Does getting defensive get you anywhere?— Seasonally varying selection in pea aphids shapes a dynamic infection polymorphism with a protective bacterial endosymbiont

Drew Smith[1], Michael O'Connor[1], Brooke Deal[1], Coleman Kotzer[1], Amanda Lee[1], Barrett Wagner[1], Jonah Joffe[2], Stephen Woloszynek[1], Kerry Oliver[3], and Jacob Russell[1]

[1]Drexel University
[2]Affiliation not available
[3]University of Georgia College of Agricultural and Environmental Sciences

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Abstract

Facultative, heritable endosymbionts are found at intermediate prevalence within most insect species, playing frequent roles in their hosts' defense against environmental pressures. Focusing on Hamiltonella defensa, a common bacterial endosymbiont of aphids, we tested the hypothesis that such pressures impose seasonal balancing selection, shaping a widespread infection polymorphism. In our studied pea aphid (Acyrthosiphon pisum) population, Hamiltonella infection frequencies ranged from 23.2% to 68.1% across a six-month longitudinal survey. Rapid spikes and declines were consistent across fields, and we estimated that selection coefficients, for Hamiltonella-infected aphids, changed sign within this single season. Prior laboratory research suggested anti-parasitoid defense as the major Hamiltonella benefit, and costs under parasitoid absence. While a prior field study supported these forces as counter-weights in a regime of seasonal balancing selection, our present survey showed no significant relationship between parasitoid wasps and Hamiltonella. Field cage experiments provided some explanation: parasitoids drove ~10% boosts to Hamiltonella frequencies that would be hard to detect under less controlled conditions. They also showed that Hamiltonella was not always costly under parasitoid exclusion, contradicting another long-held prediction. Instead, our longitudinal survey – and two overwintering studies - showed temperature to be the strongest predictor of Hamiltonella infection, matching some lab discoveries, and suggesting thermally sensitive costs and benefits, unrelated to parasitism, can shape this symbiont's prevalence. These results add to a growing body of evidence arguing for rapid, seasonal adaptation in multivoltine organisms. For many insects, such adaptation may be mediated through the diverse impacts of heritable symbionts on host phenotypes.

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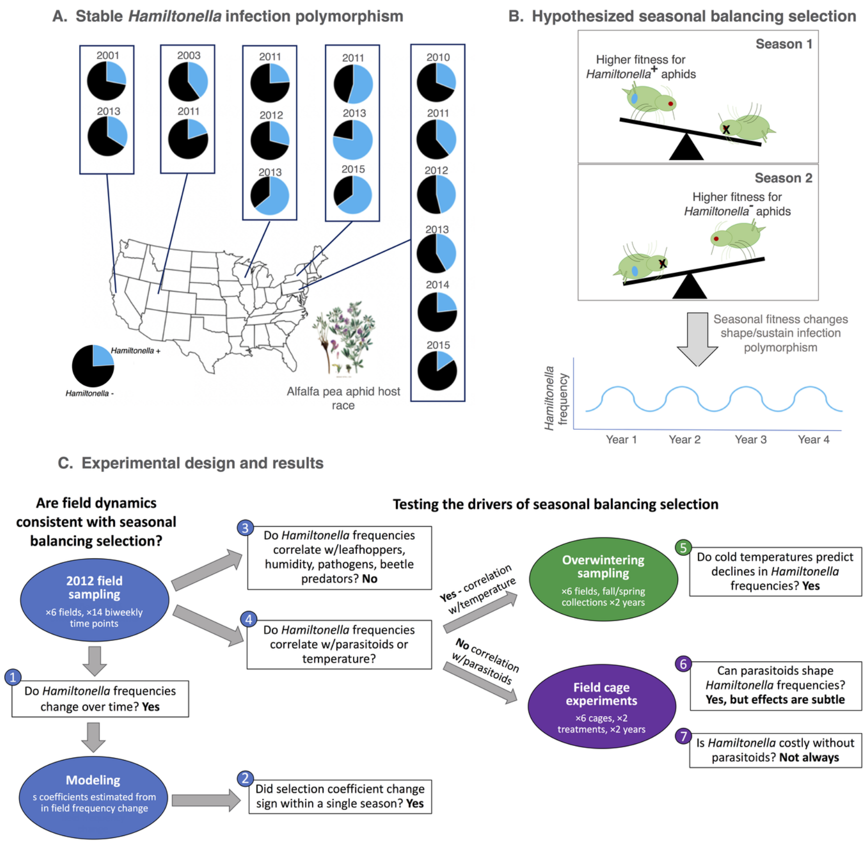
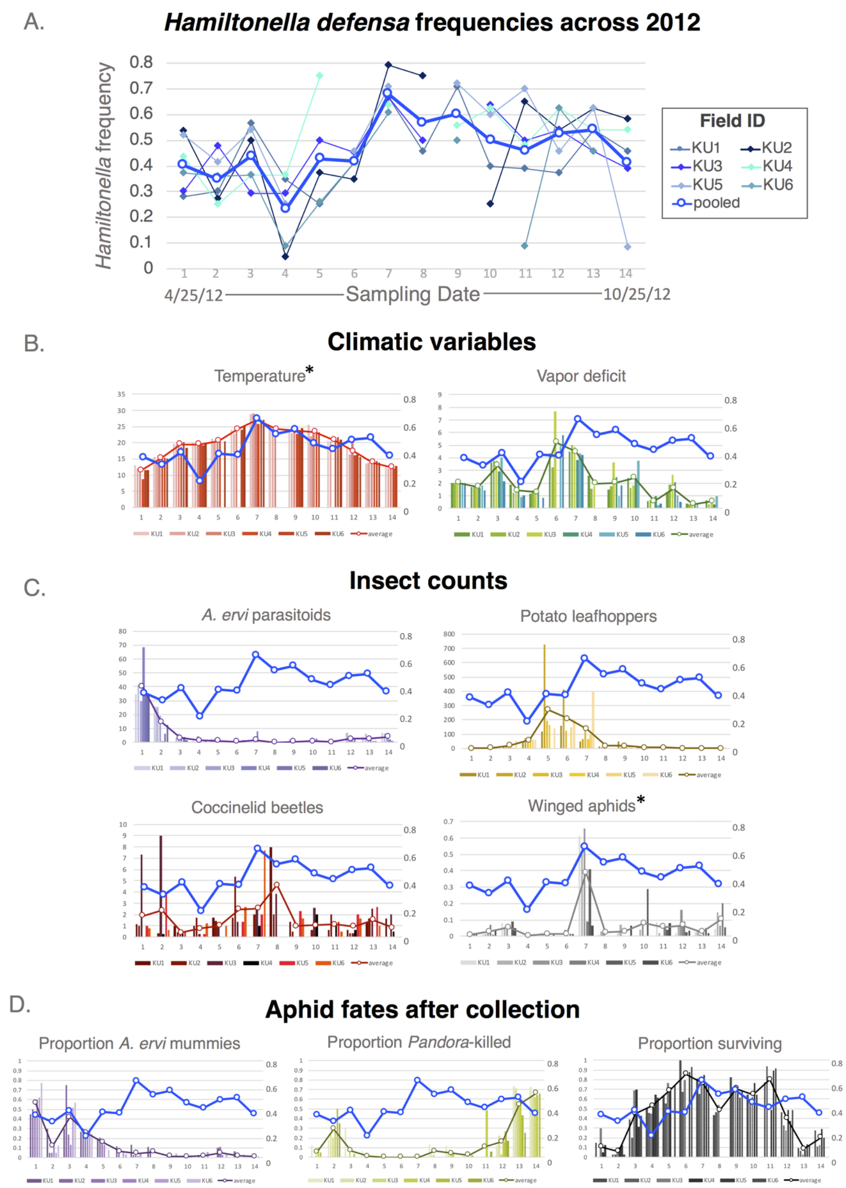
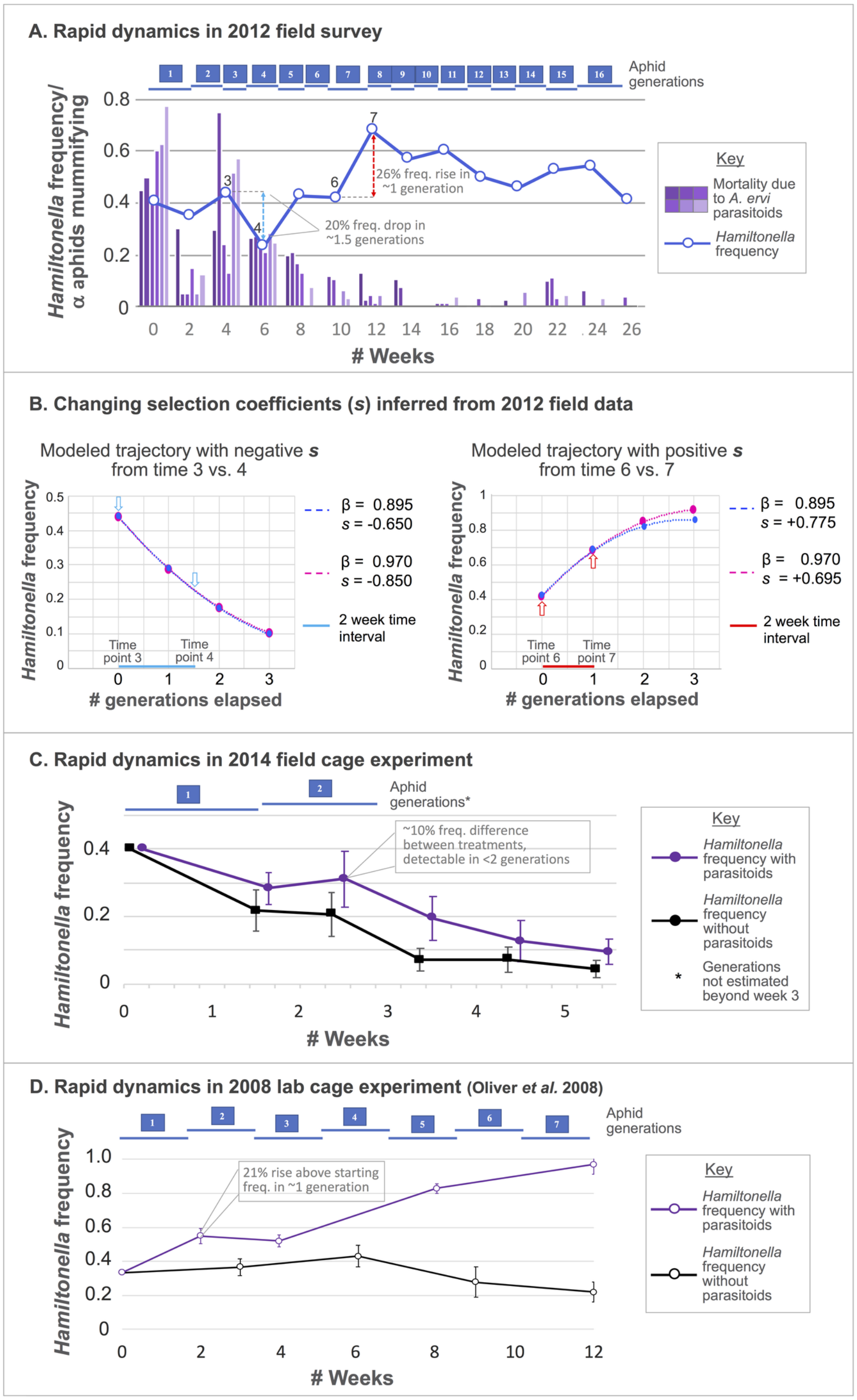
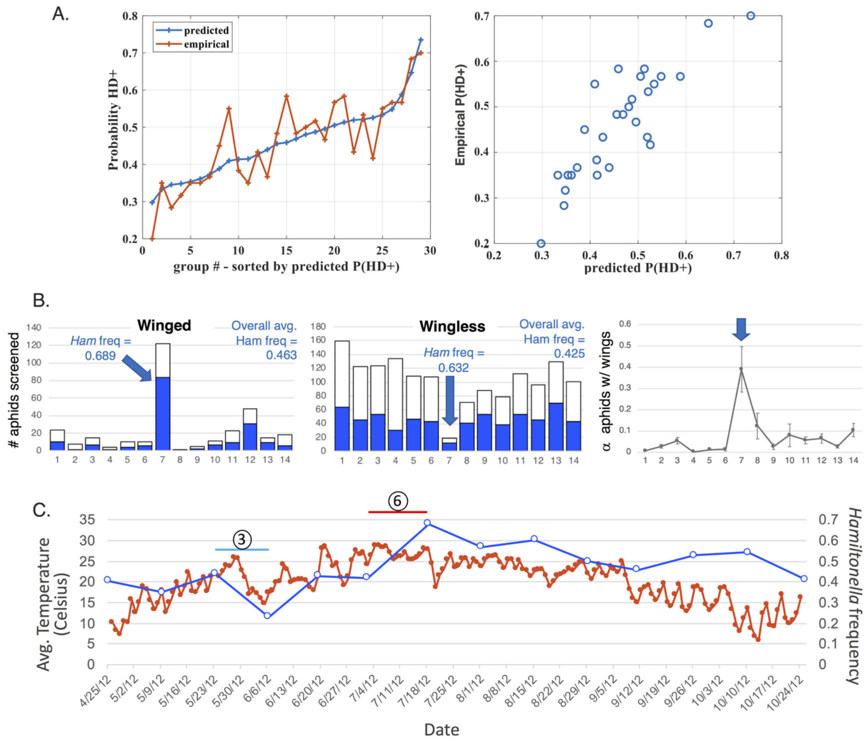
`field cage paper v38b-no changes.docx` available at https://authorea.com/users/337970/articles/467666-does-getting-defensive-get-you-anywhere-seasonally-varying-selection-in-pea-aphids-shapes-a-dynamic-infection-polymorphism-with-a-protective-bacterial-endosymbiont

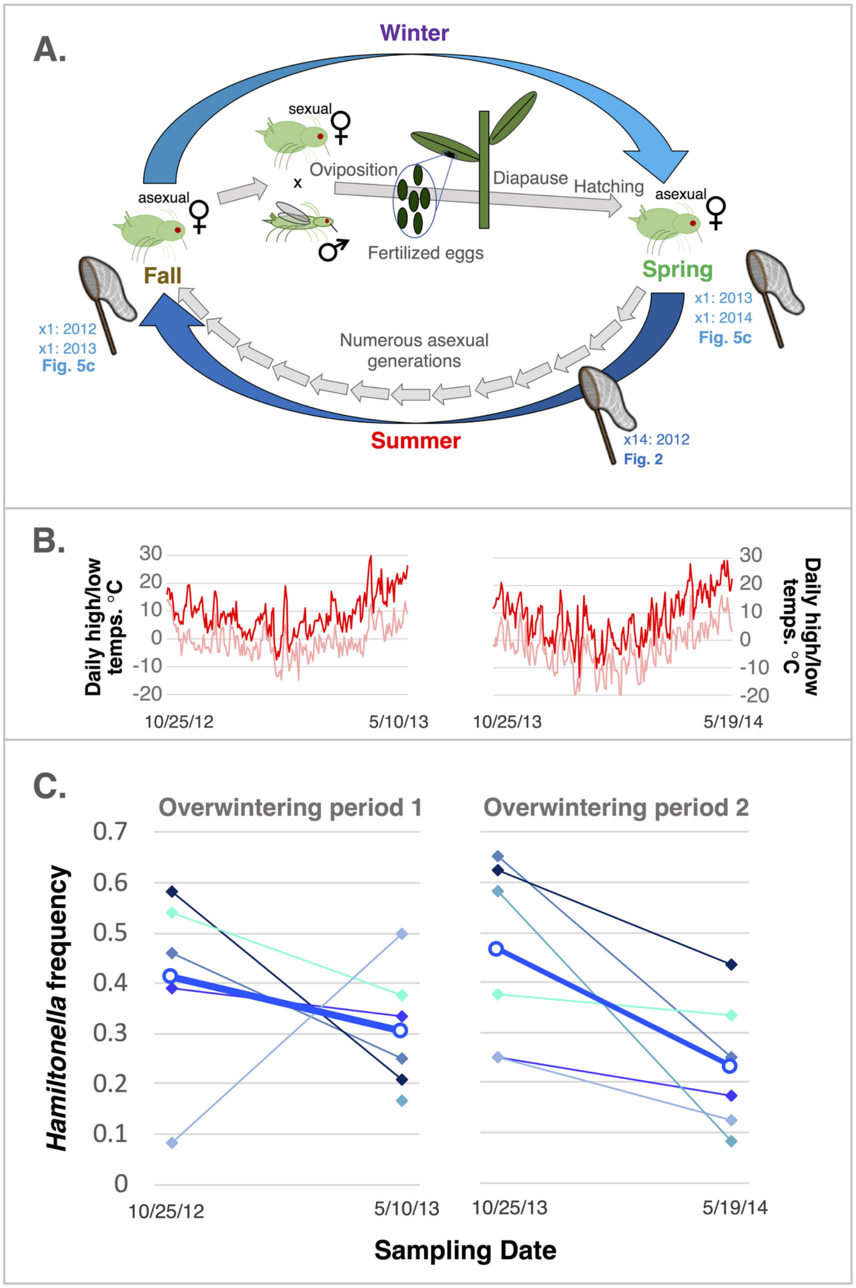


A. Stable Hamiltonella infection polymorphism
B. Hypothesized seasonal balancing selection
Season 1
Higher fitness for Hamiltonella+ aphids
Season 2
Higher fitness for Hamiltonella- aphids
Seasonal fitness changes shape/sustain infection polymorphism
Hamiltonella frequency
Year 1
Year 2
Year 3
Year 4
Hamiltonella +
Hamiltonella -
Alfalfa pea aphid host race
C. Experimental design and results
Are field dynamics consistent with seasonal balancing selection?
Testing the drivers of seasonal balancing selection
2012 field sampling
×6 fields, ×14 biweekly time points
1 Do Hamiltonella frequencies change over time? Yes
Modeling
s coefficients estimated from in field frequency change
2 Did selection coefficient change sign within a single season? Yes
3 Do Hamiltonella frequencies correlate w/leafhoppers, humidity, pathogens, beetle predators? No
4 Do Hamiltonella frequencies correlate w/parasitoids or temperature?
Yes - correlation w/temperature
No correlation w/parasitoids
Overwintering sampling
×6 fields, fall/spring collections ×2 years
5 Do cold temperatures predict declines in Hamiltonella frequencies? Yes
Field cage experiments
×6 cages, ×2 treatments, ×2 years
6 Can parasitoids shape Hamiltonella frequencies? Yes, but effects are subtle
7 Is Hamiltonella costly without parasitoids? Not always

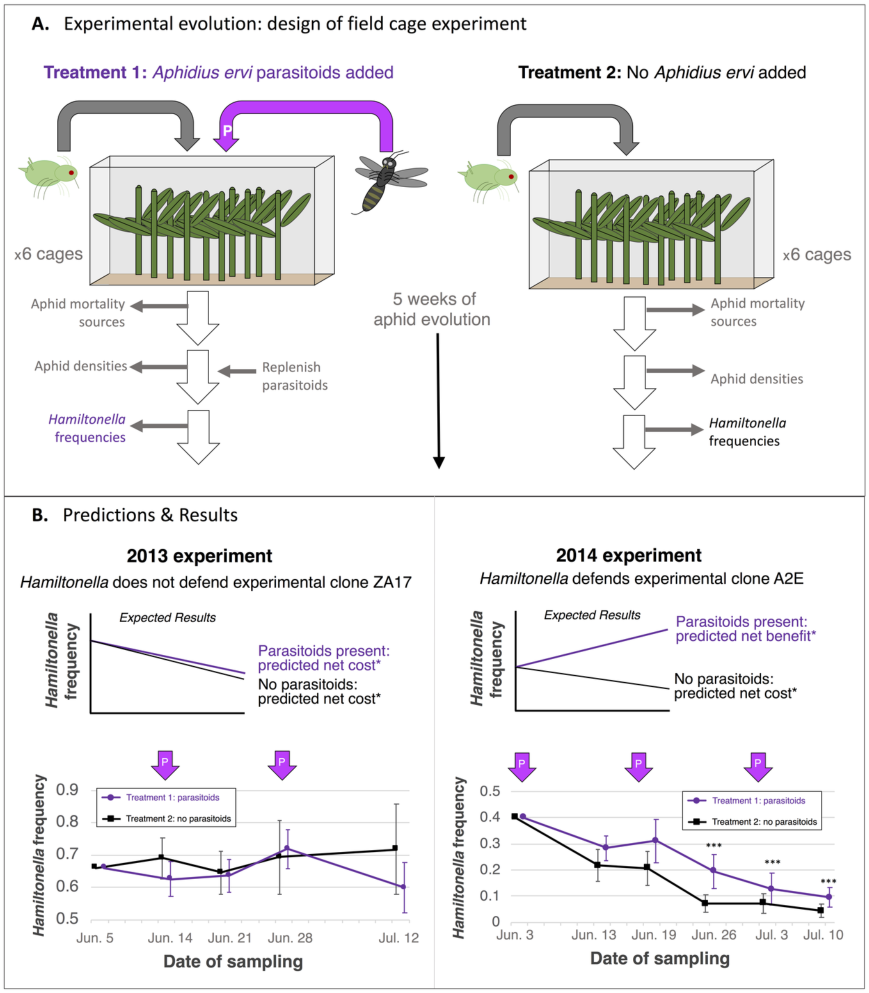

A.
Hamiltonella defensa frequencies across 2012
Hamiltonella frequency
Sampling Date
4/25/12
10/25/12
Field ID
KU1
KU2
KU3
KU4
KU5
KU6
pooled
B.
Climatic variables
Temperature*
Vapor deficit
C.
Insect counts
A. ervi parasitoids
Potato leafhoppers
Coccinelid beetles
Winged aphids*
D.
Aphid fates after collection
Proportion A. ervi mummies
Proportion Pandora-killed
Proportion surviving


A. Rapid dynamics in 2012 field survey
Aphid generations
Hamiltonella frequency/
α aphids mummifying
26% freq. rise in ~1 generation
20% freq. drop in ~1.5 generations
Weeks
Key
Mortality due to A. ervi parasitoids
Hamiltonella frequency
B. Changing selection coefficients (s) inferred from 2012 field data
Modeled trajectory with negative s from time 3 vs. 4
Hamiltonella frequency
β = 0.895
s = -0.650
β = 0.970
s = -0.850
2 week time interval
Time point 3
Time point 4
generations elapsed
Modeled trajectory with positive s from time 6 vs. 7
β = 0.895
s = +0.775
β = 0.970
s = +0.695
2 week time interval
Time point 6
Time point 7
generations elapsed
C. Rapid dynamics in 2014 field cage experiment
Aphid generations*
~10% freq. difference between treatments, detectable in <2 generations
Hamiltonella frequency
Weeks
Key
Hamiltonella frequency with parasitoids
Hamiltonella frequency without parasitoids
* Generations not estimated beyond week 3
D. Rapid dynamics in 2008 lab cage experiment (Oliver et al. 2008)
Aphid generations
21% rise above starting freq. in ~1 generation
Hamiltonella frequency
Weeks
Key
Hamiltonella frequency with parasitoids
Hamiltonella frequency without parasitoids


A.
predicted
empirical
Probability HD+
group # - sorted by predicted P(HD+)
Empirical P(HD+)
predicted P(HD+)
B.
aphids screened
Winged
Ham freq = 0.689
Overall avg. Ham freq = 0.463
Wingless
Ham freq = 0.632
Overall avg. Ham freq = 0.425
α aphids w/ wings
C.
Avg. Temperature (Celsius)
Hamiltonella frequency
Date
3
6



A.
Winter
sexual ♀
Oviposition
x
asexual ♀
♂
Fertilized eggs
Diapause
Hatching
asexual ♀
Fall
Spring
x1: 2012
x1: 2013
Fig. 5c
x1: 2013
x1: 2014
Fig. 5c
Numerous asexual
generations
Summer
x14: 2012
Fig. 2
B.
Daily high/low temps. °C
30
20
10
0
-10
-20
10/25/12
5/10/13
10/25/13
5/19/14
C.
Overwintering period 1
Overwintering period 2
Hamiltonella frequency
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
10/25/12
5/10/13
10/25/13
5/19/14
Sampling Date


A. Experimental evolution: design of field cage experiment
Treatment 1: Aphidius ervi parasitoids added
Treatment 2: No Aphidius ervi added
x6 cages
x6 cages
5 weeks of
aphid evolution
Aphid mortality
sources
Aphid densities
Replenish
parasitoids
Hamiltonella
frequencies
Aphid mortality
sources
Aphid densities
Hamiltonella
frequencies
B. Predictions & Results
2013 experiment
Hamiltonella does not defend experimental clone ZA17
Expected Results
Hamiltonella frequency
Parasitoids present:
predicted net cost*
No parasitoids:
predicted net cost*
2014 experiment
Hamiltonella defends experimental clone A2E
Expected Results
Parasitoids present:
predicted net benefit*
No parasitoids:
predicted net cost*
Treatment 1: parasitoids
Treatment 2: no parasitoids
Hamiltonella frequency
Jun. 5
Jun. 14
Jun. 21
Jun. 28
Jul. 12
Date of sampling
Jun. 3
Jun. 13
Jun. 19
Jun. 26
Jul. 3
Jul. 10
Date of sampling