Insect Outbreaks, Host-Pathogen Interactions, and Induced Plant Defenses

Bret D. Elderd[1]
Department of Biological Sciences
Louisiana State University
Baton Rouge, Louisiana, USA

Brian J. Rehill[2]
Chemistry Department
United States Naval Academy
Annapolis, Maryland, USA

Greg Dwyer[3]
Department of Ecology and Evolution
University of Chicago
Chicago, Illinois, USA

September 30, 2009

[1]elderd@lsu.edu
[2]rehill@usna.edu
[3]Corresponding author: gdwyer@uchicago.edu

Report Documentation Page

Form Approved
OMB No. 0704-0188

Public reporting burden for the collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to a penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number.

1. REPORT DATE **30 SEP 2009**	2. REPORT TYPE	3. DATES COVERED **00-00-2009 to 00-00-2009**
4. TITLE AND SUBTITLE **Insect Outbreaks, Host-Pathogen Interactions, and Induced Plant Defenses**		5a. CONTRACT NUMBER
		5b. GRANT NUMBER
		5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)		5d. PROJECT NUMBER
		5e. TASK NUMBER
		5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) **United States Naval Academy (USNA),Chemistry Department,Annapolis,MD,21402**		8. PERFORMING ORGANIZATION REPORT NUMBER
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)		10. SPONSOR/MONITOR'S ACRONYM(S)
		11. SPONSOR/MONITOR'S REPORT NUMBER(S)
12. DISTRIBUTION/AVAILABILITY STATEMENT **Approved for public release; distribution unlimited**		
13. SUPPLEMENTARY NOTES		
14. ABSTRACT		
15. SUBJECT TERMS		

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT **unclassified**	b. ABSTRACT **unclassified**	c. THIS PAGE **unclassified**	**Same as Report (SAR)**	**12**	

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std Z39-18

Ecologists have long debated whether insect-herbivore population dynamics are driven by "top-down" forces[1], such as predators and pathogens, or "bottom-up" forces[2], such as induced plant defenses. In the case of outbreaking forest insects, theoretical ecologists have argued that top-down forces are most important[3], because mathematical models that incorporate only predators and pathogens explain time series of outbreaks from the field[4]. Proponents of bottom-up forces concede that induced plant defenses often have only weak direct effects, but nevertheless argue that the models that do not consider plant quality are inaccurate because they do not include interactions between induced defenses and insect pathogens[5,6]. Such interactions reduce average infection risk in the laboratory[7], and should therefore reduce infection rates during outbreaks in nature, when induced defenses are highest[8], but data from the field have shown that infection rates instead peak during outbreaks[9–11]. Here we reconcile models, experiments, and field data, first by using a field experiment to show that, in the outbreaking North American gypsy moth, induced plant defenses affect pathogen transmission mainly by reducing variability in infection risk rather than average infection risk, which has the effect of increasing infection rates during outbreaks, not reducing them[12]. We then show that this reduction in variability has important implications for insect population dynamics, because models in which variability in pathogen infection risk is determined by induced plant defenses produce realistic outbreaks, whereas standard models, when accurately parameterized, instead predict unrealistic stable population dynamics. Our work makes clear that top-down and bottom-up forces may interact in complex ways, contrary to traditional either-or viewpoints, and suggests that induction of plant defenses may augment the effectiveness of baculoviruses in reducing the damage caused by forest pests[13].

Pathogens of outbreaking insects are often baculoviruses, fatal diseases transmitted when host larvae accidentally consume foliage contaminated by virus from infectious cadavers of other larvae[14]. Infection occurs as a result of virus consumption, and so plant defensive compounds consumed together with infectious viral particles can alter the infection process within the insect[6]. Consumption of plant defensive compounds has therefore been shown

to reduce average infection risk in the laboratory[6]. Because defensive compounds are often induced by defoliation[15], and because defoliation and thus induced-defense concentrations increase with insect densities[8], the laboratory data imply that baculovirus infection rates in the field should decline with increasing insect density[7]. In nature, however, infection rates instead increase with density, causing population collapses at outbreak peaks[9–11]. One explanation for this contradiction is that conditions in laboratory experiments differ greatly from those in nature[6], and laboratory experiments therefore may be irrelevant to processes in nature[16]. To date, however, there have been no convincing experimental tests of whether induced defenses affect baculovirus transmission in the field.

To carry out such a test, we used the gypsy moth (*Lymantria dispar*), its baculovirus, and one of the gypsy moth's main host trees in North America, the red oak, *Quercus rubra*[17]. A previous study relied on experimental defoliation, without successfully causing induction[18]. It therefore appears that defoliation must be quite severe for induction to occur, yet severe defoliation would remove so much leaf material that it would be impossible to measure virus transmission in the field. Accordingly, we instead induced red oak defenses by spraying branches with jasmonic acid or "JA"[19]. JA is a plant-signalling compound that in seedling red oaks increases hydrolyzable tannin concentrations[20], an induced defense[8] that strongly affects average gypsy-moth infection risk in the laboratory[21]. Reassuringly, our JA treatment induced hydrolyzable tannins to the same extent as defoliation in nature[8] (Table 1, note that control branches were sprayed with an identical solution that lacked JA, and that induction affected only sprayed branches, not entire trees, see Supplemental Information). We then mimicked natural transmission by first adding virus-infected larvae to the branches, allowing the larvae to die, and then allowing uninfected larvae to feed on the branches for one week, a period of time short enough to ensure only one round of transmission (see Supplemental Information for details).

This experiment showed that the main effect of induction is that it reduces variability in infection risk. To see this, we used the mathematical theory of epidemics[12] to quantify the effects of induction on average infection risk and variability in infection risk in our experiment. According to the theory, the fraction infected i in our experiments can be expressed as,

$$-\log(1-i) = \frac{1}{C^2}\log\left(1+C^2\bar{\nu}P_0T\right). \quad (1)$$

Here T is the length of the experiment, and P_0 is the virus density, which is constant in our experiments. By fitting equation (1) to our data, we estimated $\bar{\nu}$, the average infection risk, meaning the average instantaneous transmission rate, and C, the variability in the infection risk, meaning the coefficient of variation of the distribution of transmission rates. Variability in infection risk is thus expressed as a distribution of transmission rates, such that some hosts have high risk and others have low risk, following a distribution with mean $\bar{\nu}$ and coefficient of variation C. To see the effects of induction on variability in infection risk, note that when variability $C > 0$, equation (1) predicts that $-\log(1-i)$ is a nonlinear function of virus density, whereas when $C \to 0$ we have $-\log(1-i) = \bar{\nu}P_0T$, so that $-\log(1-i)$ is a linear function of virus density.

The reduction in variability in infection risk due to induction is then visually apparent in our data, in that transmission on non-induced branches is a nonlinear function of virus density (fig. 1A), while transmission on induced branches is linear (fig. 1B). Induction thus reduced variability in infection risk C from a high level to near zero (fig. 1C), but the reduction in average infection risk was relatively small. Average infection risk and variability in infection risk were similarly lower on induced foliage in the laboratory (Supplementary Information), suggesting that the effects seen in our field experiment were partly due to changes in innate susceptibility. Feeding rates were also higher on induced foliage (Supplementary Information), however, and differences in variability between treatments in the laboratory were much lower than in the field, emphasizing that induction can affect transmission in nature through multiple mechanisms.

Our field experiment thus showed that induced defenses can affect baculovirus transmission in nature in ways not apparent in laboratory experiments. To understand the consequences of our experimental results for baculovirus epidemics and insect outbreaks, we used our estimates of average infection risk $\bar{\nu}$ and variability in infection risk C in mathematical models (Supplemental Information). First, a standard result from the theory of epidemics states that reductions in variability in infection risk can lead to increased epidemic intensity[12]. Accordingly, inserting our estimates of average infection risk $\bar{\nu}$ and variability C into an epidemic model shows that, rather than reducing the virus's ability to cause population collapse, the main effect of induced defenses is to cause higher infection rates at high density (fig. 1D),

in agreement with data from epidemics in both gypsy moths[10] and other forest-defoliating insects[9,11].

To understand the consequences of this effect for outbreaks, we modified existing outbreak models to allow for induced defenses (Supplementary Information). In current models, outbreaks are terminated by epidemics of a specialist pathogen, such as a baculovirus, while inter-outbreak populations are held in check by generalist predators or parasitoids[4,22]. With the inclusion of environmentally driven stochasticity, the models can show long-period, large-amplitude cycles that recur at irregular intervals, matching the key features of outbreaks in nature. It is often the case, however, that models that incorporate the wrong mechanism can accurately reproduce observational data[23], and so experimental tests of the models are crucial. In particular, the models only show realistic outbreaks for intermediate values of variability in infection risk C, but our data show that, on non-induced foliage, variability $C > 1$, which is high enough to guarantee that the host population in the models approaches a stable, point equilibrium (fig. 2A), whereas on induced foliage $C << 1$, which is low enough to produce unstable oscillations in the models (not shown, see Dwyer, et al. 2000). Neither a stable equilibrium nor unstable oscillations is consistent with the occurrence of regular outbreaks, and our data therefore show that existing models have an important flaw. If we instead allow induced plant defenses to drive changes in variability, however, the resulting models produce stable cycles with a long period and a large amplitude (fig. 2B), and allowing for generalist predators and stochasticity produces cycles that are as irregular as outbreaks in nature (fig. 2C, D). Induced defenses may thus play a crucial role in driving outbreaks, by reducing the stabilizing effects of variability in infection risk enough to allow cycles to occur (the evolution of host resistance can play a similar role[24], and we suspect that both mechanisms are important in nature).

The theory of insect population dynamics, whether classical[25,26] or recent[4,22], has long focused on natural enemies, but our data have demonstrated that natural enemies may interact with resource quality in ways that have a significant effect on population dynamics. Meanwhile, inferences about the effects of induced defenses that rely only on laboratory experiments have led to conclusions that are contradicted by observations from the field[27], but basing models on field experiments has instead allowed us to reconcile experimental and ob-

servational data. More concretely, baculoviruses are widely used to control pest populations of forest insects[13], and previous work implied that induced defenses would interfere with control by reducing average infection risk[7]. In contrast, our work suggests that induced defenses can increase infection rates and thus enhance control by reducing variability in infection risk. A realistic theory of insect population dynamics, which incorporates interactions between host plants and natural enemies, may thus have an important role to play in forest-pest management.

References

1. Hairston, N. G., Smith, F. E., and Slobodkin, L. B. The American Naturalist **94**(879), 421–425 (1960).

2. Hunter, M. D. and Price, P. W. Ecology **73**, 724–732 (1992).

3. Liebhold, A. and Kamata, N. Population Ecology **42**, 205–209 (2000).

4. Dwyer, G., Dushoff, J., and Yee, S. H. Nature **430**, 341–345 (2004).

5. Foster, M. A., Schultz, J. C., and Hunter, M. D. Journal of Animal Ecology **61**, 509–520 (1992).

6. Cory, J. S. and Hoover, K. Trends In Ecology & Evolution **21**(5), 278–286 (2006).

7. Hunter, M. D. and Schultz, J. C. Oecologia **94**(2), 195–203 May (1993).

8. Schultz, J. and Baldwin, I. T. Science **217**(4555), 149–150 (1982).

9. Moreau, G., Lucarotti, C. J., Kettela, E. G., Thurston, G. S., Holmes, S., Weaver, C., Levin, D. B., and Morin, B. Biological Control **33**, 65–73 (2005).

10. Woods, S. A. and Elkinton, J. S. Journal Of Invertebrate Pathology **50**, 151–157 (1987).

11. Otvos, I. S., Cunningham, J. C., and Friskie, L. M. Canadian Entomologist **119**, 697–706 (1987).

12. Anderson, R. M. and May, R. M. Infectious diseases of humans: Dynamics and control. Oxford University Press, (1991).

13. Moreau, G. and Lucarotti, C. J. Forestry Chronicle **83**, 105–112 (2007).

14. Cory, J. S. and Myers, J. H. Annual Reviews of Ecology and Systematics **34**, 239–272 (2003).

15. Karban, R. and Baldwin, I. T. Induced responses to herbivory. University of Chicago Press, Chicago, IL, New York, (1997).

16. Dwyer, G., Firestone, J., and Stevens, T. E. The American Naturalist **165**, 16–31 (2005).

17. Barbosa, P. and Krischik, V. A. The American Naturalist **130**, 53–69 (1987).

18. D'Amico, V., Elkinton, J., Dwyer, G., Willis, R., and Montgomery, M. E. Ecology **79**, 1104–1110 (1998).

19. Baldwin, I. T. Proceedings of the National Academy of the United States of America **95**, 8113–8118 (1998).

20. Allison, S. D. and Schultz, J. C. Journal Of Chemical Ecology **30**(7), 1363–1379 (2004).

21. Keating, S. T., McCarthy, W. J., and Yendol, W. G. Journal of Invertebrate Pathology **54**, 165–174 (1989).

22. Bjornstad, O. N., Christelle, R., and Liebhold, A. M. Ecology **in press** (2009).

23. Box, G. E. P. In Robustness in Statistics, Launer, R. L. and Wilkinson, G. N., editors, 202–236. Academic Press (1979).

24. Elderd, B., Dushoff, J., and Dwyer, G. The American Naturalist **172**(6), 829–842 (2008).

25. Anderson, R. M. and May, R. M. Science **210**, 658–661 (1980).

26. Varley, G. C., Gradwell, G. R., and Hassell, M. P. Insect population ecology: an analytical approach. Blackwell Scientific Publications: Oxford, (1973).

27. Schultz, J. C., Foster, M. A., and Montgomery, M. E. In Population dynamics of forest insects, Watt, A. D., Leather, S. R., Hunter, M. D., and Kidd, N. A. C., editors, 621–637. Intercept Ltd (1990).

28. Dwyer, G., Dushoff, J., Elkinton, J. S., and Levin, S. A. The American Naturalist **156**, 105–120 (2000).

29. Johnson, D. M., Liebhold, A. M., and Bjornstad, O. N. Journal of Animal Ecology **74**, 882–892 (2005).

Induction method	Treatment	Pre-treatment conc'n (%)	Post-treatment conc'n (%)
Jasmonic-acid spray	Spray	19.72 ± 0.82	27.80 ± 0.82
	Control	20.74 ± 0.98	18.09 ± 0.62
Natural defoliation[8]	Defoliation	23.30 ± 1.0	27.05 ± 1.4
	Control	23.36 ± 0.9	19.54 ± 0.9

Table 1: Effects of experimental JA spray and natural defoliation on percent hydrolyzable tannin concentration in red-oak foliage. For the JA experiment, the interaction between treatment and week after spraying was statistically significant at the $p < 10^{-4}$ level (see Supplemental Information), and natural defoliation similarly had a statistically significant effect[8]. Pre-treatment concentrations were significantly different (experimentals: $t = 2.70$, df $= 52$, $p = 0.0093$; controls: $t = 2.15$, df $= 49$, $p = 0.0365$) because of natural variability, but post-treatment concentrations were virtually identical within a treatment (treatments: $t = 0.440$, df $= 52$, $p = 0.662$; controls: $t = 1.311$, df $= 49$, $p = 0.1958$).

Figure Legends

Figure 1: Effects of induction on baculovirus transmission and epidemics. AIC analysis (see Supplemental Information) showed that our data provide extremely strong evidence that transmission on control trees (A) is nonlinear, while transmission on induced trees (B) is linear (symbols indicate data, and lines indicate best-fit version of equation (1)). (C) Underlying distributions of transmission rates on control branches (bold line), and induced branches (light line). For clarity here we assume that the distribution of transmission rates is log-normal, but equation (1) makes no distributional assumptions[28]. For induced branches, there is virtually no heterogeneity, and so the distribution is essentially a vertical line. (D) Effects of induction on infection risk in full epidemics, as predicted by inserting parameter estimates from our analyses into an epidemic model.

Figure 2: Host-pathogen models with and without induction, compared to data from gypsy moth outbreaks. The model without induced defenses shows damped oscillations (A), which are inconsistent with outbreaks, whereas the model with induced defenses shows realistic long-period, large-amplitude cycles (B). These cycles are more regular than cycles in nature (C)[29], but allowing for generalist predators[4] produces cycles that are as irregular as cycles in nature (note that the data only give information about the timing of outbreaks, not the amplitude, see Supplementary Information for more details).

Figure 1:

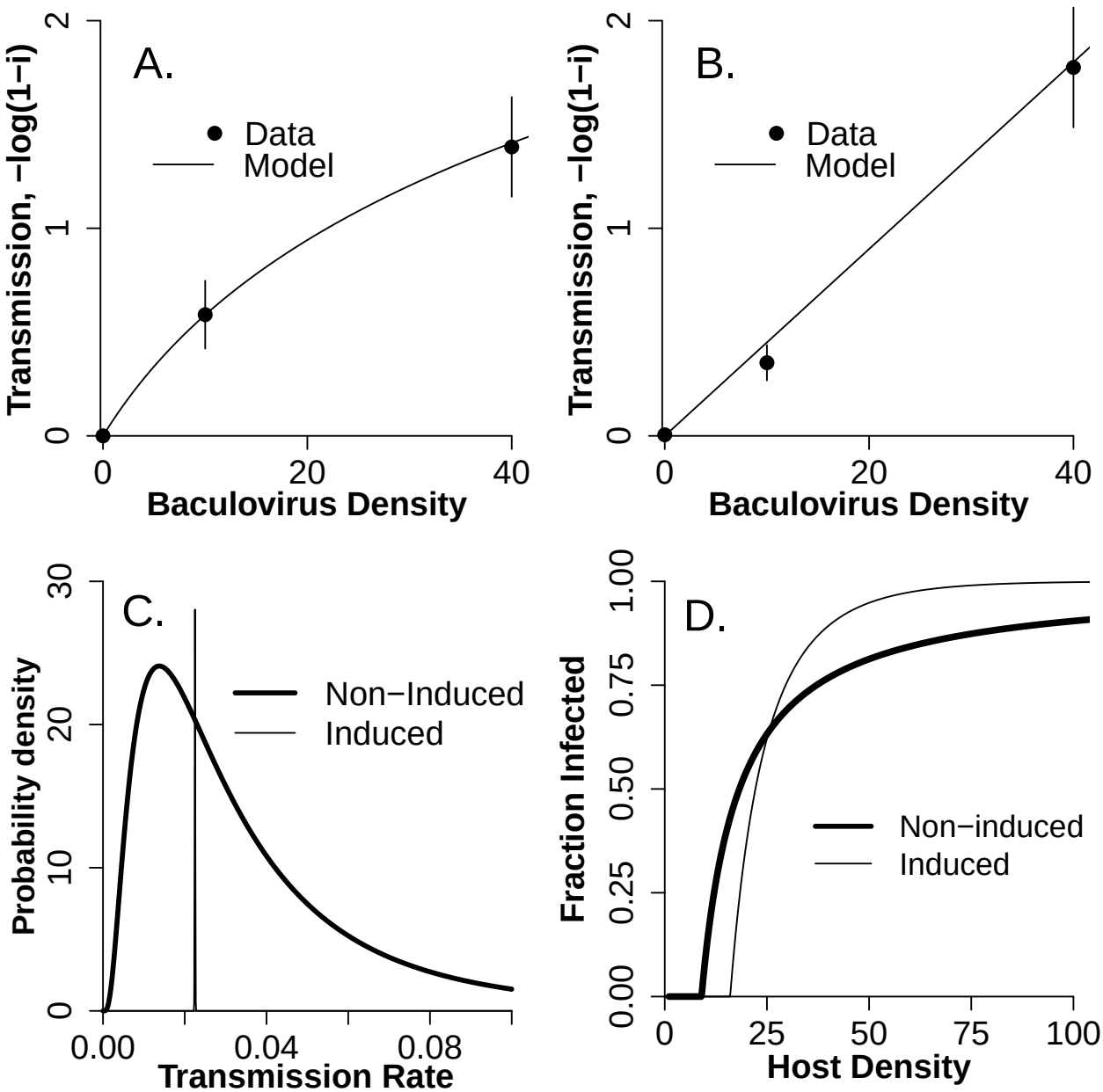
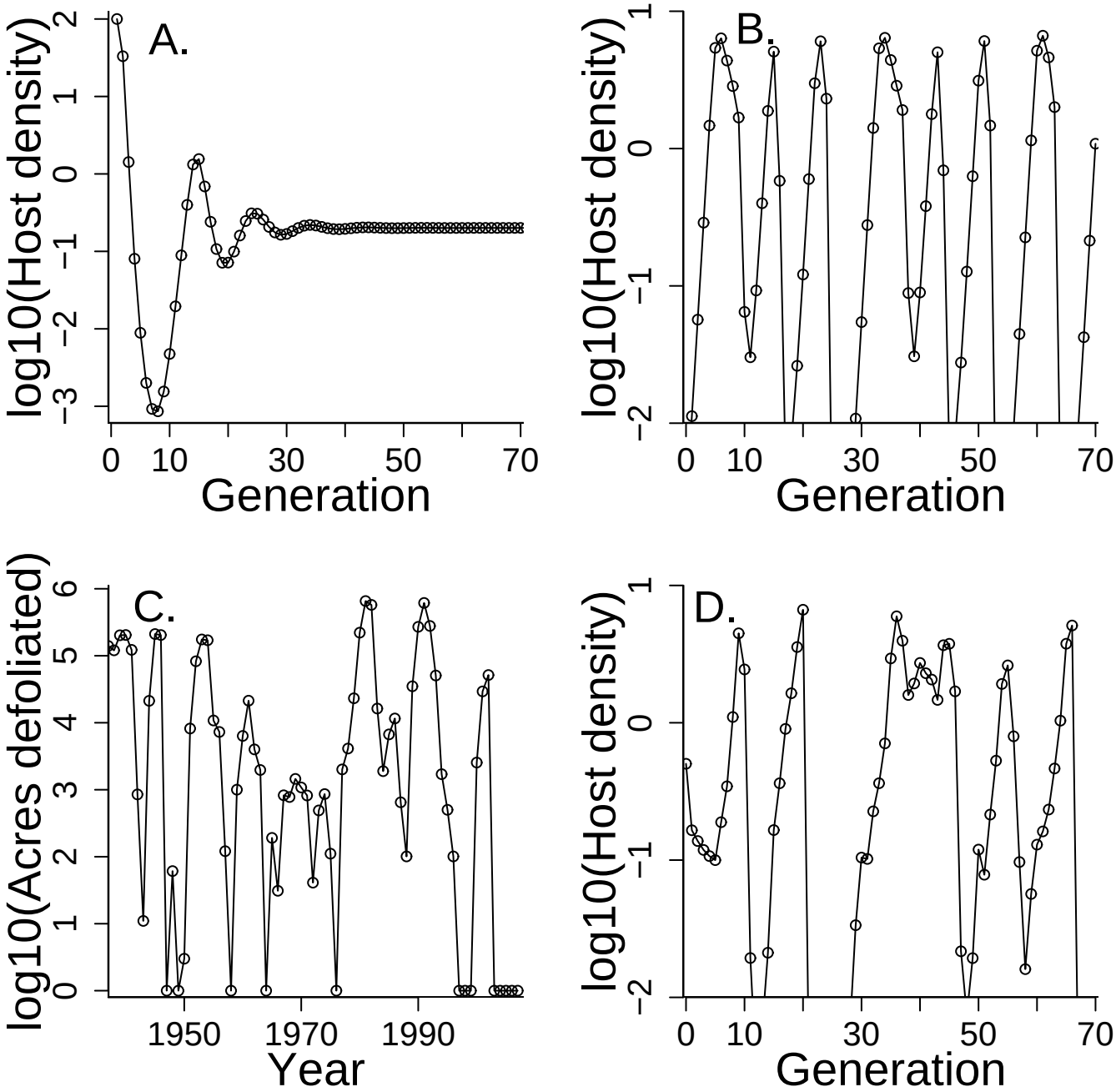

A.
Data
Model
Transmission, −log(1−i)
Baculovirus Density
B.
Data
Model
Transmission, −log(1−i)
Baculovirus Density
C.
Non−Induced
Induced
Probability density
Transmission Rate
D.
Non−induced
Induced
Fraction Infected
Host Density

Figure 2:


A.
log10(Host density)
Generation
B.
log10(Host density)
Generation
C.
log10(Acres defoliated)
Year
D.
log10(Host density)
Generation