capacity of the commodity across equivalent 2-h exposures at 60 °F. These data support the conclusion that the "observed tolerance" of this life stage is due, in large part, to internal feeding behavior and physical avoidance of MB, which increases in concentration toward the fruit periphery.

Multivariate Analysis: Negotiating the design space over 43 – 51F. A multifactorial experimental design was generated and the results were analyzed using Design Expert 7.0 (Stat-Ease, Inc.). A three-factor central composite design was employed,[4,5] which contained three levels (-1, 0, 1) of the three factors, x_1–x_3, and six replicates of the center-point. Conditions of dose, temperature, and duration were chosen to accommodate, or span, those applicable to standard industrial practice with respect toward methyl bromide schedules for the import of cherries (APHIS T101-r-1 & T101-s-1) and export of cherries to Japan and Australia (temp-ºF, initial dose-mg/L, time-h): >72, 32, 2; 72-63, 40, 2; 63-54, 48, 2; 54-43, 64, 2. The design involved a total of 34 experiments run in a randomized sequence (Tables 1 and 3), and the modeled response (y) was survivability, which was expressed as a percentage of adult emergence after a treatment relative to estimates of numbers treated based on emergence from non-treated controls.

Table 1. Three factors and three factor levels used in the central composite multivariate experimental design.

Factor (units)	Factor levels		
	-1	0[a]	1
x_1: dose (mg/L)	40	48	56
x_2: temp (°F)	43	47	51
x_3: duration (h)	1	2	3

[a] 0 = center point

A full second-order quadratic expression was fitted to data on insecticidal efficacy of MB versus SWD in cherries; it contained 10 parameters including linear and quadratic dependencies on each factor and all possible two-factor interactions:

$$y = \beta_0 + \beta_1 x_1 + \beta_{11} x_1^2 + \beta_2 x_2 + \beta_{22} x_2^2 + \beta_3 x_3 + \beta_{33} x_3^2 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3$$

Each parameter of this full second-order model includes a coefficient: β_0, a constant or offset term; β_1, β_2, β_3 estimate the linear effects of the factors; β_{11}, β_{22}, β_{33} estimate the quadratic (curvature) effects of the factors; and β_{12}, β_{13}, β_{23}, estimate the interaction effects between every pair of two factors. Equation 1 represents the optimized model, which was developed to negotiate the design space with greater accuracy between factor levels: 43-47°F, and 47-51°F; it fitted the data with a correlation coefficient (R^2) of = 0.95 (adjusted R^2 = 0.94) and predicted SWD mortality with a correlation coefficient (R^2) of = 0.90 (Table 4 and 5).

$$y = 0.35 - 3.64x_1 + 1.08x_1^2 - 1.59x_2 + 1.38x_2^2 - 6.35x_3 + 4.16x_3^2 + 1.02x_1x_2 + 4.06x_1x_3 + 1.57\, x_2x_3 \qquad (1)$$

Table 3. The experimental conditions and modeled response of SWD mortality.

run	dose (mg/L)	Temp (°C)	duration (h)	CT exposure (mg h/L)	treated specimens	survivability (%)
1	40	51	3	97.3	375 ± 146	0.000
2	48	47	2	86.5	341 ± 112	0.293
3	40	47	2	70.7	341 ± 112	2.933
4	48	47	2	86.5	341 ± 112	0.000
5	48	47	2	86.5	341 ± 112	0.293
6	56	47	2	100.9	341 ± 112	0.000
7	40	47	2	70.7	341 ± 112	2.346
8	56	43	3	149.3	794 ± 37	0.000
9	40	43	3	105.1	794 ± 37	0.252
10	40	43	3	105.1	794 ± 37	1.008
11	48	47	3	131.8	341 ± 112	0.000
12	56	51	1	54.1	375 ± 146	4.533
13	56	51	1	54.1	375 ± 146	3.733
14	56	43	3	149.3	794 ± 37	0.000
15	40	51	3	97.3	375 ± 146	0.000
16	48	51	2	91.0	375 ± 146	0.533
17	48	47	1	43.3	341 ± 112	9.091
18	56	47	2	100.9	341 ± 112	0.000
19	48	47	2	86.5	341 ± 112	0.587
20	56	51	3	152.4	375 ± 146	0.000
21	40	43	1	36.3	794 ± 37	24.937
22	56	51	3	152.4	375 ± 146	0.000
23	48	47	1	43.3	341 ± 112	8.504
24	48	47	2	86.5	341 ± 112	1.466
25	40	43	1	36.3	794 ± 37	29.597
26	40	51	1	37.5	375 ± 146	15.733
27	40	51	1	37.5	375 ± 146	18.133
28	56	43	1	53.1	794 ± 37	8.060
29	48	43	2	86.8	794 ± 37	1.511
30	48	47	3	131.8	341 ± 112	0.000
31	48	43	2	86.8	794 ± 37	3.778
32	48	51	2	91.0	794 ± 37	0.630
33	48	47	2	86.5	341 ± 112	0.293
34	56	43	1	53.1	375 ± 146	5.867
					Σ 16,464 ± 634	

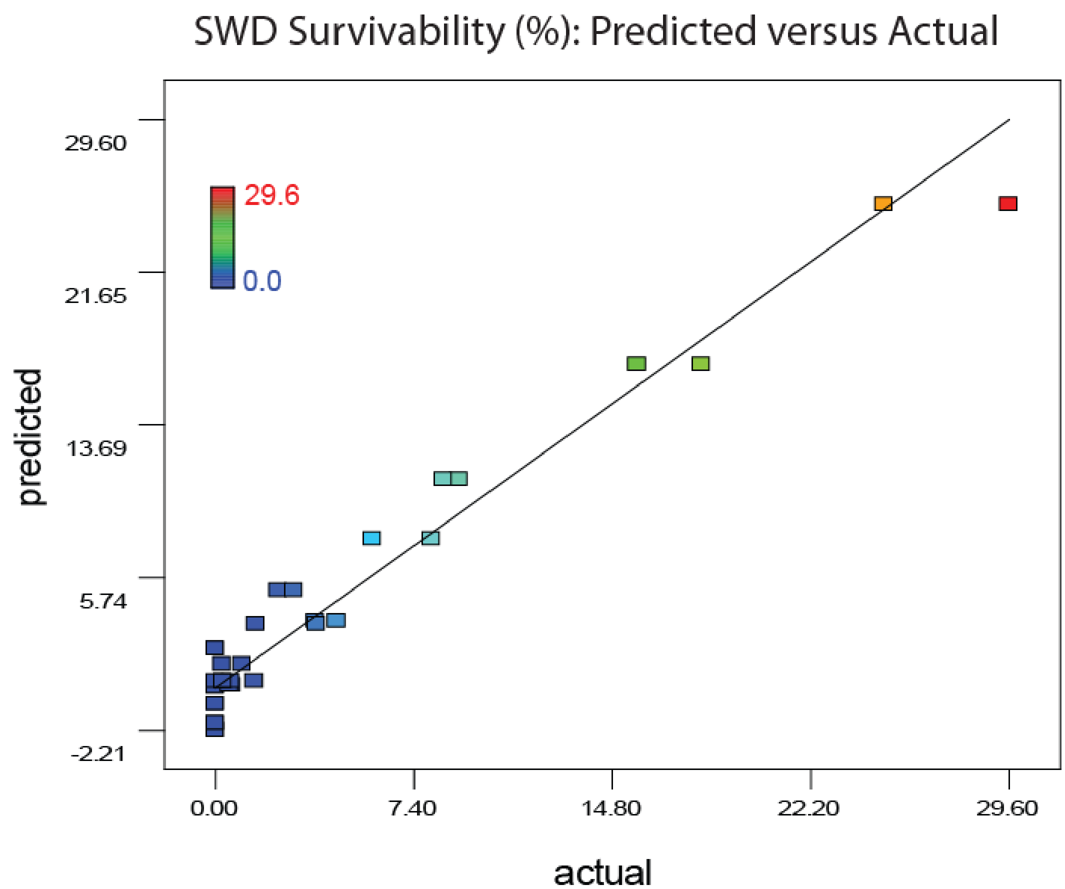

Figure 5. The quadratic model, which was optimized to fit the data on SWD mortality, can also be used for to estimate the success of a fumigation event.

The coefficients (β_x) were tested for significance against the null hypothesis ($\beta_x = 0$), that the factor was unimportant in determining survivability (Table 5). At the 95% level of confidence, SWD survivability depended linearly on the dose (β1), temperature (β2), and duration (β3), interactively on dose-temperature (β12), dose-duration (β13) and temperature-duration (β23), and quadratically on duration-duration (β33).

Table 4. ANOVA statistical analysis of the agreement between the model (equation 2) and the data regarding the survivability of the most tolerant life stage of SWD, the "large" internal feeding larvae (ca. 96-120 h old at time of fumigation).

source	sum of squares[a]	df	mean square[b]	F-value[c]	p-value[d] Prob > F
model	1706.94	9	189.66	56.59	< 0.0001
residual	80.43	24	3.35		
lack of fit	59.44	5	11.89	10.76	< 0.0001

a total for the sum of squares for the terms in the model
b estimate of variance, models sum of squares / model degrees of freedom
c comparison of term variance (mean square) with residual variance (res. mean square)
d probability of seeing observed F value if the null hypothesis is true (no factor effect)

Table 5. ANOVA statistical tests for single parameters of the quadratic model (equation 2) fit to the data on SWD mortality.

parameter coefficient	factor effect	estimate	standard error(1df)	sum of squares [a]	F value [b]	p-Value [c,d] prob > F
β_0	intercept	0.35	0.055			
β_1	dose	-3.64	0.041	7.12	78.95	**< 0.0001**
β_2	temp	-1.59	0.041	1.47	15.01	**< 0.0007**
β_3	duration	-6.35	0.041	36.37	240.37	**< 0.0001**
β_{12}	dose-temp	1.02	0.046	0.18	4.93	**0.0360**
β_{13}	dose-duration	4.06	0.046	4.46	78.67	**< 0.0001**
β_{23}	temp-duration	1.57	0.046	0.44	11.72	**0.0022**
β_{11}	dose-dose	1.08	0.079	0.074	1.87	0.1839
β_{22}	temp-temp	1.38	0.079	0.39	3.03	0.0948
β_{33}	duration-duration	4.16	0.079	3.07	27.69	**< 0.0001**

a *n* of experiments / 4 x squared factor effect
b comparison of term variance (mean square) with residual variance (res. mean square)
c probability of seeing observed F value if the null hypothesis is true (no factor effect)
d "prob > F" values < 0.05 tests as significant at the 95% confidence level

Table 6. Initial doses and "CT" products required in commercial scenarios to achieve Probit 9 control of SWD (right column) were generated based on predictions derived from a multivariate model (left column).

		multivariate prediction		commercial evaluation	
		Dose (± 0.7mg/I	"CT"[a] (mg h/L)	Dose (± 2 mg/L)	"CT"[b] (mg h/L)
51 °F	2 h	48.2	88.1	54.1	88.1
	3 h	35.1	93.3	41.7	93.3
50 °F	2 h	49.6	90.2	55.3	90.2
	3 h	35.5	94.3	42.1	94.3
49 °F	2 h	50.7	92.2	56.6	92.2
	3 h	35.6	94.5	42.2	94.5
48 °F	2 h	51.7	93.9	57.6	93.9
	3 h	35.8	95.1	42.5	95.1
47 °F	2 h	52.5	95.6	58.7	95.6
	3 h	36.0	95.7	42.7	95.7
46 °F	2 h	53.3	97.0	54.6	97.0
	3 h	36.7	97.6	43.6	97.6
45 °F	2 h	54.0	98.2	60.3	98.2
	3 h	37.0	98.4	43.9	98.4
44 °F	2 h	54.6	99.3	60.9	99.3
	3 h	41.3	109.8	49.0	109.8
43 °F	2 h	55.2	100.4	61.8	100.4
	3 h	44.6	118.7	53.0	118.7

a sorption profile (time, % headspace loss of MB): 0.5 h, 7.5%; 1 h, 9.5%; 2 h, 14.5%, 3 h, 17.5%
b sorption profile (time, % headspace loss of MB): 0.5 h, 10.0%; 1 h, 20.0%; 2 h, 34.0%, 3 h, 44.0%

Confirmatory fumigations. MB fumigations were conducted to confirm the mortality of all SWD life stages in cherry export to Australia and Japan The minimum allowable exposure of this schedule between 54-63F, denoted by a shaded box, is a concentration-time "CxT" product of 64.5 mghL^{-1} based on the standard indices used by APHIS to account for percentage fumigant loss through time (75% at 30 min, 63% at 1h, 50% at 2h, and 38% at 3h). These projected losses, which are used to generate the concentration-time cross product "CT" exposure minimum for prescribed fumigations, accommodate chamber leakage and sorption to fruit. The differential sorption of MB between replicate fumigations was used to establish a range of "CxT's" encompassing the target/confirmatory exposure minimum (Table 7). Data indicates that >88 mgh/L exposures of SWD to MB in cherry loads packaged for export resulted in the mortality of all ~ 55,000 test specimens.

Table 7. MB exposures at 54F for each fumigation replicate indexed relative to minimum requirements.

# treated specimens	Applied (mg/L)	1/2 h [MB]	1 h [MB]	2 h [MB]	3 h [MB]	% Sorp.	CxT (mgh/L)	survivors
Min.	48.0	36.0	30.0	24.0	-	50.0	64.5	
3854 ± 210	45.5	36.6	32.8	27.4	-	39.7	68.1	27
3854 ± 210	47.5	37.3	33.3	27.6	-	41.8	69.5	14
4340 ± 275	49.9	38.4	33.3	27.9	-	44.1	70.6	10
4340 ± 275	51.5	39.7	34.2	28.3	-	45.1	72.6	3
4170 ± 468	48.5	43.7	39.4	33.8	-	30.3	80.4	1
3020 ± 324	42.2	35.5	32.5	27.8	25.4	42.2	84.6	4
4170 ± 468	36.8	32.7	30.4	26.3	-	28.6	86.6	1
4256 ± 334	53.3	48.7	43.3	32.4	-	39.2	86.7	0
4470 ± 239	53.8	48.1	44.4	33.2	-	38.3	87.4	0
3020 ± 324	41.5	33.3	30.5	26.1	24.3	41.5	88.1	0
4125 ± 310	55.4	48.4	43.8	37.9	-	31.5	89.9	0
4256 ± 334	57.6	53.4	44.2	34.2	-	40.6	91.4	0
4125 ± 310	57.1	50.4	45.3	36.8	-	35.6	91.9	0
4470 ± 239	57.6	52.9	47.6	36.4	-	36.8	94.8	0
3741 ± 102	38.2	35.3	33.5	29.0	26.3	31.7	94.9	0
4581 ± 467	44.9	39.1	33.9	29.1	25.6	43.7	98.0	0
4581 ± 467	44.7	37.3	34.2	29.8	26.3	41.2	98.4	0
2984 ± 198	62.2	55.4	49.2	41.0	-	34.1	100.7	0
3741 ± 102	41.2	39.8	35.6	31.2	29.1	29.4	102.7	0
3761 ± 273	47.5	41.4	37.7	29.8	25.9	45.5	103.6	0
2984 ± 198	64.1	57.6	51.2	42.2	-	34.0	104.3	0
3761 ± 273	44.3	40.7	37.4	32.2	27.1	38.8	105.2	0

Σ 87,144 ± 1449.6

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Executive Summary. This research is ongoing and a final report will be sent to WTFRC upon completion.