

2014

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Prendeville, Holly R.; Tenhumberg, Brigitte; and Pilson, Diana, "Effects of Virus on Plant Fecundity and Population Dynamics" (2014). *Brigitte Tenhumberg Papers*. 3.

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Effects of Virus on Plant Fecundity and Population Dynamics

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Abstract

Microorganisms are ubiquitous and thought to regulate host populations. Although microorganisms can be pathogenic and affect components of fitness, few studies have examined their effects on wild plant populations. As individual traits might not contribute equally to changes in population growth rate, it is essential to examine the entire life cycle to determine how microorganisms affect host population dynamics. In this study, we used data from common garden experiments with plants from three *Cucurbita pepo* populations exposed to three virus treatments. These data were used to parameterize a deterministic matrix model, which allowed us to estimate the effect of virus on components of fitness and population growth rate. Virus did not reduce fruit number, but population growth rates varied among virus treatments and wild *C. pepo* populations. The effect of virus on population growth rate depended on virus species and wild *C. pepo* population. Contributions of life-history transitions and life-history traits to population growth rates varied among populations and virus treatments. However, this population–virus interaction was not evident when examining individual components of fitness. Thus, caution must be used when interpreting the effects of changes in individual traits, as single traits do not always predict population-level change accurately.

Keywords: *Cucumber mosaic virus*, *Cucurbita pepo*, deterministic matrix model, life-history traits, plant–virus interactions, population growth rate, *Zucchini yellow mosaic virus*

Introduction

Microorganisms, including bacteria, fungi and viruses, are ubiquitous in wild plant populations (MacClement & Richards, 1956; Saikkonen et al., 1998; Vidhyasekaran, 2002; Muthukumar et al., 2009; Roossinck et al., 2010; Biddle et al., 2012), but few studies have examined the effects of microorganisms on wild plant populations (although see Alexander & Antonovics, 1988; Raybould et al., 2001; Godfree et al., 2007). Even though wild plants differ from crops, much of our understanding of natural plant–microorganism interactions is inferred from investigations of microorganisms in agricultural crops. In agricultural crops, microorganisms can stunt plant growth, cause deformity, and reduce survival and yield (Walkey, 1991; Jarosz & Davelos, 1995; Gianessi et al., 2002). From these data, it is often assumed that microorganisms typically reduce plant fitness in wild populations. Clearly, microorganisms can have devastating effects in wild populations (e.g., chestnut blight: Anagnostakis, 1987; sudden oak death: Rizzo & Garbelotto, 2003). However, recent work indicates that microorganisms, particularly viruses, are not always pathogenic (as reviewed by Roossinck, 2013), which highlights how little is known about the effects of microorganisms on wild plant fitness, population size, or dynamics (Cooper & Jones, 2006).

The effect of microorganisms might be more variable or less severe in wild populations for several reasons. First, genetic diversity within an agricultural field is typically lower than within wild plant populations. In addition, genetic vari-

ation for resistance might vary among natural populations (Raybould et al., 2001). Thus, only some wild populations, or some individuals within a population, may be susceptible to colonizing microorganisms. In addition, average resistance in wild populations might be higher than in crops because resistance to pathogens may have been lost during selection for other agronomic properties in commercial lines. Secondly, perhaps because of the genetic diversity present in wild populations, pathogenic microorganisms that are common in managed plants can be rare in wild systems (Zettler et al., 1978; Davis & Mizuki, 1987; Ullman et al., 1991; Pallett et al., 2002; Kawakami et al., 2007; Prendeville et al., 2012). Finally, in crops, virus infections typically cause visually apparent symptoms, whereas virus infection in wild plant populations is frequently unapparent (Thurston et al., 2001; Remold, 2002; Prendeville et al., 2012). While infections with no visible symptoms may still result in reduced plant fitness, such plants are probably able to tolerate infection better than stunted plants with deformed fruits.

In addition, recent work with both agricultural and wild plants suggests that microorganisms may have complicated effects on plant fitness. For example, microorganisms have been shown to have negative effects (Friess & Maillet, 1996; Malmstrom et al., 2006), no effect (Jarosz & Burdon, 1992; Malmstrom et al., 2005), or even positive effects on components of plant fitness (Ferris et al., 1989; Remold, 2002; Eviner & Chapin, 2003; Bradley et al., 2008; Xu et al., 2008; Roossinck, 2011). Two studies that have examined the effects of microor-

ganisms on wild plant populations found multiple outcomes (i.e., microorganism extinction, plant and microorganism extinction, and coexistence; Alexander & Antonovics, 1988; Godfree et al., 2007), suggesting that plant–microorganism interactions are more complex and dynamic than previously thought. Furthermore, some effects of microorganism infection are only observed in certain environments. For example, several crop plants are more drought-tolerant when infected with *Cucumber mosaic virus* (CMV; Xie et al., 1994; Xu et al., 2008), suggesting that the cost or benefit of virus infection might vary with water availability. If the effects of microorganisms on yield or fitness frequently depend on environmental context, it might not be surprising that the effects of microorganisms often appear idiosyncratic.

Another reason the effects of microorganisms might appear variable is that most studies have only examined these effects on components of fitness, such as survival, growth or fecundity (Maskell et al., 1999; Kollmann et al., 2007; Bradley et al., 2008; van Mølken & Stuefer, 2011). Then, on the basis of reduced survival or offspring production, authors conclude that microorganisms regulate the size or dynamics of host populations (Milligan & Cosper, 1994; Thrall & Burdon, 1997; but see Holmes, 1982). However, this conclusion could be incorrect. For example, plant populations are not always limited by seed production (Bergelson, 1994; Alexander & Mihail, 2000), suggesting that microorganisms that reduce fecundity might have little effect on population size.

Thus, when evaluating the effects of microorganisms on population size or dynamics, the entire life cycle must be considered. Without prior knowledge, it is not always clear which life-history traits (i.e., seed dormancy, germination timing, survival, or seed production) have the largest effects on population growth rate (Caswell, 1996). In addition, abiotic and biotic factors, such as drought or microorganisms, may vary in their effects on different life-history characters, and thus scale differently to population size or growth rate. One method of integrating across the life history is to use matrix models, which link the entire life history to population growth and dynamics (Caswell, 1989, 2001).

In the work presented here, we examine the effects of two virus species on fecundity and population growth rate in three populations of wild squash, *Cucurbita pepo*. We used data from a common garden experiment to determine if virus inoculation affects fecundity, and parameterize deterministic matrix models to evaluate the effect of virus on population growth rate in wild *C. pepo*. These results allowed us to determine whether changes in fecundity predicted changes in population growth rate. Next, we used life table response experiments (LTREs) to determine if the contributions of either life-history transitions or life-history traits to population growth rate varied among populations or among virus treatments.

Materials and Methods

Study system

Wild squash (*Cucurbita pepo* L. var. *ozarkana* D. Decker and *Cucurbita pepo* L. var. *texana* (Scheele) D. Decker) is found in central and southwestern USA and throughout Mexico. This an-

nual herbaceous vine grows in floodplains, disturbed areas, agricultural fields, and roadside ditches. As wild *C. pepo* is a disturbance specialist, population growth rates are extremely variable and populations go through boom-and-bust cycles (see supporting information following the references: Notes S1, fig. S1a,b). Wild *C. pepo* is monoecious and requires insect-mediated pollination for reproduction. Flower production occurs over several weeks; however, individual flowers last for < 1 d, opening at dawn and closing around noon. Plants produce buoyant gourds (hard-shelled fruits), which are sometimes dispersed by water (Wilson, 1993). Seeds can remain viable within gourds for > 1 yr, a stage referred to as the gourd bank. However, gourds must open before seeds can germinate. Seeds can remain viable within the soil for multiple years, a stage referred to as the seedbank. Germination starts in spring, seedlings establish, and then 4–8 wk later flowering begins. Flower and gourd production can continue until either the first frost or severe drought.

We collected gourds from three populations growing in different habitats in Mississippi, near the towns of Onward, Vaiden, and Eagle Lake (hereafter referred to as Eagle). Onward is 24 km north of Eagle and 130 km southwest of Vaiden. The Onward population (*C. pepo* ssp. *texana*) grows adjacent to a road, so plants experience disturbance and road runoff. In the Vaiden population, wild *C. pepo* ssp. *texana* grows in an abandoned pasture near an ephemeral creek and in competition with grasses. The Eagle population grows in an agricultural field in which peanuts (and occasionally corn) are usually grown. In comparison to the other two populations, wild *C. pepo* ssp. *ozarkana* from the Eagle population germinates later and grows larger before reproduction (H. R. Prendeville, pers. obs.).

Wild *C. pepo* is susceptible to virus, and virus prevalence varies among populations, years, and virus species (Prendeville et al., 2012). Prevalence ranges from 0% to 100%, with a median infection rate of 25% (Quemada et al., 2008; Prendeville et al., 2012). In 80% of virus infections, wild *C. pepo* did not develop any visually apparent symptoms (Prendeville et al., 2012). Viruses that infect wild *C. pepo* include *Zucchini yellow mosaic virus* (ZYMV, Potyviridae) and *Cucumber mosaic virus* (CMV, Bromoviridae; Provvidenti et al., 1978; Fuchs & Gonsalves, 1999). The host range of ZYMV is moderate (c. 10 host plant families), and ZYMV infects mostly cucurbits. Conversely, the host range of CMV is very broad (at least 85 plant families; reviewed by Palukaitis et al., 1992). Aphids non-persistently transmit both ZYMV and CMV. Virus infection can drastically reduce yield in cultivated squash by stunting growth; causing malformation of leaves, flowers, and fruits; reducing fruit production; and occasionally causing death (Walkey, 1991; Fuchs & Gonsalves, 1995; Gianessi et al., 2002). In wild *C. pepo*, viruses can reduce flower, fruit, and seed production as well as plant biomass (Fuchs et al., 2004; Laughlin et al., 2009). However, it is not known if reduced fruit and seed number leads to reduced population growth rate.

Demographic model

We constructed stage-structured matrix models (Lefkovich models) that considered the following life-history stages: adult flowering plants (A), gourds containing viable seeds on

or in the soil (gourd bank, G), and dormant seeds on or in the soil (seedbank, S; fig. 1a,b). Using this deterministic matrix model, we estimated the population growth rate, λ , which is the dominant eigenvalue of the matrix (fig. 1b). We used an annual time step, as *C. pepo* is an annual species, and represented reproduction as a birth-pulse process, because within a year gourds are produced only from summer to fall (Caswell, 2001). A population census was carried out in the summer after plants had flowered, but before gourds were produced (a prebreeding census). Following the population census, adults produced gourds that entered the gourd bank (A to G), opened and released seeds that entered the seedbank (A to S), or opened and released seeds that germinated and survived to flower (A to A). Gourds present in one census remained in the gourd bank at the next census (G to G), opened and released

seeds to the seedbank (G to S), or opened and released seeds that germinated and survived to flower (G to A). Finally, seeds present in one census remained in the seedbank (S to S) at the next census or germinated and survived to flower (S to A).

Each of these demographic transitions is the product of one or more life-history characters (lower-level parameters; fig. 1a-c). Following the population census, flowering adults (A) produce g gourds; these gourds avoid rodent consumption with probability r . These < 1-yr-old gourds open with probability o_0 and release s seeds per gourd, or remain in the gourd bank with probability $1 - o_0$. Released seeds are dormant and enter the seedbank with a probability d , or germinate with a probability b_0 . Seeds that germinate survive to flower with probability f . Gourds in the gourd bank (G) survive to the next year with probability v and avoid rodent herbivory with probability r . These gourds, which are > 1 yr old, open with probability o_1 , or remain in the gourd bank with probability $1 - o_1$. Older gourds that open release s seeds per gourd, of which a proportion a remain viable. Viable seeds (> 1 yr old) enter the seedbank with probability d or germinate with probability b_1 . Seeds that germinate survive to flower with probability f . Finally, seeds in the seedbank (> 1 yr old; S) remain dormant with probability d , or germinate with probability b_1 and then survive to flower with probability f . We performed field experiments in order to estimate each life-history trait contributing to each of these demographic transitions.

Model parameterization: germination, survival, gourd production, and seed production

On 28 March 2007, we planted a common garden experiment at the Delta Conservation Demonstration Center in Metcalfe, Mississippi using seeds collected from naturally growing plants with unknown virus status. Although seed transmission of ZYMV and CMV is rare (Lecoq et al., 1998; Simmons et al., 2011), maternal effects resulting from virus infection may affect offspring traits (Roberts, 1983; Shattuck, 1993). We mixed seeds collected within a population to randomly distribute potential maternal effects among virus treatments. Using a randomized block design, we planted seeds from three Mississippi populations (Onward, Eagle, and Vaiden) and three virus treatments (inoculated with ZYMV or CMV, or uninoculated). In each of 24 spatial blocks, each population \times virus inoculation treatment (ZYMV, CMV) was replicated once, but each population \times uninoculated treatment was replicated twice. Thus, each block consisted of 12 planting locations for a total of 288 planting locations and each planting location was separated by 6 m. Experimental plants experienced competition from other species present in the field. At each planting location, four seeds were sown from one of the three populations (Onward, Eagle, or Vaiden). If multiple seeds germinated at a location, seedlings were either transplanted to locations with no germination or thinned to one plant. Since germination and seedling establishment rates were low, some planting locations were unoccupied by a plant, resulting in an incomplete block design.

The timing of natural virus infection is not known in wild populations. However, in cultivated squash and other crops in the southeastern US, aphids and subsequent virus infections

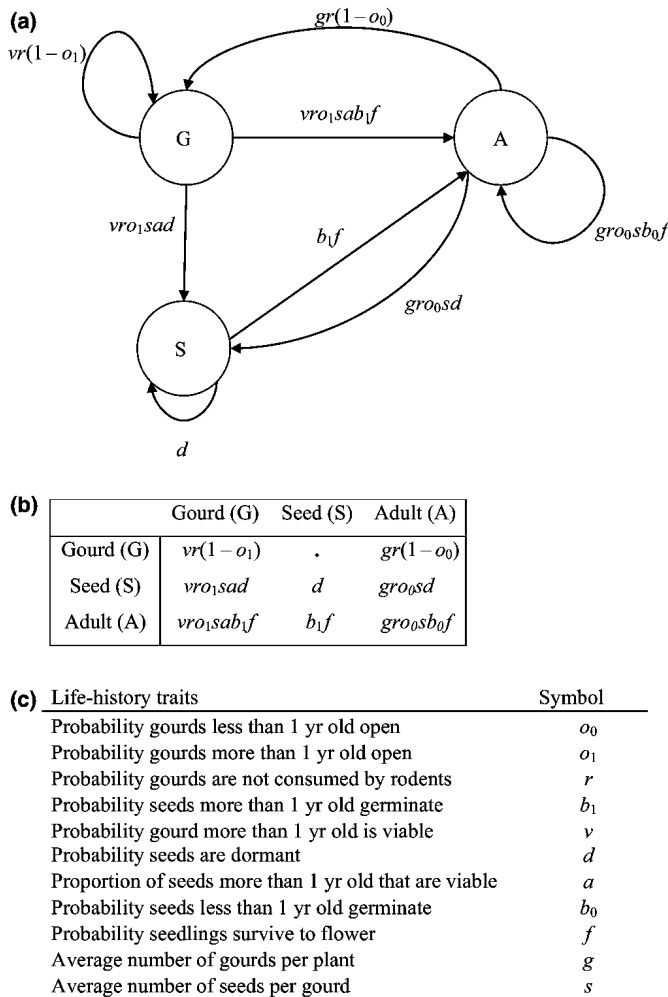


Figure 1. Life-history parameters used in the deterministic matrix model to project population growth of wild *Cucurbita pepo*. (a) Life-history diagram of wild *C. pepo* with arrows indicating life-history transitions within and between stages: adult plants (A), gourd bank (G), and seedbank (S). (b) Transition matrix for wild *C. pepo* with life-history parameters multiplied to calculate each matrix element. (c) Symbols of life-history parameters defined.

are common in summer and fall (Chalfant et al., 1977; Wosula et al., 2013). For this reason, we inoculated when plants were established (*c.* 75 leaves on average) in July. Virus inoculation occurred by rubbing two to three new leaves with *c.* 1 ml of phosphate buffer with celite and homogenized squash leaf tissue infected with either CMV (10 July) or ZYMV (14–15 July). Virus inocula were provided by Rosario Provvidenti's laboratory at Cornell University and individually maintained on cucurbit crops.

The common garden was managed to ensure plant persistence and to limit virus spread. In May 2007, precipitation was below normal. To simulate normal precipitation and to improve seedling establishment and survival, we flood irrigated the field once in June. Precipitation increased later in the growing season, but remained below the long-term average for the area (National Climatic Data Center, 2009). On 28–31 May and 31 July, we sprayed plants with Sevin (Bayer Crop-Science, Research Triangle Park, North Carolina, USA) to limit aphid populations and virus spread. The impact of the pesticide on pollinators was limited by spraying in the evening when pollinators were not active. Also, wild *C. pepo* flowers are only open in the morning of a single day; thus, pesticide was not applied to surfaces with which pollinators are frequently in contact. Finally, rows between plants were mowed to provide access to experimental plants.

We monitored each planting location ($n = 96$ locations per population; four seeds per location) daily to estimate the germination rate of seeds < 1 yr old (b_0 ; fig. 1a,b, table 1). We used a generalized linear model with a Gaussian error distribution to analyze the effect of population on b_0 , which was arcsine-transformed before analysis. Following transplanting to locations without seedlings, or thinning, seedlings were monitored daily for survival to flowering (f). We used a generalized linear model with a binomial error distribution to analyze the effect of population on f . Parameters b_0 and f , as well those described later, were estimated as least-square means derived from generalized linear models (back-transformed when appropriate) using the *glm* package in R software version 2.15.1 (R Development Core Team, 2012). Germination was estimated from seeds that had been collected from plants with unknown virus status, and some plants flowered before the virus treatments were applied. For these reasons, b_0 and f were estimated for each population, but not for each virus treatment. Thus, to the extent that germination and survival to flowering are influenced by virus infection, our model underestimates the effect of virus on population growth rate.

In November, following plant death, gourds were collected and brought to the laboratory. From these data, we estimated g , the number of gourds with viable (*i.e.*, filled) seeds per plant, and s , the average number of viable seeds per gourd. In nature, gourds can be buried, relocated to unsuitable habitats after flood dispersal, or consumed by mammals, particularly rodents (H. R. Prendeville, pers. obs.). Thus, in nature the numbers of gourds and seeds that contribute to future population growth are probably lower than estimates from this common garden experiment. However, these overestimates were probably similar across all populations and virus treatments.

We used generalized linear models to analyze the effect of virus treatment, population, and the virus \times population interaction on gourd number per plant (g) and average seed number per gourd (s). Gourd number and seeds per gourd were log-transformed to meet the assumptions of normality. In these analyses, we treated virus treatment, population, and the virus \times population interaction as fixed effects (PROC GLIMMIX, SAS 9.3 for Windows; SAS, 2010). The average number of seeds per gourd differed among populations; thus we conducted *post hoc* comparisons of virus treatments with a Tukey-Kramer adjustment for multiple comparisons.

Model parameterization: seed and gourd survival and dormancy

To assay gourd opening and loss rates, we placed gourds that had been produced the previous fall and collected from plants with unknown virus status on the ground next to the common garden experiment. Using a dome of chicken wire, we individually caged 15 gourds per population (Vaiden and Eagle) in February 2006, and 15–20 additional gourds per population (Vaiden, Eagle, and Onward) in March 2007. Cages were tacked to the ground with wire stakes. During the 2006 and 2007 growing seasons, we monitored gourd integrity on a weekly basis, which allowed us to estimate the proportion of gourds that opened within 1 yr (o_0) for both cohorts, the proportion of gourds that opened in > 1 yr (o_1) for the 2006 cohort, and the probability that a gourd > 1 yr old is viable (v). In this experiment, rodents consumed gourds and these data were used to estimate the probability that a gourd avoids rodent herbivory (r). Because we had a limited number of gourds, we were not able to determine if gourd dormancy traits vary among populations. For this reason, we pooled data across populations. We used a generalized linear model with a binomial error distribution to estimate o_0 , o_1 , v , and r . Once a gourd opened, all seeds were collected and stained with tetrazolium to assess seed viability, a . In addition, gourds that were still intact in April 2008 were opened manually and seeds were tested for viability. Following arcsine transformation, we used a generalized linear model with a Gaussian error distribution to estimate a , the proportion of seeds in gourds > 1 yr old that remain viable. Although o_1 , v , and a probably decline with time, in our model we assumed that these parameters are constant.

To assess seed dormancy, we buried 20 open-topped mesh baskets (20 cm \times 20 cm \times 10 cm deep) for each population and planting time (February 2006: Eagle, Vaiden; March 2007: Eagle, Vaiden, Onward). In each basket, 50 seeds (produced the previous season) were buried *c.* 1 cm deep. To prevent seeds from dispersing outside of the basket, the open top of each basket was *c.* 0.5 cm higher than the soil surface. During the growing seasons in 2006 and 2007, we monitored germination on a weekly basis. Following germination, seedlings were cut at the stem to limit soil disturbance. In August 2006, we randomly collected four baskets per population that had been buried in February 2006 to estimate seed viability. As all of these seeds were viable, we did not repeat this procedure with baskets buried in 2007. Data from the remaining seed baskets

Table 1. Estimates of life-history parameters (symbols defined in fig. 1c) used to estimate each element in the deterministic matrix model to project population growth of wild *Cucurbita pepo* from three populations (Onward, Eagle, and Vaiden) with three virus treatments (No, uninoculated; CMV, inoculated with *Cucumber mosaic virus*; ZYMV, inoculated with *Zucchini yellow mosaic virus*)

Life-history symbol	Population	Population effect	Virus	Virus effect	Population × virus	Parameter value	SE
<i>g</i>	Onward	ns	None	ns	ns	6.49	1.37, 1.73
			CMV			4.75	1.28, 1.75
			ZYMV			4.12	1.16, 1.61
	Eagle		None			4.53	1.09, 1.43
			CMV			3.59	0.99, 1.36
			ZYMV			4.81	1.39, 1.95
	Vaiden		None			7.31	2.05, 2.86
			CMV			4.28	1.18, 1.62
			ZYMV			11.04	3.18, 4.46
<i>s</i>	Onward	***	None	ns	ns	105.11	10.79, 12.03
			CMV			83.92	10.71, 12.28
			ZYMV			95.11	13.75, 16.07
	Eagle		None			120.87	15.43, 17.68
			CMV			101.18	15.80, 18.72
			ZYMV			119.45	17.92, 21.08
	Vaiden		None			60.65	8.47, 9.85
			CMV			54.89	7.43, 8.59
			ZYMV			63.17	8.82, 10.26
<i>d</i>	Onward	*		na	na	0.16	0.062, 0.074
	Eagle		0.09			0.29, 0.034	
	Vaiden		0.009			0.011, 0.014	
<i>b₀</i>	Onward	***		na	na	0.13	0.024, 0.026
	Eagle		0.05			0.015, 0.018	
	Vaiden		0.20			0.030, 0.059	
<i>f</i>	Onward	**		na	na	0.77	0.04
	Eagle		0.56			0.05	
	Vaiden		0.65			0.05	
<i>o₀</i>		ns		na	na	0.09	0.06
<i>o₁</i>		ns		na	na	0.20	0.07
<i>r</i>		ns		na	na	0.48	0.05
<i>b₁</i>		ns		na	na	0.29	0.10, 0.12
<i>v</i>		ns		na	na	0.43	0.10
<i>a</i>		ns		na	na	0.13	0.061, 0.078

For each parameter estimate, the statistically significant effects of population, virus, or the population × virus interaction are indicated by: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.007$; ns, $P > 0.05$; na, not analyzed. Parameter estimates are least-square means from generalized linear models with appropriate error distributions. When data were transformed before analysis, we present back-transformed least-square means and standard errors; since these standard errors are not symmetric, both errors are presented. *g*, average number of gourds per plant; *s*, average number of seeds per gourd; *d*, probability seeds are dormant; *b₀*, probability seeds less than 1 year old germinate; *f*, probability seedlings survive to flower; *o₀*, probability gourds less than 1 year old open; *o₁*, probability gourds more than 1 year old open; *r*, probability gourds are not consumed by rodents; *b₁*, probability seeds more than 1 year old germinate; *v*, probability gourd more than 1 year old is viable; *a*, proportion of seeds more than 1 year old that are viable.

were used to estimate the germination rate of seeds > 1 yr old, *b₁*. In May 2007, following germination, we randomly collected four baskets per population from the 2006 cohort. The remaining baskets (buried in 2006 and 2007) were collected in late winter and early spring of 2008 (Eagle, $N = 11, 17$; Vaiden, $N = 9, 17$; and Onward, $N = n/a, 11$; animals destroyed some baskets so not all were recovered). Seeds were sieved from the soil and stained with tetrazolium to assay seed viability; these data allowed us to estimate the probability, *d*, that seeds > 1 yr old remain viable but dormant. Using a generalized linear model with a Gaussian error distribution, we analyzed the effect of population on *b₁* and *d* following arcsine transformation.

In nature, estimating seed survival and dormancy is difficult because many seeds are lost before entering the soil

through predispersal seed predation and by dispersing to sites inappropriate for germination and establishment. Thus, our experiment probably overestimated seed survival; however, these overestimates were probably similar across populations.

Analysis of demographic model

Virus effects on λ . To examine the effect of virus treatment (CMV-inoculated, ZYMV-inoculated, uninoculated) on population growth rate, we parameterized stage-structured matrix models to calculate population growth rate for each virus treatment and population (Onward, Eagle, Vaiden) combination (tables 1, S1). To evaluate differences in population growth rate, λ , between virus treatments within each population, we used randomization tests (Sokal & Rohlf, 1995; Caswell, 2001; detailed methods in Notes S2).

Contributions to observed differences in λ among virus-inoculated treatments compared with uninoculated plants within each population. We conducted LTREs to quantify the contribution of each life-history transition and life-history trait (lower-level parameter) to observed differences in population growth rate among virus treatments within a population (Caswell, 1996, 2001; Levin et al., 1996). Contributions of each life-history transition and life-history trait were determined by comparing the population matrix of a virus treatment in a particular population to the population matrix associated with uninoculated plants from the same population (detailed methods in Notes S2).

Additional retrospective and prospective analyses were conducted with detailed methods and results presented in the Notes S2. We conducted additional LTREs to determine the contributions of life-history traits to observed differences in population growth rate among populations and virus treatments by comparing each population-virus combination with an overall mean of uninoculated plants. We also conducted elasticity and sensitivity analyses to examine the effects of changes in life-history transitions and life-history traits on future population growth rate (de Kroon et al., 1986). All calculations and analyses of population growth rates were completed using R software version 2.15.1 (R Development Core Team, 2012).

Results

Gourd and seed production (common garden experiment)

The average number of gourds per plant did not vary among virus treatments ($F_{2,163} = 1.15$, $P = 0.320$) or among populations ($F_{2,163} = 0.72$, $P = 0.486$; table 1). In addition, the effect of virus treatment on the average number of gourds per plant did not vary among populations (virus \times population interaction: $F_{4,163} = 0.66$, $P = 0.618$; table 1). Similarly, the average number of seeds per gourd did not differ among virus treatments ($F_{2,131} = 1.14$, $P = 0.323$; table 1) and the effect of virus treatment did not vary among populations (virus \times population: $F_{4,131} = 0.08$, $P = 0.987$). However, the average number of seeds per gourd differed among populations ($F_{2,131} = 14.6$, $P < 0.0001$; table 1). Post hoc comparisons indicated that fewer seeds per gourd were produced from Vaiden plants (59.3 seeds per gourd) than either Onward (94.3 seeds per gourd; $F_{1,131} = 15.86$, $P = 0.0001$) or Eagle (113.3 seeds per gourd; $F_{1,131} = 26.75$, $P < 0.0001$) plants. The number of seeds per gourd did not differ between the Onward and Eagle populations ($F_{1,131} = 2.36$, $P = 0.127$).

Population growth rates

Population growth rates of wild *C. pepo* varied among populations and virus treatments (fig. 2a–c; table S2a,b). Plants from the Eagle population had lower population growth rates than plants from either the Onward or Vaiden populations, which did not differ from one another (table S2a). In the Onward population, inoculation with CMV and ZYMV reduced the population growth rate relative to uninoculated

plants, but differences were not statistically significant ($P = 0.244$, $P = 1.00$, respectively; fig. 2a). In the Eagle population, virus had no effect on population growth rate (fig. 2b, table S2b). In the Vaiden population, population growth rate was reduced in CMV-inoculated plants relative to ZYMV-inoculated plants ($P = 0.008$) and was not significantly different from uninoculated plants ($P = 0.126$). In Vaiden, the λ of ZYMV-inoculated plants was greater than that of uninoculated plants ($P = 0.062$, fig. 2c).

Contributions to observed differences in λ among virus-inoculated treatments compared with uninoculated plants within each population

Effect of virus infection on life-history transitions. In comparison to uninoculated plants across all populations, inoculation with CMV and ZYMV had the greatest effect on contributions from adult-to-adult transition (fig. 2d–f). The reduction in population growth rate of plants from the Onward and Eagle populations inoculated with CMV or ZYMV was a result of lower contributions from the adult-to-adult, adult-to-seedbank, and adult-to-gourd bank transitions, in comparison to uninoculated plants (fig. 2d,e). In Vaiden the reduction in the population growth rate caused by CMV inoculation and the increase caused by ZYMV inoculation is mainly the result of contributions of the adult-to-adult transition (fig. 2f).

Effect of virus infection on life-history traits. The number of gourds per plant (g) and seeds per gourd (s) both contributed to differences in λ between CMV-inoculated and uninoculated plants, and between ZYMV-inoculated and uninoculated plants (fig. 2g–i). In Onward, both the number of gourds per plant (g) and the number of seeds per gourd (s) contributed to reduced population growth rate in CMV- and ZYMV-inoculated plants, although the magnitude of these contributions differed between the viruses (fig. 2g). In Eagle, inoculation with CMV reduced contributions of both the number of gourds (g) and seeds per gourd (s), whereas inoculation with ZYMV led to a minor increase in contributions from the number of gourds per plant (g ; fig. 2h). In Vaiden, gourds per plant (g) and, to a lesser extent, seeds per gourd (s) contributed to a reduced population growth rate in CMV-inoculated plants and increased the population growth rate in ZYMV-inoculated plants (fig. 2i). Our experimental design does not allow us to evaluate the contributions of other life-history traits. Additional LTREs comparing each population-virus combination with an overall mean of uninoculated plants found that virus, population, and the interaction of these factors affected the contribution of many life-history traits to population growth rate (Notes S2, fig. S2a–c).

The stable stage structure differs among populations (Notes S2, fig. S3). At the stable age distribution, the Vaiden population contains very few seeds, which is probably the result of high germination and low dormancy in this population. Inoculation with virus had very little effect on the stable age distribution (fig. S3). Sensitivity and elasticity analyses indicated that populations and virus treatments differed in terms of which demographic transitions and life-history traits most

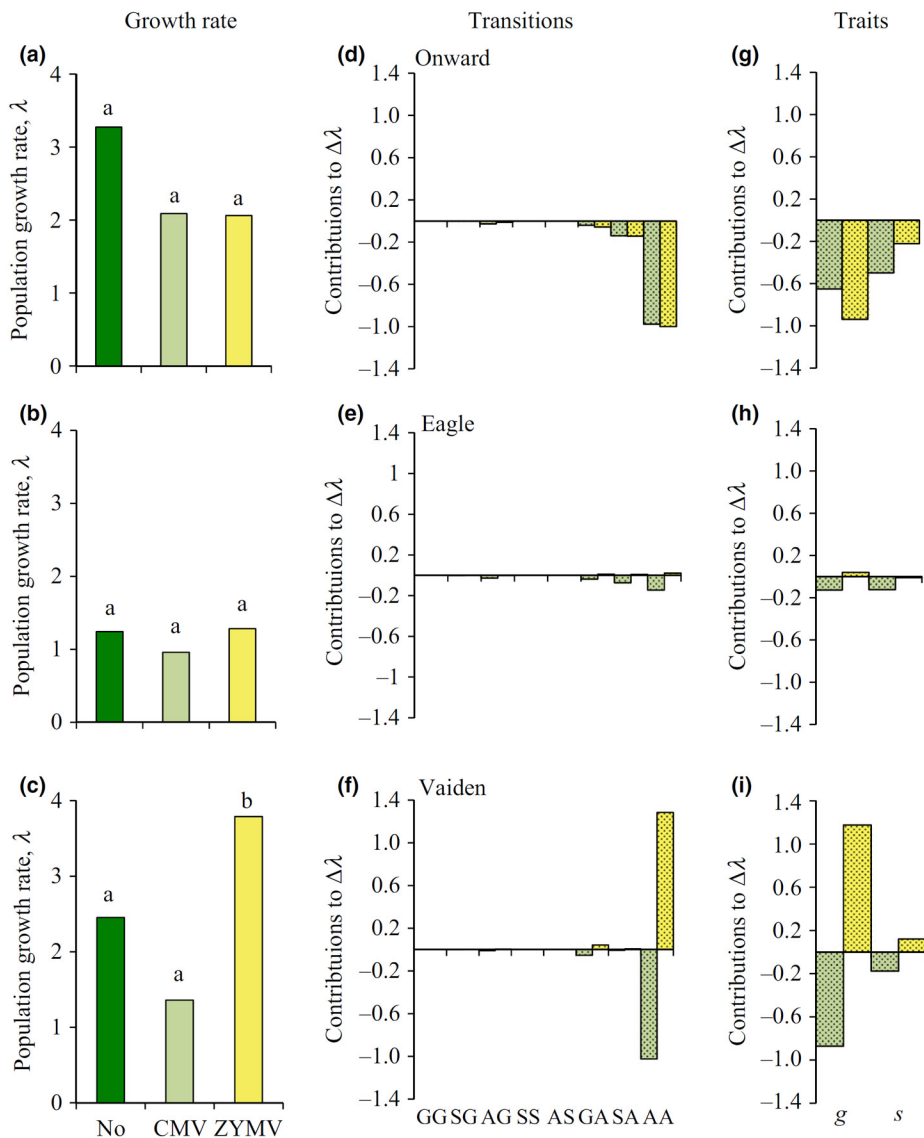


Figure 2. Population growth rates (λ) of wild *Cucurbita pepo* from three populations (a, Onward; b, Eagle; c, Vaiden) subjected to three virus treatments: No, uninoculated plants; CMV, plants inoculated with *Cucumber mosaic virus*; and ZYMV, plants inoculated with *Zucchini yellow mosaic virus*. Different letters indicate significant differences between virus treatments within a population as estimated by randomization tests (P -values in table S2). Life table response experiments quantify the contribution of each life-history transition (d–f) and life-history traits (g–i) to observed differences in population growth rate between a virus inoculation treatment and uninoculated plants within each population. A, adult plants; G, gourd bank; S, seedbank; g, average number of gourds per plant; s, average number of seeds per gourd; light green speckled bars, CMV; yellow speckled bars, ZYMV.

affected future population growth rate (Notes S2, figs. S4–S7). In general, sensitivities involving the adult stage were greater than those that did not include the adult stage, and the patterns of sensitivities across demographic transitions were similar in Onward and Eagle, which differed from Vaiden.

Discussion

The effect of virus inoculation on population growth rate in wild *C. pepo* depends on both virus species and plant population. ZYMV inoculation reduced the population growth rate in Onward, had no effect in Eagle, and increased the population growth rate in Vaiden. CMV inoculation reduced the population growth rate in Onward and Vaiden, but had no effect in Eagle. We observed these effects on population growth rate even though virus infection did not significantly reduce fecundity (gourds per plant, seeds per gourd) in our common garden experiment. Thus, multiple small changes in life-history traits together contributed to significant population-level effects.

The effect of virus on population growth rate may vary among populations because these populations have historically experienced different frequencies of virus infection. If populations are regularly exposed to viruses, tolerance to infection may evolve (Pagán et al., 2008). For example, if the Vaiden and Eagle populations have historically been exposed to ZYMV, then these populations may have evolved tolerance to this virus, and such past exposure could explain why population growth rate is not reduced when plants from Vaiden and Eagle are inoculated with ZYMV. By contrast, CMV reduces the population growth rate in Onward and Vaiden, suggesting that past exposure to CMV in these populations has not been frequent enough (or genetic variation is not present) to allow the evolution of tolerance to CMV. These hypotheses are difficult to evaluate without more complete information about the history of the virus in these populations. However, we do know that virus pressure varies among these populations and that some viruses are more common than others (Prendeville et al., 2012).

In the absence of virus inoculation, population growth rate also varied among wild *C. pepo* populations. The pop-

ulation growth rate of the Eagle population was lower than that of the Onward and Vaiden populations, which did not differ from each other. However, in the absence of virus inoculation, differences in fecundity among populations did not predict differences in the population growth rate. For instance, fecundity and the population growth rate were greatest in the Onward population, whereas in the Eagle population fecundity was relatively high (i.e., ranked second), but the population growth rate was the lowest, and in the Vaiden population fecundity was the lowest, but the population growth rate was greater than the Eagle population. Thus, the contributions of other life-history traits in conjunction with fecundity are required to predict population growth rate. In the Eagle population, germination of new seeds and survival to flowering were both low, and these traits both reduced the population growth rate. By contrast, in the Vaiden population, high germination of new seeds and relatively high survival contributed to high population growth rate, even though these plants had lower fecundity. These results illustrate the importance of examining the entire life cycle when evaluating differences in population-level characteristics.

Individual demographic transitions and life-history traits did not always predict population growth rate. Our results are thus consistent with other studies that have shown that life-history traits, particularly fecundity, do not always scale up to affect populations (Bergelson, 1994; Alexander & Mikhail, 2000; Kolb, 2011). Furthermore, LTREs indicated that contributions of fecundity to population growth rate differed by virus treatment. Clearly, using an individual component of fitness to infer the effect of a factor (e.g., virus infection, population identity) on population growth may be inappropriate (Caswell, 1989). For instance, gourd production is frequently used to infer population-level effects in studies of *C. pepo* (Laughlin et al., 2009; Sasu et al., 2009). However, in the work presented here, population growth rate, but not gourd production, differed among viruses and populations. Thus, predictions based on gourd production alone would have been incorrect.

In contrast to our study, in which virus inoculation caused a (nonsignificant) reduction in fecundity of 27–37%, other studies of wild *C. pepo* have found that virus infection can reduce fecundity up to 80–100% (Fuchs et al., 2004; Laughlin et al., 2009). However, in these studies, plants were infected at a much smaller size. For instance, Laughlin et al. (2009) inoculated plants with four leaves or fewer and before transplanting into field, whereas we inoculated well-established plants with c. 75 leaves. In other species, the timing of virus infection in relation to plant development is known to mediate the effect of virus on plant populations. In particular, plants are more severely affected by virus if infected at a small size (Pagán et al., 2007). In agricultural crops, it is clear that the timing of virus infection varies from year to year (Rowell et al., 1999), and it is likely that the timing of infection varies in wild populations as well. Thus, the consequences of virus infection for individual fitness and population growth probably vary over time.

In a broader context, results from this work along with others (Godfree et al., 2007; Biddle et al., 2012) inform predictions on the effects of novel traits, such as transgenes, on wild

populations. One ecological risk associated with the use of transgenic crops is the introgression of transgenes into wild populations. Because it is known that novel traits acquired through hybridization can affect range expansion or competitive ability in invasive species (Ellstrand & Schierenbeck, 2000), there is concern that transgenic traits that introgress from crops into wild populations will allow weedy plants to become more invasive (Pilson & Prendeville, 2004). In the US, squash (also *C. pepo*) with transgenic virus resistance was released for commercial production in 1994 (USDA/APHIS, 1994). Commercial squash is grown within the native range of wild *C. pepo* (Wilson, 1993) and nontransgenic crop alleles have been detected in wild populations (Decker, 1988; Wilson, 1990, 1993; Decker-Walters et al., 2002). Thus, transgenic virus resistance may introgress into wild populations in the future. If virus infection is common (Prendeville et al., 2012), and if wild populations are frequently limited by virus, transgenic virus resistance may allow wild populations to grow more rapidly. Our results suggest that CMV resistance would be beneficial to wild populations, while the benefit of ZYMV resistance would depend on the population. Conversely, indirect costs of transgenic resistance (Sasu et al., 2009) or pleiotropic effects of the transgene (Prendeville & Pilson, 2009) could slow introgression of transgenic resistance. Evaluating the potential consequences of transgenic resistance in wild populations is difficult, and minimally requires examining population-level effects of virus infection (as we have presented here), identifying any direct or indirect costs of transgenic resistance, and surveying wild populations for virus prevalence.

The work presented here adds to the growing body of evidence that virus infection is not consistently detrimental to plants (Godfree et al., 2007; Pagán et al., 2008; Xu et al., 2008; Roossnick, 2012). By examining the entire life cycle of wild *C. pepo*, we found that the plant traits affected by virus inoculation varied among populations and between virus species. Furthermore, these idiosyncratic effects on the population growth rate would not have been apparent if we had quantified only plant fecundity. Whether viruses, bacteria, and fungi living on plants typically have such idiosyncratic effects on plant populations is an open question. However, it is clear that when evaluating the potential ecological risks associated with transgenes moving from crops into wild populations, it is necessary to quantify effects through the entire life cycle.

Acknowledgments – We appreciate the expertise of the staff of the Delta Conservation and Demonstration Center and the ability to use their land and equipment. A. Baldwin, W. Blaylock, E. Berryman, H. Boone, C. Davis, R. Stout Evans, A. Firestone, V. Fyke, S. R. Liddy, B. Miller, T. J. Morris, A. Steadman, C. Weathers, X. Ye, and many others helped in the field and the laboratory. Members of the Galloway laboratory at the University of Virginia provided comments that improved the manuscript. This work was funded by USDA-BRAG #05-03806 to D.P. and T. Jack Morris, University of Nebraska (UNL) SBS Special Funds to H.R.P., and a US-Department of Education GAANN (#P200A040126 to UNL) fellowship to H.R.P.

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Supporting Information (following)

- Figure S1** Population growth rates of wild *Cucurbita pepo* estimated from the literature.
- Figure S2** Contributions of life-history traits to λ for each virus treatment, each wild *Cucurbita pepo* population, and each population \times virus treatment combination.
- Figure S3** Stable stage distribution indicates the proportion of wild *Cucurbita pepo* population in each stage with each virus treatment.
- Figure S4** Sensitivity values for each matrix element by each wild *Cucurbita pepo* population and virus treatment.
- Figure S5** Sensitivity values for each life-history trait by wild *Cucurbita pepo* population and virus treatment.
- Figure S6** Elasticity values of demographic transitions for each wild *Cucurbita pepo* population and virus treatment combination.
- Figure S7** Elasticity values for each life-history trait for each wild *Cucurbita pepo* population and virus treatment.
- Table S1** Demographic matrix for each wild *Cucurbita pepo* population and virus treatment used to estimate population growth rate
- Table S2** Results of randomization tests comparing population growth rates between virus treatments within each population, and populations within each virus treatment
- Notes S1** Population growth rates of wild *Cucurbita pepo* estimated from the literature.
- Notes S2** 2007 Common garden experiment – perturbation analysis.

Supporting Information Figs S1-S7, Tables S1 & S2 and Notes S1 & S2

Notes S1

Population growth rates of wild *Cucurbita pepo* estimated from literature

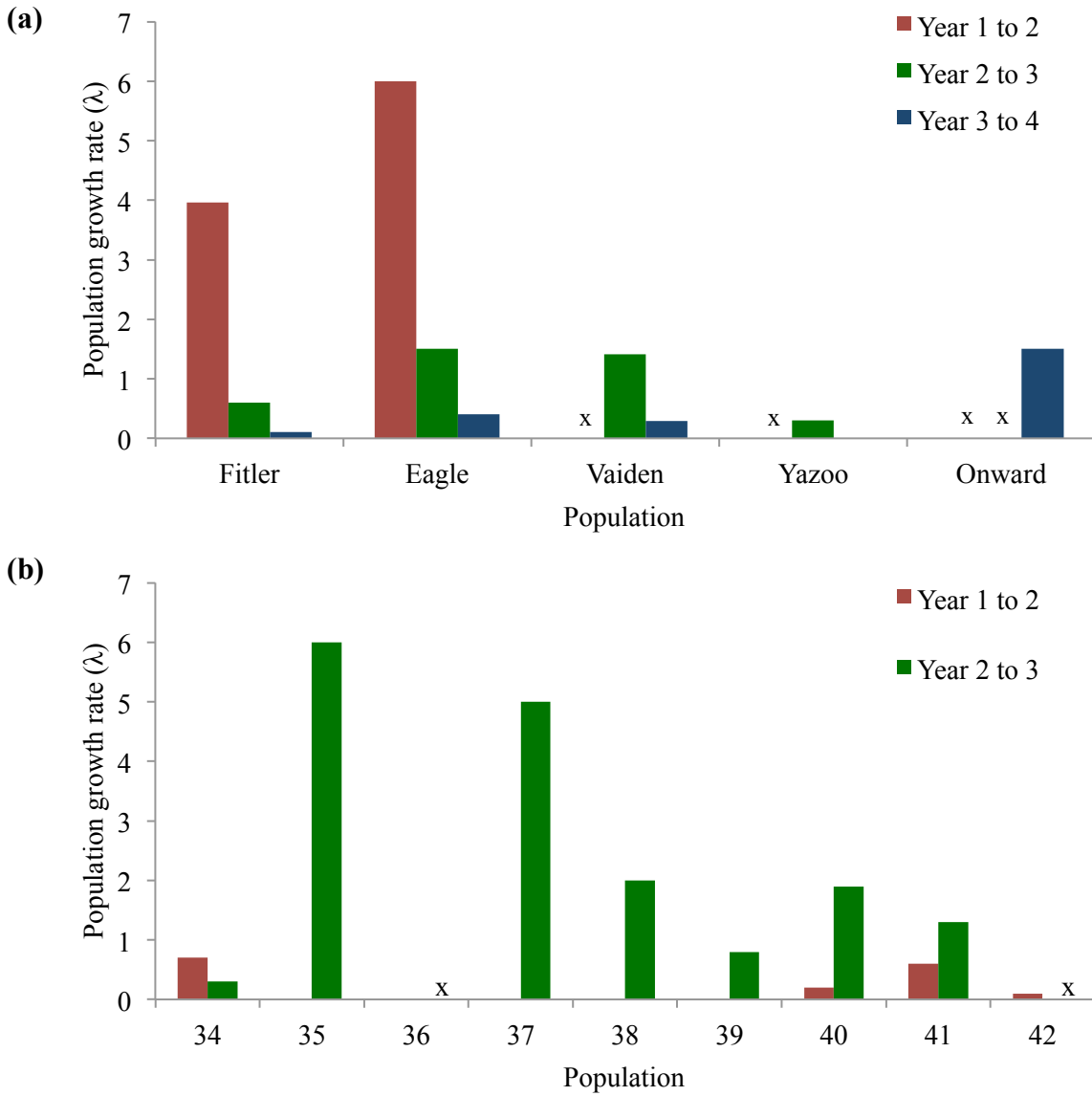
Methods

We compiled census data of wild *Cucurbita pepo* populations from the literature (Quemada *et al.*, 2008; Prendeville *et al.*, 2012) to determine if populations growth rates from wild populations are comparable to those calculated from our deterministic matrix model that was parameterized with data from a common garden experiment. Using census data of wild *C. pepo* populations, we estimated population growth rates by dividing the number of wild *C. pepo* plants in year $t-1$ by the number in year t . This method was used to estimate population growth rates for 14 wild *C. pepo* populations that were each monitored over 2-4 growing seasons. Population sizes were estimated in 2004-2007 for populations in Mississippi near the towns of Eagle Lake (Eagle), Vaiden, Onward, Yazoo City (Yazoo), and Fitler (Prendeville *et al.*, 2012) and in 2000-2002 for nine populations in Missouri and Illinois (Quemada *et al.*, 2008).

Results

Population growth rates ranged from 0-6, with an average of 1.35 and a median of 0.6. Seeds and gourds from three of these 14 populations (Onward, Eagle, Vaiden) were used in the common garden experiments presented here. In these three populations λ ranged from 0-6, with an average $\lambda=1.78$ and a median $\lambda=1.45$. In many years, virus was present in these populations and virus prevalence ranged from 11-53% of the population when present (Quemada *et al.*, 2008; Prendeville *et al.*, 2012). As a disturbance specialist with a gourd bank and seedbank, wild *C. pepo* goes through boom and bust cycles as illustrated here (Figure S1).

Figure S1. Population growth rates of wild *Cucurbita pepo* estimated from published population counts (a) Prendeville *et al.*, 2012 and (b) Quemada *et al.*, 2008. Population identification coincides with publications. No bars indicate the λ was zero (i.e. extinction) and 'x' indicates the λ was not estimated since population size was not documented in consecutive years.



Notes S2

2007 Common garden experiment-Perturbation analysis

Methods

Randomization tests. To evaluate differences in population growth rate, λ , between virus treatments within each population, we used randomization tests (Sokal & Rohlf, 1995; Caswell, 2001). Using data from the two groups being compared (e.g. CMV-inoculated vs. uninoculated within a population), we permuted data 5000 times. For each permutation, we constructed two new matrices, calculated the associated λ -values, and computed the test statistic $\theta = |\lambda^{(A)} - \lambda^{(B)}|$, where A and B indicate the permuted matrices. Then, we calculated the probability that the observed difference in population growth rate between the two groups is significant using the following formula: $[\theta \geq \theta_{obs} | H_0] = (\#\{\theta^{(i)} \geq \theta_{obs}\} + 1)/(5000 + 1)$, where H_0 is the null hypothesis, and $(\#\{\theta^{(i)} \geq \theta_{obs}\})$ indicates the number of times the difference in λ -values of a permuted data set was larger than the observed difference in λ -values.

Contributions to observed differences in λ among virus-inoculated treatments compared to uninoculated plants within each population. We conducted life table response experiments to quantify the contribution of each life history transition and trait to observed differences in population growth rate among virus treatments within a population. Let A^m be the population matrix associated with a virus treatment in a particular population, with entries α_{ij}^m , and let A^r be the population matrix associated with uninoculated plants from the same population, which we used as the reference matrix. The difference between the λ s of the virus treatment (λ^m) and the reference matrix (λ^r) is $\lambda^m - \lambda^r = \alpha^m$. Following the contribution of life history transitions to differences in population growth rate between inoculated and uninoculated plants is estimated

by: $\tilde{\alpha}^m = \sum_k (\alpha_{ij}^m - \alpha_{ij}^r) \frac{\partial \lambda}{\partial \alpha_{ij}} \Big|_{\frac{1}{2}(A^m + A^r)}$ and the contribution of the lower-level parameter $(x)_k$ to

differences in population growth rate between inoculated and uninoculated plants is estimated

by: $\tilde{\alpha}^m = \sum_k (x_k^m - x_k^r) \frac{\partial \lambda}{\partial \alpha_{ij}} \Big|_{\frac{1}{2}(A^m + A^r)}$.

Contributions to observed differences in λ among virus-inoculated treatments compared to uninoculated plants averaged over all populations. We conducted life table response experiments (LTREs) to quantify the contribution of each life history trait (lower-level parameter) to observed differences in λ among populations, virus treatments, and population*virus combinations (Caswell, 1996; 2001; Levin *et al.*, 1996). Let $A^{(ij)}$ be the population matrix associated with virus treatment and population combination ij , and let $A^{No\ virus}$ be the reference matrix, no virus treatment averaged over all populations. The difference in λ between the population*virus treatment combination and the reference matrix is: $\lambda^{ij} = \lambda^{(No\ virus)} + \alpha^i + \delta^j + \alpha\delta^{ij}$, where α^i (virus) and δ^j (population) are the treatment main effects and $\alpha\delta^{ij}$ are the interaction effects. Following Caswell (1996), the contribution of the lower-level parameter (x_k^{ij}) to differences in λ among virus treatments, populations, and virus*population combinations are estimated by:

$$\begin{aligned}\tilde{\alpha}^{(i)} &= \sum_k \left(x_k^{(i)} - x_k^{(No\ virus)} \right) \frac{\partial \lambda}{\partial x_k} \Big|_{\frac{1}{2}(A^i + A^{No\ virus})}, \\ \tilde{\delta}^{(j)} &= \sum_k \left(x_k^{(j)} - x_k^{(No\ virus)} \right) \frac{\partial \lambda}{\partial x_k} \Big|_{\frac{1}{2}(A^j + A^{No\ virus})}, \text{ and} \\ \tilde{\alpha\delta}^{(i)} &= \sum_k \left(x_k^{(ij)} - x_k^{(No\ virus)} \right) \frac{\partial \lambda}{\partial x_k} \Big|_{\frac{1}{2}(A^{ij} + A^{No\ virus})}, \text{ respectively.}\end{aligned}$$

All calculations and analyses of population growth rates were completed using the R software version 2.15.1 (R Development Core Team, 2012).

Additional perturbation analyses

Stable stage distribution. We calculated the stable stage distribution to determine if the stable stage structure differs among populations and virus treatments. Stable stage distribution is the eigenvector corresponding to the dominant eigenvalue.

Effects of changes in life history transitions and traits on future λ s. To examine the effect of changes in life history transitions and traits on future population growth, we conducted perturbation analyses. We evaluated the sensitivity of λ to additive, infinitesimal small changes in each life history transition (prospective analysis, Caswell, 2001). Sensitivity (S) is the local slope of the asymptotic population growth rate, λ , as a function of a matrix element, α_{ij} , holding

all other parameters constant, $S = \frac{\partial \lambda}{\partial a_{ij}}$. Sensitivity quantifies the dependence of λ on the matrix element in order to understand how small changes in life history traits due to abiotic and biotic factors may affect the growth of a population. Since each matrix element is composed of a number of life history traits (see Modeling; Table 1), we also conducted lower level sensitivity analyses (LLS). LLS determines the effect of an infinitesimal small, additive change in a parameter, x , on λ , $LLS = \sum_{i,j} \frac{\partial \lambda}{\partial a_{ij}} \frac{\alpha_{ij}}{\partial x}$. In addition, we calculated elasticity matrices, E , to examine the effect of a small proportional change in each demographic transition on (α_{ij}) on λ : $E = \left(\frac{\alpha_{ij}}{\lambda} \frac{\partial \lambda}{\partial \alpha_{ij}} \right)$ (de Kroon *et al.*, 1986; Caswell, 2001, p. 226). We calculated lower level elasticities (LLE) to determine the effect on the population growth rate to proportional change in each life history trait, x . LLE is $\left(\frac{x}{\lambda} \frac{\partial \lambda}{\partial x} \right)$ do not sum to 1 since λ is not expected to be a homogeneous function of x (Caswell, 2001).

Results

Demographic matrices for each wild *C. pepo* population and virus treatment (Table S1).

Randomization tests. See Main Manuscript (Fig. 2a-c and Table S2a,b).

Life table response experiments

Contributions to observed differences in λ among virus-inoculated treatments compared to uninoculated plants for each population. See Main Manuscript (Fig. 2d-i).

Contributions to observed differences in λ among virus treatments compared to uninoculated plants averaged over all populations.

Main effect of virus infection – The number of gourds per plant (g) and average seeds per gourd (s) both contributed to the differences in the λ between CMV-inoculated plants and both uninoculated and ZYMV-inoculated plants (Fig. S2a).

Main effect of population – Although the λ of the Eagle population was lower than in either the Onward or Vaiden populations, the life history traits that contributed to these differences were not the same. In particular, the λ s of the Eagle and Vaiden populations differed because germination of <1-year old seeds (b_0), number of gourds per plant (g), number of seeds per gourd (s), and dormant seeds (d) contributed differently to λ s (Fig. S2b). For example, relative to the average matrix, the number of gourds per plant (g) reduced the λ of the Eagle population, but increased the λ of the Vaiden population. In contrast, the λ s of the Eagle and Onward populations differed because germination of <1-year old seeds (b_0), the probability of surviving to flower (f), and dormant seeds (d) contributed differently to λ . In addition, although the λ s of the Onward and Vaiden populations do not differ from one another, the probability that new seeds germinate (b_0), the probability that seedlings survive to flower (f), the number of gourds per plant (g), the number of seeds per gourd (s), and the probability a seed remains dormant (d) all contribute differently to λ (although the magnitude these contributions varies among life history traits; Fig. S7b). For example, relative to the average matrix the number of seeds per gourd has a greater contribution to the λ of the Onward population in comparison to Vaiden.

Effect of virus*population interaction – The contributions of the number of gourds per plant (g), number of seeds per gourd (s), and germination <1-year old seeds (b_0) to λ depended on particular combinations of virus treatment and population, over and above the effect of either virus treatment or population alone (Fig. S2c). For example, in addition to the main effects of virus and population, the number of gourds per plant contributed to lower λ in Eagle plants regardless of virus treatment, lower λ in Onward plants inoculated with CMV and ZYMV, lower λ in Vaiden plants inoculated with CMV, but higher λ in uninoculated Vaiden plants and Vaiden plants inoculated with ZYMV (Fig. S2c).

Additional perturbation analyses

Stable stage distribution. The stable stage structure differs among populations (Fig. S3). At the stable age distribution the Vaiden population contains very few seeds, which is likely the result of high germination and low dormancy in this population. Inoculation with virus had very little effect on the stable age distribution (Fig. S3).

Effects of changes in demographic transitions on future λ . The sensitivity of λ to changes to each life history transition varied among populations and virus treatments (Fig. S4). Across all virus treatments in the Onward and Eagle populations the λ depends on the seedbank-to-adult transition and the adult-to-adult transition (Fig. S4a,b). However, for the Vaiden population across all virus treatments the λ primarily depends on the adult-to-adult transition (Fig. S4c).

Sensitivity of λ to perturbations in most life history traits differed among populations and slightly varied among virus treatments (Fig. S5). For all populations and virus treatments, changes in the λ were most sensitive to in the probability a gourd opens (o_0) and germination of new seeds (b_0). Interestingly, λ was not sensitive to additive changes in the average number of gourds per plant (g), average number of seeds per gourd (s), or gourd viability (v ; Fig. S5).

Elasticity analyses indicated that the effect of potential changes in demographic transitions on future λ differs among populations and varies slightly in magnitude among virus treatments (Fig. S6). In Vaiden, a small perturbation to the adult-to-adult transition has the largest effect on the λ , followed by the gourd-to-adult and adult-to-gourd transitions. In Onward, the effect of the adult-to-adult transition is reduced (relative to Vaiden) and the seed-to-adult, gourd-to-adult, adult-to-gourd, and seed-to-gourd transitions all have minor effects on λ . Finally, in Eagle, the effect of the adult-to-adult transition is reduced even further (relative to Vaiden) and the effects of the seed-to-adult, gourd-to-adult, adult-to-gourd, and seed-to-gourd transitions are all larger than in Onward. Thus, the effect of transitions to and from dormant stages (i.e. seedbank and gourd bank) on potential future λ is greatest in the Eagle population, less in the Onward population, and least in the Vaiden population.

Among all populations and virus treatments, elasticity analysis indicates that λ is dependent on many lower level parameters (life history traits; Fig. S7). In particular, λ is dependent on those life history traits used to estimate the probability an adult produces a new adult in the next year. Although λ did not differ between virus treatments in Eagle, elasticities for many lower level parameters differed between uninoculated and virus-inoculated treatments.

Table S1. Demographic matrix for each wild *Cucurbita pepo* population ((a, d, g): Onward, (b, e, h): Eagle, (c, f, i): Vaiden) and virus treatment ((a-c): No: uninoculated plants, (d-f): CMV: plants inoculated with *Cucumber mosaic virus*, (g-i): ZYMV: plants inoculated with *Zucchini yellow mosaic virus*) used to estimate population growth rate.

		(a) No				(d) CMV				(g) ZYMV		
		Gourd	Seed	Adult		Gourd	Seed	Adult		Gourd	Seed	Adult
Onward	Gourd	0.154	.	2.84	Gourd	0.154	.	2.08	Gourd	0.154	.	1.80
	Seed	0.118	0.160	4.56	Seed	0.094	0.160	2.67	Seed	0.106	0.160	2.62
	Adult	0.162	0.220	2.80	Adult	0.129	0.220	1.64	Adult	0.146	0.220	1.61
Eagle	Gourd	0.154	.	1.98	Gourd	0.154	.	1.57	Gourd	0.154	.	2.10
	Seed	0.079	0.090	2.13	Seed	0.066	0.090	1.42	Seed	0.078	0.090	2.24
	Adult	0.136	0.160	0.677	Adult	0.114	0.160	0.45	Adult	0.134	0.160	0.71
Vaiden	Gourd	0.154	.	3.20	Gourd	0.154	.	1.87	Gourd	0.154	.	4.83
	Seed	0.004	0.010	0.17	Seed	0.004	0.009	0.09	Seed	0.004	0.010	0.27
	Adult	0.078	0.180	2.33	Adult	0.071	0.185	1.24	Adult	0.081	0.180	3.67

Table S2. Results of randomization tests comparing population growth rates (λ) between (a) virus treatments (No: uninoculated plants, CMV: plants inoculated with *Cucumber mosaic virus*, and ZYMV: plants inoculated with *Zucchini yellow mosaic virus*) within each population, and (b) populations (Onward, Eagle, Vaiden) within each virus treatment.

(a)	No	Onward-Eagle	0.021	(b)	Onward	No-CMV	0.244
		Onward-Vaiden	0.595			No-ZYMV	1.000
		Eagle-Vaiden	0.151			CMV-ZYMV	0.152
CMV	Onward-Eagle	0.048	Eagle	No-CMV	0.744		
		Onward-Vaiden			0.355	No-ZYMV	0.966
		Eagle-Vaiden			0.402	CMV-ZYMV	0.715
ZYMV	Onward-Eagle	0.013	Vaiden	No-CMV	0.126		
		Onward-Vaiden			0.731	No-ZYMV	0.062
		Eagle-Vaiden			0.041	CMV-ZYMV	0.008

Figure S2. Contributions of life history traits (abbreviations in Fig. 1c) to λ for (a) each virus treatment (No: uninoculated plants, CMV: plants inoculated with *Cucumber mosaic virus*, and ZYMV: plants inoculated with *Zucchini yellow mosaic virus*), (b) each wild *Cucurbita pepo* population (Onward, Eagle, and Vaiden), and (c) each population*virus treatment combination. Note: contributions to differences in λ can only be estimated for parameters that differ among virus treatments (g, s) or populations (b_0, f, g, s, d).

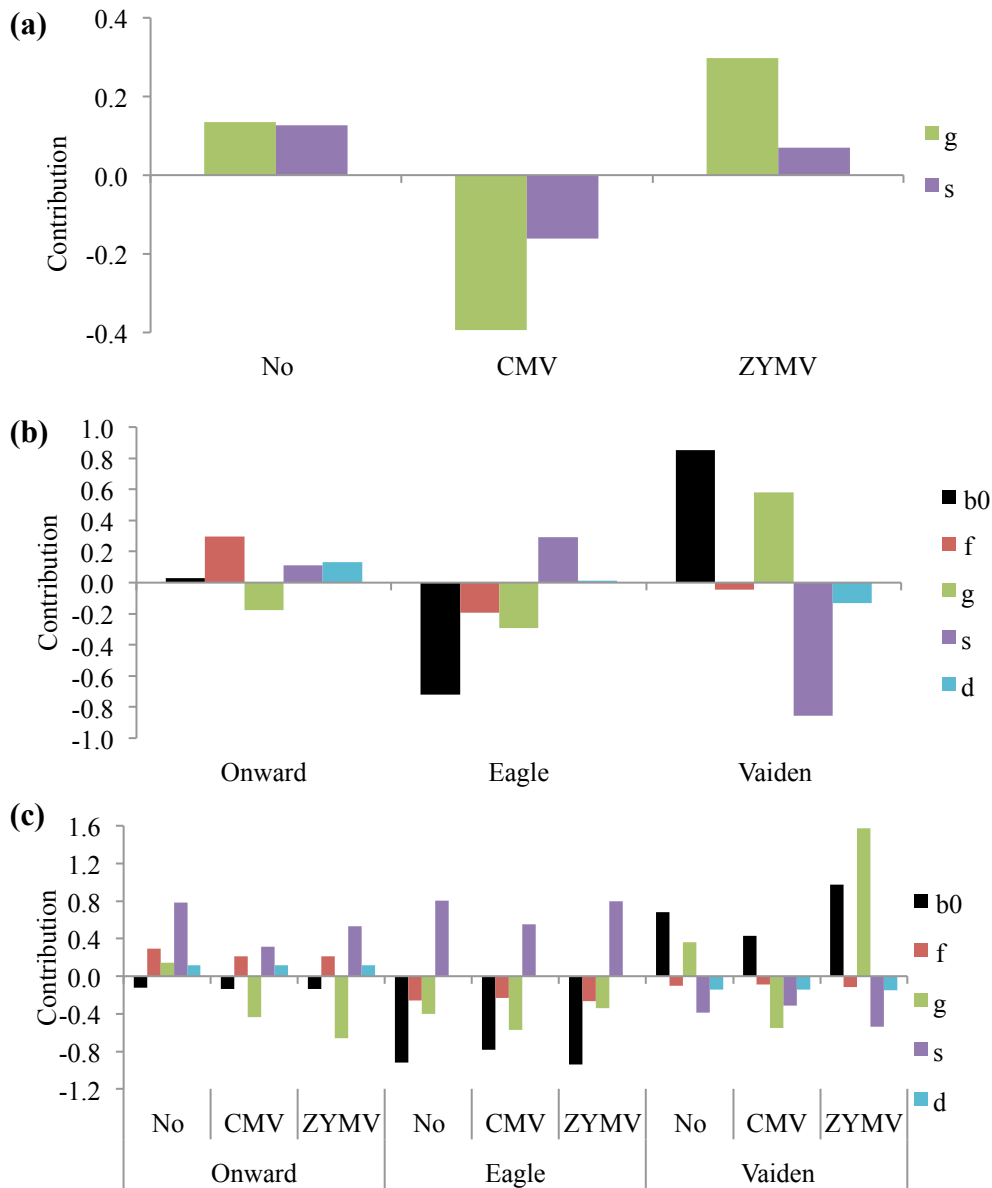


Figure S3. Stable stage distribution indicates the proportion of wild *Cucurbita pepo* population (Onward, Eagle, and Vaiden) in each stage (A: adult, S: seedbank, G: gourd bank) with each virus treatment (No: uninoculated plants, CMV: plants inoculated with *Cucumber mosaic virus*, or ZYMV: plants inoculated with *Zucchini yellow mosaic virus*).

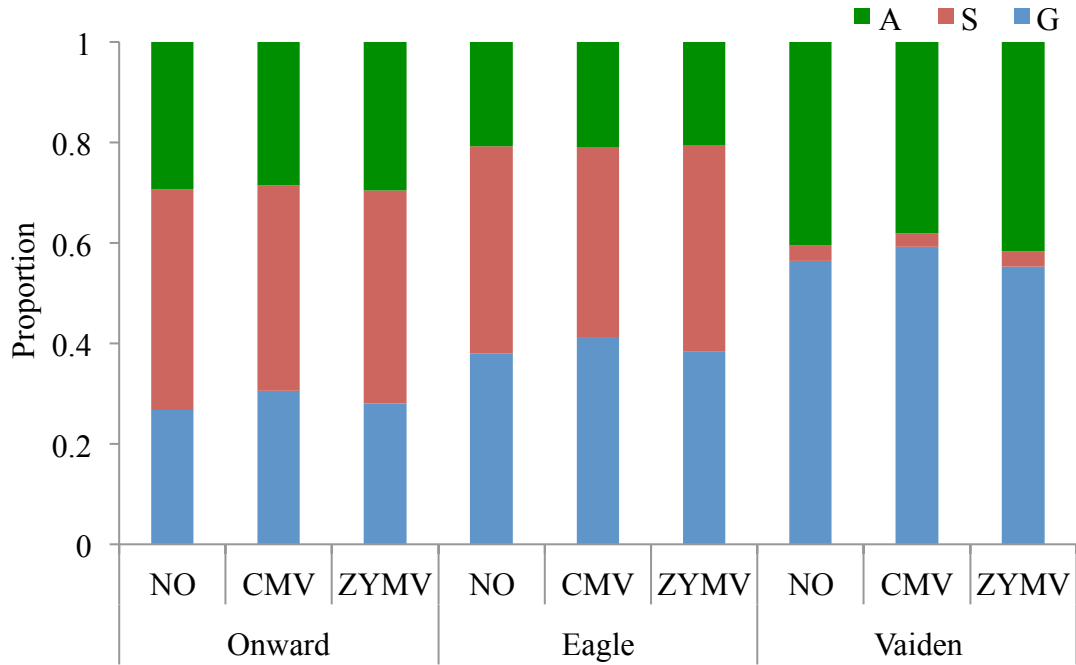


Figure S4. Sensitivity values for each matrix element by each wild *Cucurbita pepo* population: (a) Onward, (b) Eagle, and (c) Vaiden and virus treatment (No: uninoculated plants, CMV: plants inoculated with *Cucumber mosaic virus*, and ZYMV: plants inoculated with *Zucchini yellow mosaic virus*). Life history transitions are presented in Figs 1a,b.

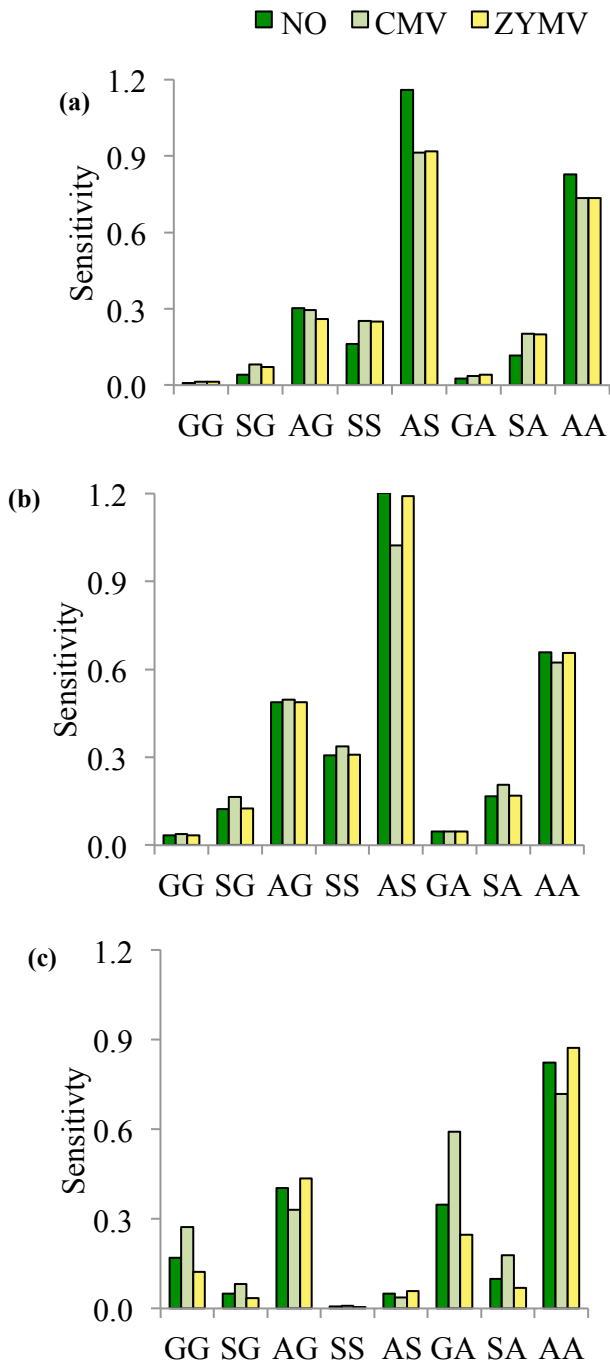


Figure S5. Sensitivity values for each life history trait (abbreviations in Fig. 1c) by wild *Cucurbita pepo* population: (a) Onward, (b) Eagle, and (c) Vaiden and virus treatment (No: uninoculated plants, CMV: plants inoculated with *Cucumber mosaic virus*, and ZYMV: plants inoculated with *Zucchini yellow mosaic virus*).

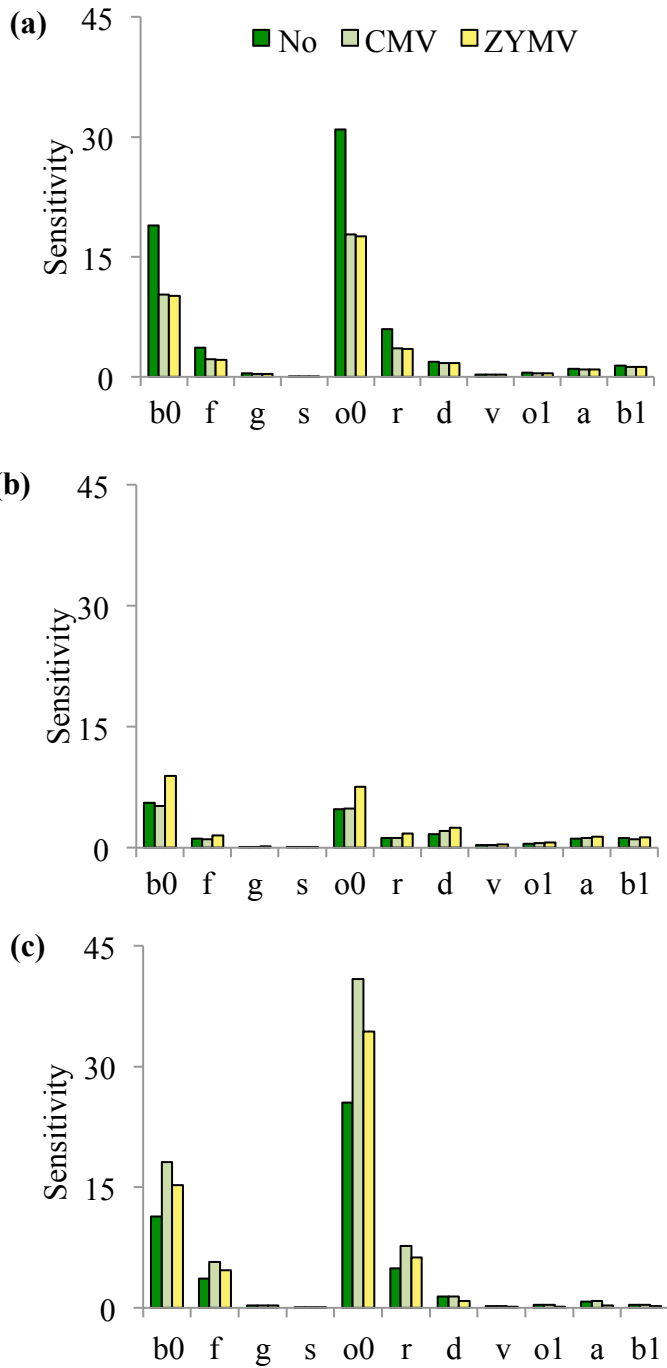


Figure S6. Elasticity values of demographic transitions (i.e. matrix transition elements) for each wild *Cucurbita pepo* population (Onward, Eagle, and Vaiden) and virus treatment combination (No: uninoculated plants, CMV: plants inoculated with *Cucumber mosaic virus*, and ZYMV: plants inoculated with *Zucchini yellow mosaic virus*). Life history transitions are represented by AA: adult produces an adult; SA: seed in seedbank develops into adult; GA: gourd in gourd bank opens to form an adult; AS: adult produces a gourd that opens and seed enters seedbank; SS: seed remains in seedbank; GS: gourd in gourd bank opens and seed enters seedbank; AG: adult produces a gourds that enters gourd bank; and GG: gourd remains in gourd bank.

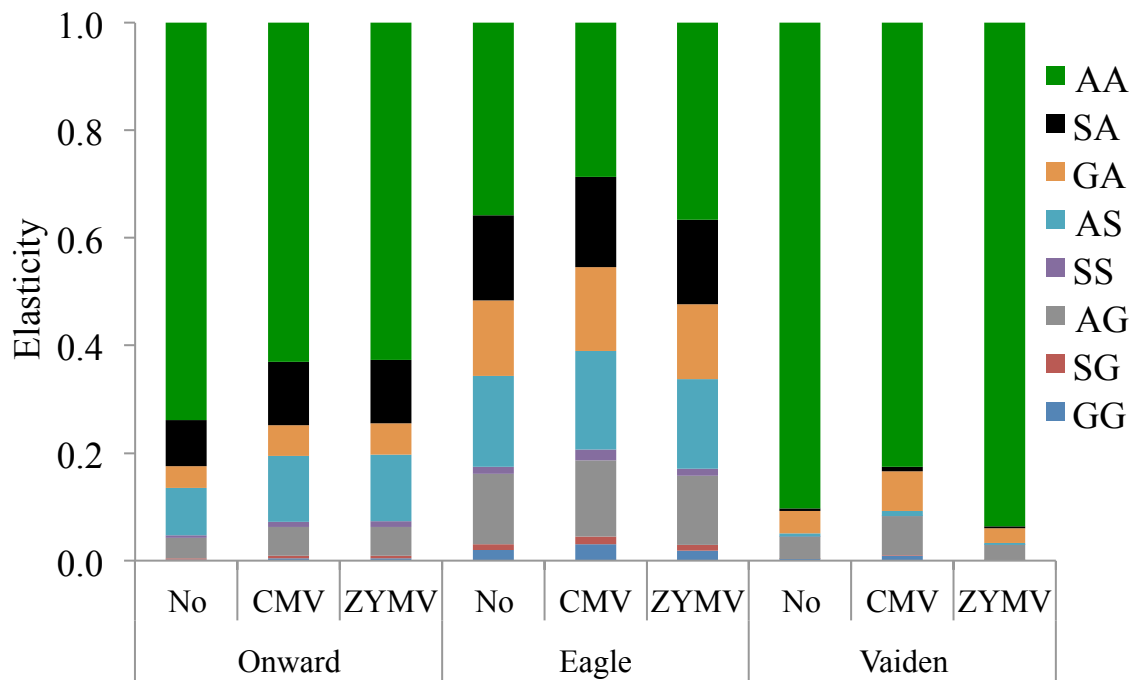
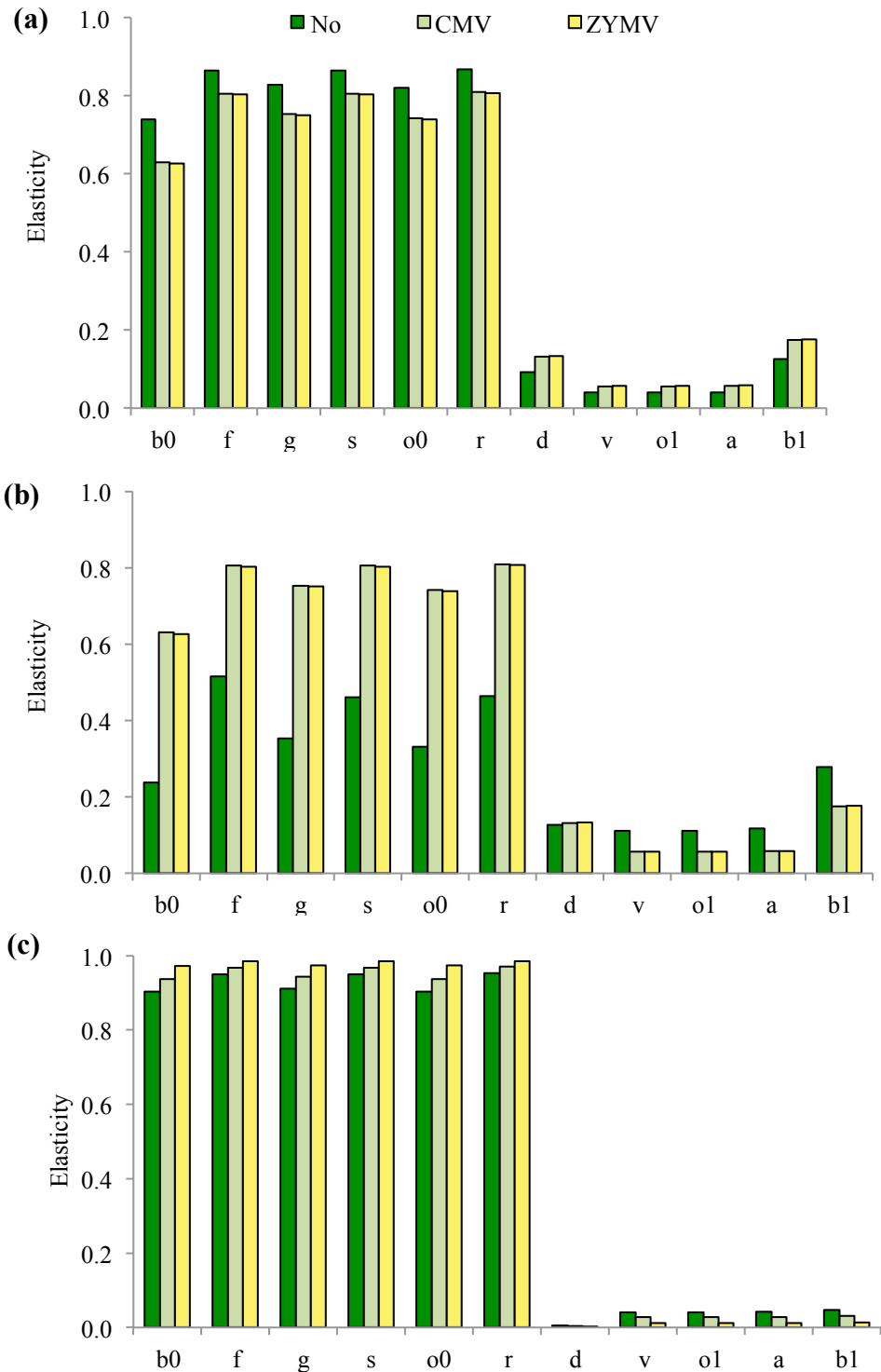


Figure S7. Elasticity values for each life history trait (abbreviations in Fig. 1c) for each wild *Cucurbita pepo* population: (a) Onward, (b) Eagle, (c) Vaiden and virus treatment (No: uninoculated plants, CMV: plants inoculated with *Cucumber mosaic virus*, and ZYMV: plants inoculated with *Zucchini yellow mosaic virus*).



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