

# Climate and Mediterranean Fruit Fly Invasion Persistence: Insights from Agent-Based Simulations

Nicholas C. Manoukis and Travis C. Collier

Agricultural Research Service - USDA

Daniel K. Inouye US Pacific Basin Agricultural Research Center  
Hilo Hawaii USA



[www.ars.usda.gov](http://www.ars.usda.gov)

Agricultural  
Research  
Service



IAEA-AW Conference, May 2017

# Outline

## Motivation

- Testing Scenarios

- Estimating Quarantine Length

## Methods

- What is an ABS?

- Simulating a Medfly

## Application

- Single Outbreaks

- Outbreaks Over Space and Time

## Conclusion

- Take-Home

# Outline

## Motivation

### Testing Scenarios

### Estimating Quarantine Length

## Methods

### What is an ABS?

### Simulating a Medfly

## Application

### Single Outbreaks

### Outbreaks Over Space and Time

## Conclusion

### Take-Home

# Recurrent “Finds” of Medfly in Free Areas

PROCEEDINGS  
— OF —  
THE ROYAL  
SOCIETY **B**

[rspb.royalsocietypublishing.org](http://rspb.royalsocietypublishing.org)

Research



**Cite this article:** Papadopoulos NT, Plant RE, Carey JR. 2013 From trickle to flood: the large-scale, cryptic invasion of California by tropical fruit flies. *Proc R Soc B* 280: 20131466. <http://dx.doi.org/10.1098/rspb.2013.1466>

Received: 10 June 2013

Accepted: 10 July 2013

## Subject Areas:

ecology, environmental science, evolution

## Keywords:

Tephritidae, invasion biology, subdetectable populations, eradication

## Author for correspondence:

James R. Carey

e-mail: [jrcarey@ucdavis.edu](mailto:jrcarey@ucdavis.edu)

## From trickle to flood: the large-scale, cryptic invasion of California by tropical fruit flies

Nikos T. Papadopoulos<sup>1</sup>, Richard E. Plant<sup>2</sup> and James R. Carey<sup>3,4</sup>

<sup>1</sup>Laboratory of Entomology and Agricultural Zoology, School of Agricultural Sciences, University of Thessaly, Phytohy Street 38446 N. Ionia (Volos), Magnisia, Greece

<sup>2</sup>Department of Plant Sciences and Biological and Agricultural Engineering, and <sup>3</sup>Department of Entomology, University of California, Davis, CA 95616, USA

<sup>4</sup>Center for the Economics and Demography of Aging, University of California, Berkeley, CA 94720, USA

Since 1954, when the first tropical tephritid fruit fly was detected in California, a total of 17 species in four genera and 11 386 individuals (adults/larvae) have been detected in the state at more than 3348 locations in 330 cities. We conclude from spatial mapping analyses of historical capture patterns and modelling that, despite the 250+ emergency eradication projects that have been directed against these pests by state and federal agencies, a minimum of five and as many as nine or more tephritid species are established and widespread, including the Mediterranean, Mexican and oriental fruit flies, and possibly the peach, guava and melon fruit flies. We outline and discuss the evidence for our conclusions, with particular attention to the incremental, chronic and insidious nature of the invasion, which involves ultra-small, barely detectable populations. We finish by considering the implications of our results for invasion biology and for science-based invasion policy.

## 1. Introduction

Tropical fruit flies (Tephritidae), such as the Mediterranean fruit fly (*Ceratitis capitata*) from Africa, the oriental fruit fly (*Bactrocera dorsalis*) from Asia and the Mexican fruit fly (*Anastrepha ludens*) from the Americas, are recognized by entomologists as among the most destructive agricultural insect pests in the world [1,2]. Because of tephritids' economic importance, US states such as California—considered by both the US Department of Agriculture (USDA) and the California Department of Food and Agriculture (CDFA) to be free of these pests, but with climates favourable to their establishment—invest heavily in measures to keep tephritids from becoming established. These steps include restricting importation of commodities that originate in regions with ongoing tephritid outbreaks, requiring post-harvest treatments for imported fruits and vegetables grown in areas where the pests are endemic or established, maintaining large-scale monitoring programmes for early detection, supporting preventive release programmes of sterile flies to pre-empt establishment, and launching eradication campaigns to eliminate pest populations once discovered.



## Question: How long to maintain Quarantine?



## NYT Retro Report

## Specific Possibilities



## “WHAT IF...”

And the VisiCalc program lets you take full advantage of the IBM® Personal Computer's memory expansion capability. You can create worksheets six times larger than those possible at the 64K level. So you can solve even the biggest

"What if? ... just change any number in your problem and instantly, the VisiCalc program recalculates all the numbers and displays the new results. So you can readily analyze the

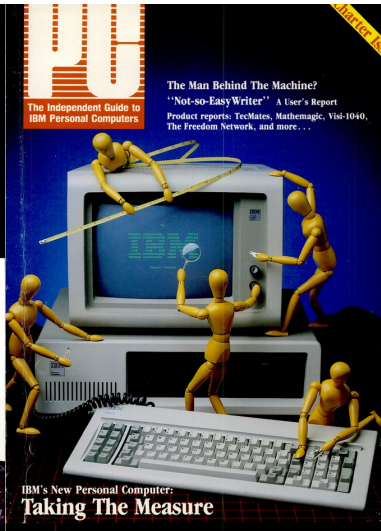
**VISICALC FROM VISICORP**  
PARAGRAPH TECHNOLOGY

VisiCalc  
VisiDraw  
VisiText  
VisiForm



*PC Magazine* Feb/Mar 1982

VISICALC FROM  
VISICORP<sup>®</sup>  
PERSONAL. SCOTTSMILE<sup>®</sup>



# Specific Possibilities

## Why?

- Fly biology varies
- Outbreaks vary
- Response varies
- Time/location  $\implies$  variation in climate/weather

# Specific Possibilities

## Why?

- Fly biology varies
- Outbreaks vary
- Response varies
- Time/location  $\implies$  variation in climate/weather

**We will use our model of invasive Medfly outbreaks to play out millions of “what ifs”**

# Outline

## Motivation

Testing Scenarios

Estimating Quarantine Length

## Methods

What is an ABS?

Simulating a Medfly

## Application

Single Outbreaks

Outbreaks Over Space and Time

## Conclusion

Take-Home

# Thermal Unit Accumulation Model

QL: 3 generations of Degree-day ( $^{\circ}D$ ) development

- Min and max temperature for development to occur ( $T_{min}$ ,  $T_{max}$ )
- $1^{\circ}D = 24$  h with the temperature  $1^{\circ}$  above  $T_{min}$
- Each stage will have a required amount of  $^{\circ}D$  for transition to the next stage

# Thermal Unit Accumulation Model

QL: 3 generations of Degree-day ( $^{\circ}D$ ) development

- Min and max temperature for development to occur ( $T_{min}$ ,  $T_{max}$ )
- $1^{\circ}D = 24$  h with the temperature  $1^{\circ}$  above  $T_{min}$
- Each stage will have a required amount of  $^{\circ}D$  for transition to the next stage



# Thermal Unit Accumulation Model

QL: 3 generations of Degree-day ( $^{\circ}D$ ) development

- Min and max temperature for development to occur ( $T_{min}$ ,  $T_{max}$ )
- $1^{\circ}D = 24$  h with the temperature  $1^{\circ}$  above  $T_{min}$
- Each stage will have a required amount of  $^{\circ}D$  for transition to the next stage

# Thermal Unit Accumulation Model

QL: 3 generations of Degree-day ( $^{\circ}D$ ) development

- Min and max temperature for development to occur ( $T_{min}$ ,  $T_{max}$ )
- $1^{\circ}D = 24$  h with the temperature  $1^{\circ}$  above  $T_{min}$
- Each stage will have a required amount of  $^{\circ}D$  for transition to the next stage

$^{\circ}D$  threshold from laboratory studies:

$$\frac{1}{d} = a + bT$$

$d$  = developmental time in days

$T$  = constant temperature

$a$  = developmental rate at  $0^{\circ}C$

$b$  = slope

$$T_{min} = -a/b$$

$K = 1/b$  = Length of time for stage transition

# Outline

## Motivation

Testing Scenarios

Estimating Quarantine Length

## Methods

**What is an ABS?**

Simulating a Medfly

## Application

Single Outbreaks

Outbreaks Over Space and Time

## Conclusion

Take-Home

# A Bottom-Up Simulation

A computer simulation of a complex system

- Representations of unique, autonomous individuals (“Agents”)
- Closed-form solutions for all behaviors/relations are not necessary
- Observe emergent behavior of a collection of agents

# A Bottom-Up Simulation

A computer simulation of a complex system

- Representations of unique, autonomous individuals (“Agents”)
- Closed-form solutions for all behaviors/relations are not necessary
- Observe emergent behavior of a collection of agents

# A Bottom-Up Simulation

A computer simulation of a complex system

- Representations of unique, autonomous individuals (“Agents”)
- Closed-form solutions for all behaviors/relations are not necessary
- Observe emergent behavior of a collection of agents

# A Bottom-Up Simulation

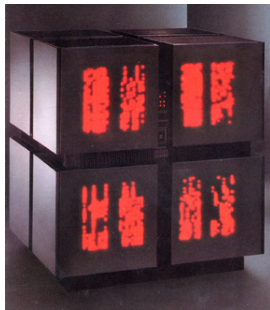
A computer simulation of a complex system

- Representations of unique, autonomous individuals (“Agents”)
- Closed-form solutions for all behaviors/relations are not necessary
- Observe emergent behavior of a collection of agents

# A Bottom-Up Simulation

A computer simulation of a complex system

- Representations of unique, autonomous individuals (“Agents”)
- Closed-form solutions for all behaviors/relations are not necessary
- Observe emergent behavior of a collection of agents



*Connection Machine ~1987, Thinking Machines Corp*



# Key Differences Between ABS and Degree-Day

## Degree Day

- Requires few parameters
- Calculation is quick
- Three generation approach seems to work

## ABS

- Demographically explicit
- More realistic (e.g. mortality)/ specific
- Stochastic
- Extreme flexibility, extensible

# Key Differences Between ABS and Degree-Day

## Degree Day

- Requires few parameters
- Calculation is quick
- Three generation approach seems to work

## ABS

- Demographically explicit
- More realistic (e.g. mortality)/ specific
- Stochastic
- Extreme flexibility, extensible

# Key Differences Between ABS and Degree-Day

## Degree Day

- Requires few parameters
- Calculation is quick
- Three generation approach seems to work

## ABS

- Demographically explicit
- More realistic (e.g. mortality)/ specific
- Stochastic
- Extreme flexibility, extensible

# Key Differences Between ABS and Degree-Day

## Degree Day

- Requires few parameters
- Calculation is quick
- Three generation approach seems to work

## ABS

- Demographically explicit
- More realistic (e.g. mortality)/ specific
- Stochastic
- Extreme flexibility, extensible

# Key Differences Between ABS and Degree-Day

## Degree Day

- Requires few parameters
- Calculation is quick
- Three generation approach seems to work

## ABS

- Demographically explicit
- More realistic (e.g. mortality)/ specific
- Stochastic
- Extreme flexibility, extensible

# Key Differences Between ABS and Degree-Day

## Degree Day

- Requires few parameters
- Calculation is quick
- Three generation approach seems to work

## ABS

- Demographically explicit
- More realistic (e.g. mortality)/ specific
  - Stochastic
  - Extreme flexibility, extensible

# Key Differences Between ABS and Degree-Day

## Degree Day

- Requires few parameters
- Calculation is quick
- Three generation approach seems to work

## ABS

- Demographically explicit
- More realistic (e.g. mortality)/ specific
- Stochastic
- Extreme flexibility, extensible

# Key Differences Between ABS and Degree-Day

## Degree Day

- Requires few parameters
- Calculation is quick
- Three generation approach seems to work

## ABS

- Demographically explicit
- More realistic (e.g. mortality)/ specific
- Stochastic
- Extreme flexibility, extensible



# Outline

## Motivation

Testing Scenarios

Estimating Quarantine Length

## Methods

What is an ABS?

**Simulating a Medfly**

## Application

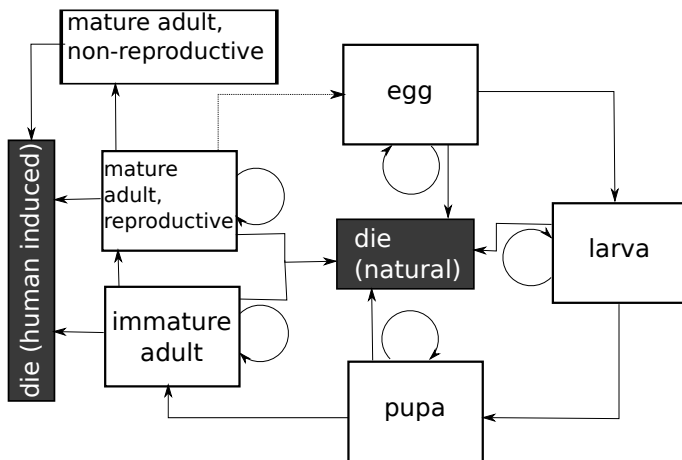
Single Outbreaks

Outbreaks Over Space and Time

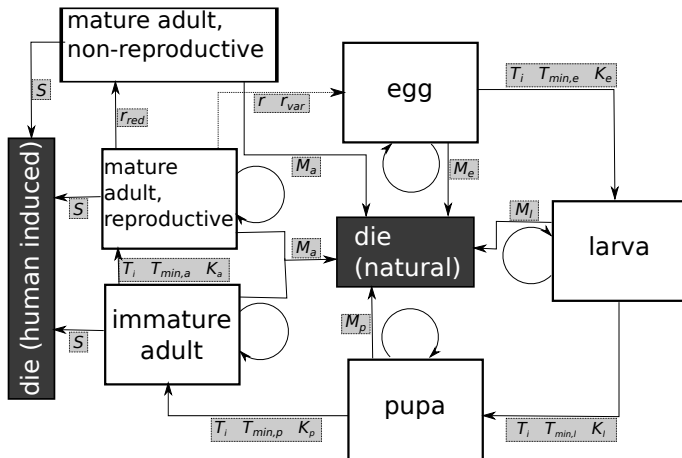
## Conclusion

Take-Home

# Medfly Agent Life Cycle



# Medfly Agent Life Cycle



# Principal Simulation Parameters: the Inputs

Key Input: Hourly temperature data ( $T$ )

Input parameter	Symbol	Input parameter	Symbol
Initial population size	$N_0$	Reproductive output	$r$
Daily mortality	$M_x$	Reproductive variance	$r_{var}$
Base temperature	$T_{min,x}$	Sterilization rate	$r_{red}$
Thermal constant	$K_x$	Human induced mortality	$S$
Developmental model	$Dm$	Time to countermeasures	$t_S$
Variation in development rate	$\gamma$	Initial age distribution	NA

# Initialization

Simulation is *demographically explicit*; it needs numbers of individuals

# Initialization

Simulation is *demographically explicit*; it needs numbers of individuals

- Approximate size of the adult female population?
  - Trapping grid: 5 Jackson traps (Trimedlure) + 5 McPhail traps (protein) per sq mi
  - Trimedlure efficiency 0.6-2.2 % (Cunningham & Couey 1986, Lance & Gates 1994).
  - Protein lure approximately as effective (Katsoyannos et al, 1999; Midgarden et al, 2004; Grout et al, 2011)
  - Overall detection efficiency 1 - 3 %
- Age structure of the population?
  - Use a stable age distribution (Carey 1982, Vargas et al 1997, 2000)

# Initialization

Simulation is *demographically explicit*; it needs numbers of individuals

- Approximate size of the adult female population?
  - Trapping grid: 5 Jackson traps (Trimedlure) + 5 McPhail traps (protein) per sq mi
  - Trimedlure efficiency 0.6-2.2 % (Cunningham & Couey 1986, Lance & Gates 1994).
  - Protein lure approximately as effective (Katsoyannos et al, 1999; Midgarden et al, 2004; Grout et al, 2011)
  - Overall detection efficiency 1 - 3 %
- Age structure of the population?
  - Use a stable age distribution (Carey 1982, Vargas et al 1997, 2000)

# Initialization

Simulation is *demographically explicit*; it needs numbers of individuals

- Approximate size of the adult female population?
  - Trapping grid: 5 Jackson traps (Trimedlure) + 5 McPhail traps (protein) per sq mi
  - Trimedlure efficiency 0.6-2.2 % (Cunningham & Couey 1986, Lance & Gates 1994).
  - Protein lure approximately as effective (Katsoyannos et al, 1999; Midgarden et al, 2004; Grout et al, 2011)
  - Overall detection efficiency 1 - 3 %
- Age structure of the population?
  - Use a stable age distribution (Carey 1982, Vargas et al 1997, 2000)



# Initialization

Simulation is *demographically explicit*; it needs numbers of individuals

- Approximate size of the adult female population?
  - Trapping grid: 5 Jackson traps (Trimedlure) + 5 McPhail traps (protein) per sq mi
  - Trimedlure efficiency 0.6-2.2 % (Cunningham & Couey 1986, Lance & Gates 1994).
  - Protein lure approximately as effective (Katsoyannos et al, 1999; Midgarden et al, 2004; Grout et al, 2011)
  - Overall detection efficiency 1 - 3 %
- Age structure of the population?
  - Use a stable age distribution (Carey 1982, Vargas et al 1997, 2000)

# Initialization

Simulation is *demographically explicit*; it needs numbers of individuals

- Approximate size of the adult female population?
  - Trapping grid: 5 Jackson traps (Trimedlure) + 5 McPhail traps (protein) per sq mi
  - Trimedlure efficiency 0.6-2.2 % (Cunningham & Couey 1986, Lance & Gates 1994).
  - Protein lure approximately as effective (Katsoyannos et al, 1999; Midgarden et al, 2004; Grout et al, 2011)
  - Overall detection efficiency 1 - 3 %
- Age structure of the population?
  - Use a stable age distribution (Carey 1982, Vargas et al 1997, 2000)

# Initialization

Simulation is *demographically explicit*; it needs numbers of individuals

- Approximate size of the adult female population?
  - Trapping grid: 5 Jackson traps (Trimedlure) + 5 McPhail traps (protein) per sq mi
  - Trimedlure efficiency 0.6-2.2 % (Cunningham & Couey 1986, Lance & Gates 1994).
  - Protein lure approximately as effective (Katsoyannos et al, 1999; Midgarden et al, 2004; Grout et al, 2011)
  - Overall detection efficiency 1 - 3 %
- Age structure of the population?
  - Use a stable age distribution (Carey 1982, Vargas et al 1997, 2000)

# Development

for all  $T_i < T_{max}$ , When

$$C + \gamma < \sum_{t=0}^i T_i - T_{min}$$

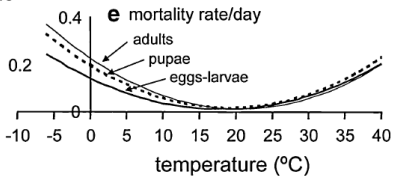
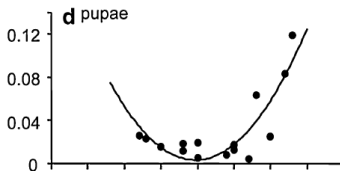
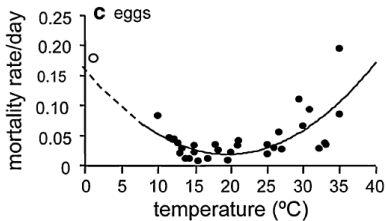
stage transition occurs.

$i$  = current time

$C$  = stage-specific thermal units needed for transition to next stage

$\gamma$  = individual variation in development time

# Mortality



From Gutierrez & Ponti (2011)



# Reproduction

Each fly lays eggs every 24 h prior to intervention ( $t_S$ ). After intervention number of reproductive flies drops due to SIT.

# Reproduction

Each fly lays eggs every 24 h prior to intervention ( $t_S$ ). After intervention number of reproductive flies drops due to SIT.

- $r$  Mean number of eggs
- $r_{var}$  Variance in the number of eggs
- $r_{red}$  Reduction in reproductive flies per unit time



# Parameter Value Ranges

---

Input parameter	Range (min-max)	References
-----------------	-----------------	------------

---

# Parameter Value Ranges

Input parameter	Range (min-max)	References
$N_0$	33 – 100	1–4

# Parameter Value Ranges

Input parameter	Range (min-max)	References
$N_0$	33 – 100	1–4
$M_e$	0.0198–0.1200	5–11
$M_l$	0.0068–0.0946	5–10
$M_p$	0.0016–0.0465	5–10, 19
$M_a$	0.0245–0.1340	7,12,13,20,21

# Parameter Value Ranges

Input parameter	Range (min-max)	References
$N_0$	33 – 100	1–4
$M_e$	0.0198–0.1200	5–11
$M_l$	0.0068–0.0946	5–10
$M_p$	0.0016–0.0465	5–10, 19
$M_a$	0.0245–0.1340	7,12,13,20,21
$S$	0.005–0.050	

# Parameter Value Ranges

Input parameter	Range (min-max)	References
$N_0$	33 – 100	1–4
$M_e$	0.0198–0.1200	5–11
$M_l$	0.0068–0.0946	5–10
$M_p$	0.0016–0.0465	5–10, 19
$M_a$	0.0245–0.1340	7,12,13,20,21
$S$	0.005–0.050	
$T_{min,e}$	9.6–12.5	5–8,11
$K_e$	27.27–33.80	5–8

# Parameter Value Ranges

Input parameter	Range (min-max)	References
$N_0$	33 – 100	1–4
$M_e$	0.0198–0.1200	5–11
$M_l$	0.0068–0.0946	5–10
$M_p$	0.0016–0.0465	5–10, 19
$M_a$	0.0245–0.1340	7,12,13,20,21
$S$	0.005–0.050	
$T_{min,e}$	9.6–12.5	5–8,11
$K_e$	27.27–33.80	5–8
$T_{min,l}$	5.0–10.8	5–8
$K_l$	94.50–186.78	5–8
$T_{min,p}$	9.1–13.8	5–7
$K_p$	123.96–169.49	5–7
$T_{min,a}$	7.9–9.9	5,6,13
$K_a$	58.20–105.71	5,6,13

# Parameter Value Ranges

Input parameter	Range (min-max)	References
$N_0$	33 – 100	1–4
$M_e$	0.0198–0.1200	5–11
$M_l$	0.0068–0.0946	5–10
$M_p$	0.0016–0.0465	5–10, 19
$M_a$	0.0245–0.1340	7,12,13,20,21
$S$	0.005–0.050	
$T_{min,e}$	9.6–12.5	5–8,11
$K_e$	27.27–33.80	5–8
$T_{min,l}$	5.0–10.8	5–8
$K_l$	94.50–186.78	5–8
$T_{min,p}$	9.1–13.8	5–7
$K_p$	123.96–169.49	5–7
$T_{min,a}$	7.9–9.9	5,6,13
$K_a$	58.20–105.71	5,6,13
$r$	5.0–35.0	1,2,7,13,14–18
$r_{red}$	0.5–1.0	22,23

# Outline

## Motivation

Testing Scenarios

Estimating Quarantine Length

## Methods

What is an ABS?

Simulating a Medfly

## Application

Single Outbreaks

Outbreaks Over Space and Time

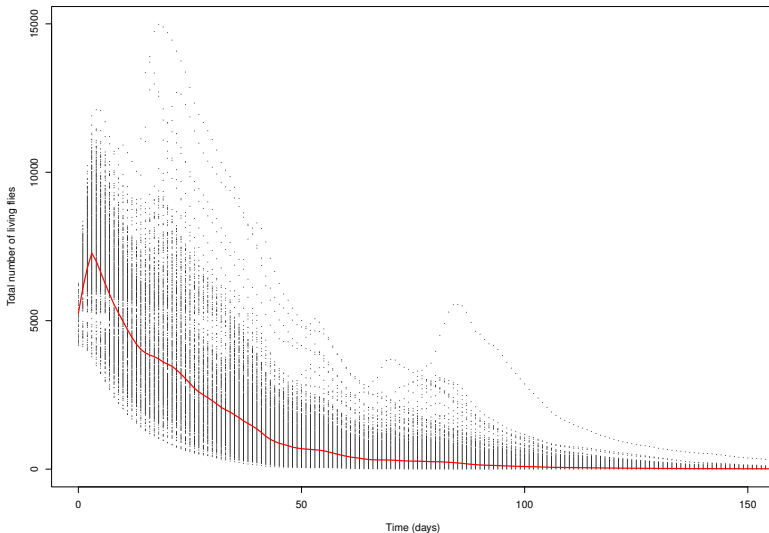
## Conclusion

Take-Home



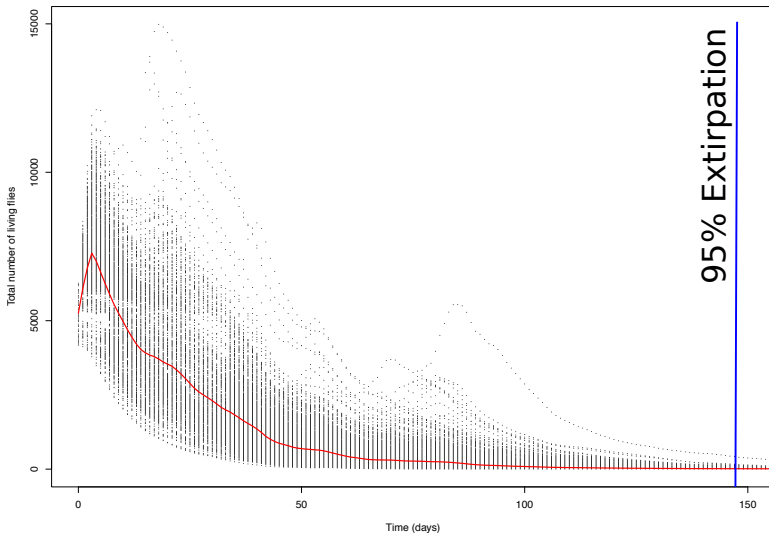
# Santa Monica Outbreak

1000 simulations of 2009 outbreak, daily numbers with mean



# Santa Monica Outbreak

1000 simulations of 2009 outbreak, daily numbers with mean



# Seven Outbreaks in CA

10,000 simulations per outbreak

# Seven Outbreaks in CA

10,000 simulations per outbreak

Outbreak	$N$ adults	QL (d)	$t_{0.95}$ (d)	$t_{0.95} - \text{QL}$
Santa Monica	3	297	231	-66
Fallbrook	4	273	206	-67
Spring Valley	2	204	200	-4
Imperial beach	1	145	172	+27
Mira mesa	6	166	181	+15
Escondido	7	337	167	-170
Cajon	21	245	227	-18
<b>Total</b>		1667	1384	-283

# General Agreement

## Interesting Differences in Details

- ABS within 20 d of DD in 3 of 7 outbreaks
- In one case (Imperial Beach) ABS suggests significantly longer quarantine
- ABS suggests significantly shorter quarantine in 3 of 7 cases
- In Escondido (09/2009) case, much shorter quarantine is suggested by ABS (170 d shorter!) Reason:

# General Agreement

## Interesting Differences in Details

- **ABS within 20 d of DD in 3 of 7 outbreaks**
- In one case (Imperial Beach) ABS suggests significantly longer quarantine
- ABS suggests significantly shorter quarantine in 3 of 7 cases
- In Escondido (09/2009) case, much shorter quarantine is suggested by ABS (170 d shorter!) Reason:

# General Agreement

## Interesting Differences in Details

- ABS within 20 d of DD in 3 of 7 outbreaks
- In one case (Imperial Beach) ABS suggests significantly longer quarantine
- ABS suggests significantly shorter quarantine in 3 of 7 cases
- In Escondido (09/2009) case, much shorter quarantine is suggested by ABS (170 d shorter!) Reason:

# General Agreement

## Interesting Differences in Details

- ABS within 20 d of DD in 3 of 7 outbreaks
- In one case (Imperial Beach) ABS suggests significantly longer quarantine
- ABS suggests significantly shorter quarantine in 3 of 7 cases
- In Escondido (09/2009) case, much shorter quarantine is suggested by ABS (170 d shorter!) Reason:



# General Agreement

## Interesting Differences in Details

- ABS within 20 d of DD in 3 of 7 outbreaks
- In one case (Imperial Beach) ABS suggests significantly longer quarantine
- ABS suggests significantly shorter quarantine in 3 of 7 cases
- In Escondido (09/2009) case, much shorter quarantine is suggested by ABS (170 d shorter!) Reason:

# General Agreement

## Interesting Differences in Details

- ABS within 20 d of DD in 3 of 7 outbreaks
- In one case (Imperial Beach) ABS suggests significantly longer quarantine
- ABS suggests significantly shorter quarantine in 3 of 7 cases
- In Escondido (09/2009) case, much shorter quarantine is suggested by ABS (170 d shorter!) Reason:

Date	Time	$T(^{\circ}C)$
11/16/09	2400	3.7
11/17/09	0100	2.4
11/17/09	0200	0.9
11/17/09	0300	0.4
11/17/09	0400	-0.3
11/17/09	0500	-0.4
11/17/09	0600	-1.1
11/17/09	0700	-0.8

# Outline

## Motivation

Testing Scenarios

Estimating Quarantine Length

## Methods

What is an ABS?

Simulating a Medfly

## Application

Single Outbreaks

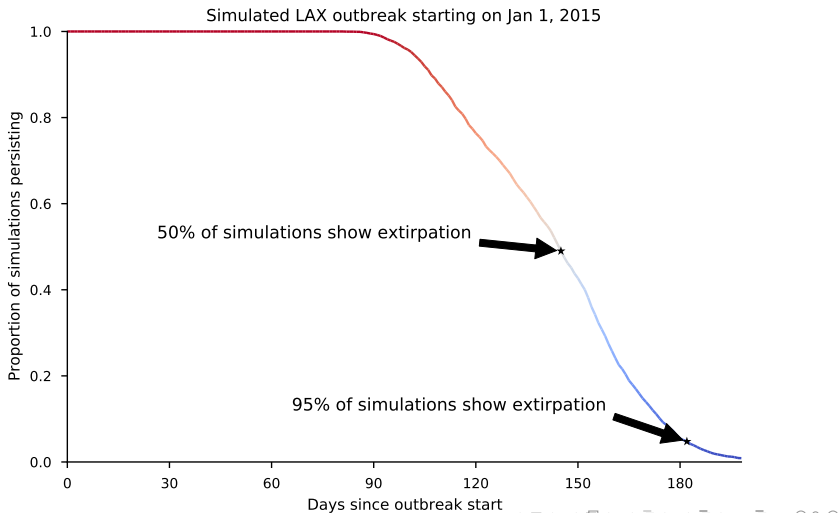
Outbreaks Over Space and Time

## Conclusion

Take-Home

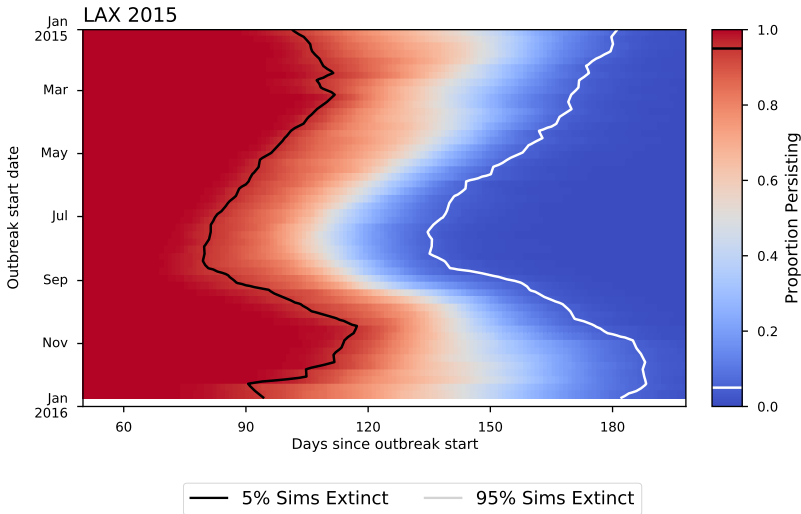
# Hypothetical Single Outbreak

Visualizing 2,500 simulations with varying parameters



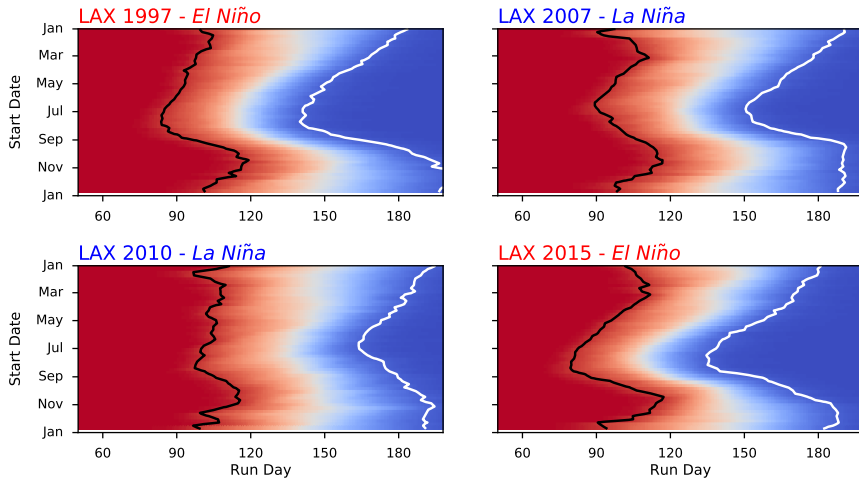
# Hypothetical Outbreaks at Different Times

2,500 outbreaks starting at each week of the year



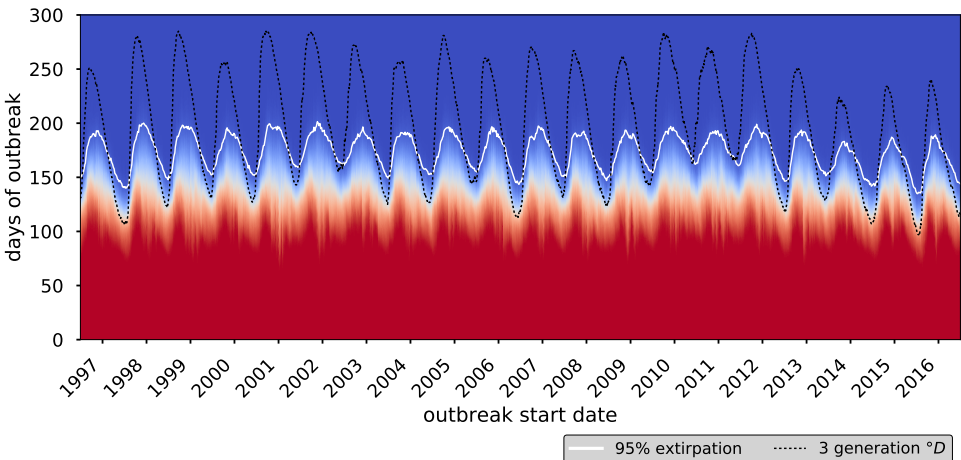
# Climate Variability

## Inter-year variation



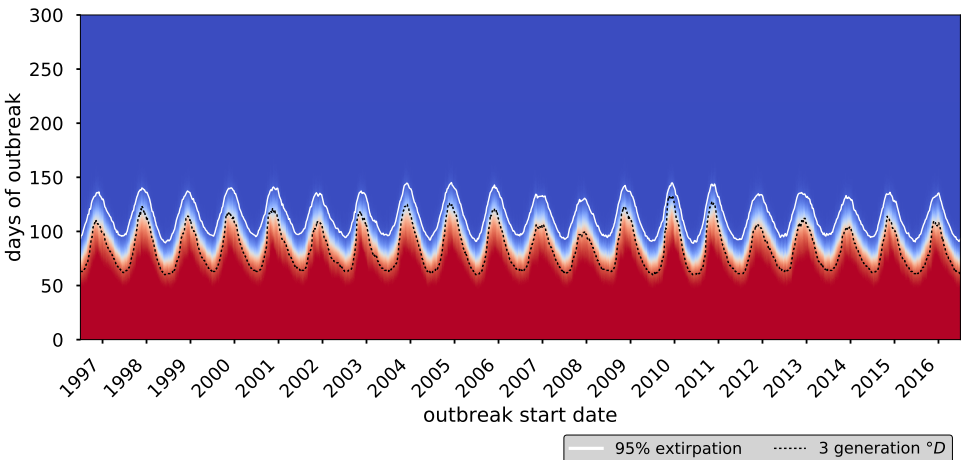
# $5.2 \times 10^6$ Simulations at Hourly Resolution

## LAX - Outbreak Persistence



# $5.2 \times 10^6$ Simulations at Hourly Resolution

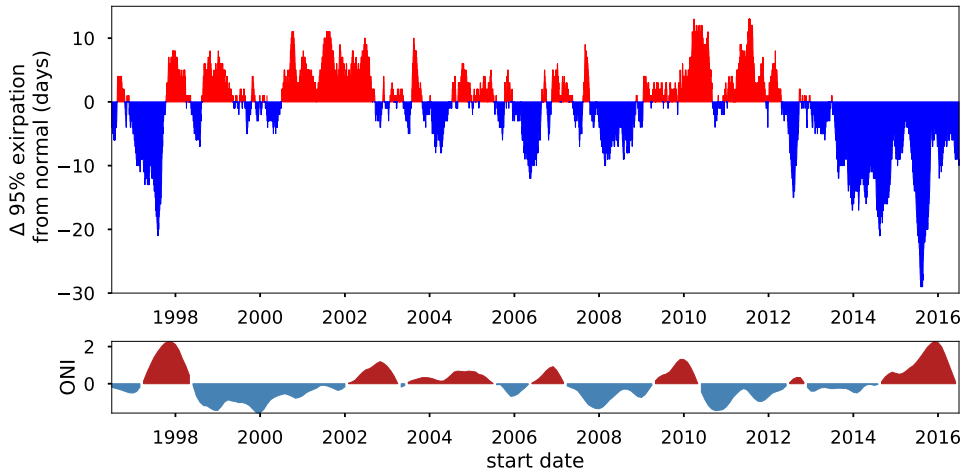
## MIA - Outbreak Persistence





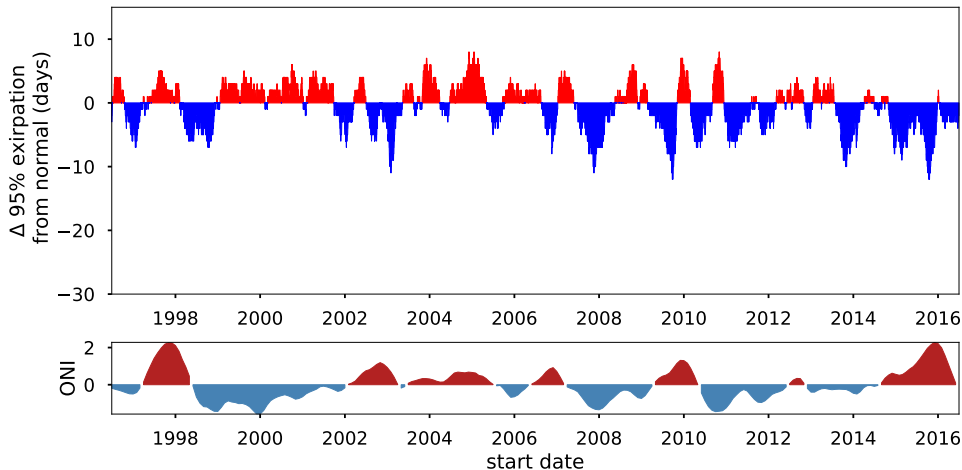
# $5.2 \times 10^6$ Simulations at Hourly Resolution

## LAX - Normals and ONI



# $5.2 \times 10^6$ Simulations at Hourly Resolution

## MIA - Normals and ONI



# Outline

## Motivation

Testing Scenarios

Estimating Quarantine Length

## Methods

What is an ABS?

Simulating a Medfly

## Application

Single Outbreaks

Outbreaks Over Space and Time

## Conclusion

Take-Home

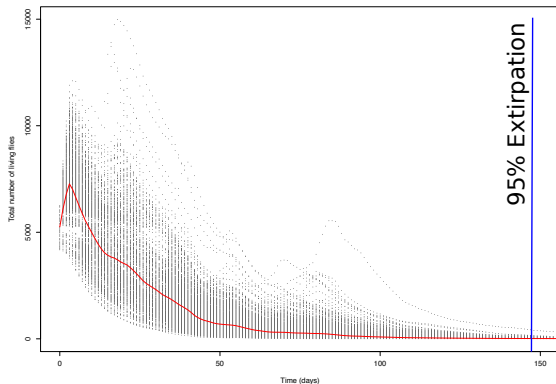
# Take-Home Message

Two outcomes to remember

# Take-Home Message

Two outcomes to remember

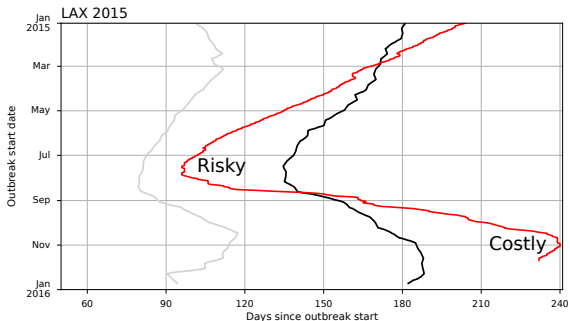
1. We simulate range of outcomes for a specific outbreak of *C. capitata*; Useful for quarantine length
2. We can ask “What if” questions across time and space to help planning



# Take-Home Message

Two outcomes to remember

1. We simulate range of outcomes for a specific outbreak of *C. capitata*; Useful for quarantine length
2. We can ask “What if” questions across time and space to help planning



— Degree-Day — 5% Sims Extinct — 95% Sims Extinct

This work was funded by USDA-ARS

