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Effects of virus on plant fecundity and population dynamics

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Summary

- 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.

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 variation 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 microorganims 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 Mölken & 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 annual 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 (Supporting Information 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 run-off. 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.).

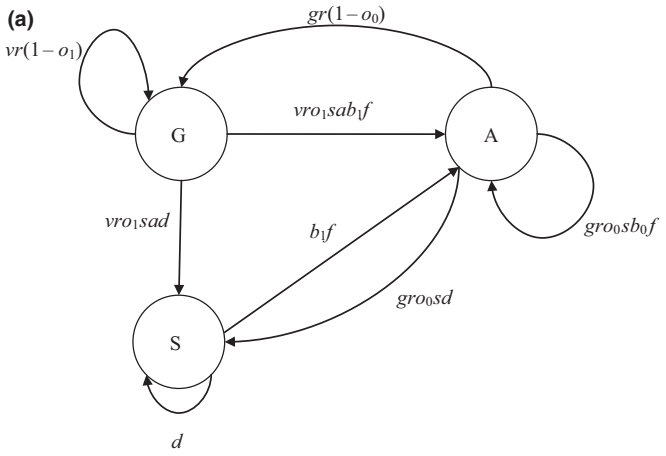
Wild *C. pepo* is susceptible to virus, and virus prevalence varies among populations, years, and virus species (Prendeville *et al.*, 2012). Prevalence ranges from 0% to 100%, with a median infection rate of 25% (Quemada *et al.*, 2008; Prendeville *et al.*, 2012). In 80% of virus infections, wild *C. 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 nonpersistently 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 (Lefkovitch 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 pre-breeding 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).

(a)



(b)

	Gourd (G)	Seed (S)	Adult (A)
Gourd (G)	$vr(1-o_1)$	.	$gr(1-o_0)$
Seed (S)	vro_1sad	d	gro_0sd
Adult (A)	vro_1sab_1f	b_1f	gro_0sb_0f

(c)

Life-history traits	Symbol
Probability gourds less than 1 yr old open	o_0
Probability gourds more than 1 yr old open	o_1
Probability gourds are not consumed by rodents	r
Probability seeds more than 1 yr old germinate	b_1
Probability gourd more than 1 yr old is viable	v
Probability seeds are dormant	d
Proportion of seeds more than 1 yr old that are viable	a
Probability seeds less than 1 yr old germinate	b_0
Probability seedlings survive to flower	f
Average number of gourds per plant	g
Average number of seeds per gourd	s

Fig. 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.

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 × virus inoculation treatment (ZYMV, CMV) was replicated once, but each population × 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 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 CropScience, Research Triangle Park, NC, 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 × 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 × 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 × 20 cm × 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 were used to estimate the germination

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
g	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
s	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
d	Onward	*		na	na	0.16	0.062, 0.074
	Eagle					0.09	0.29, 0.034
	Vaiden					0.009	0.011, 0.014
b_0	Onward	***		na	na	0.13	0.024, 0.026
	Eagle					0.05	0.015, 0.018
	Vaiden					0.20	0.030, 0.059
f	Onward	**		na	na	0.77	0.04
	Eagle					0.56	0.05
	Vaiden					0.65	0.05
o_0		ns		na	na	0.09	0.06
o_1		ns		na	na	0.20	0.07
r		ns		na	na	0.48	0.05
b_1		ns		na	na	0.29	0.10, 0.12
v		ns		na	na	0.43	0.10
a		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: *, $P < 0.05$; **, $P < 0.01$; ***, $P \leq 0.007$; 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 ; b_0, probability seeds less than 1 year old germinate ; f, probability seedlings survive to flower ; o_0, probability gourds less than 1 year old open; o_1, probability gourds more than 1 year old open ; r, probability gourds are not consumed by rodents ; b_1, 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.

rate of seeds > 1 yr old, b_1. 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 b_1 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 × 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 × 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

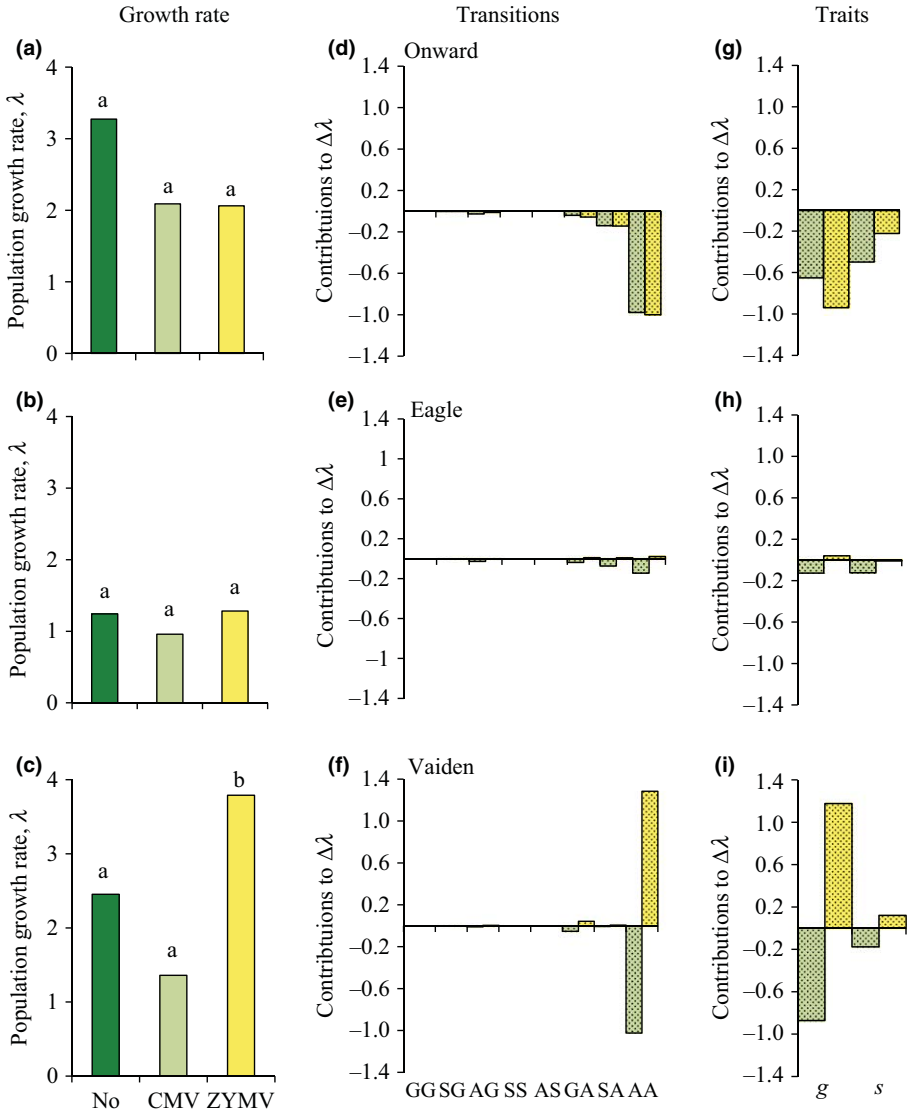


Fig. 2 Population growth rates (λ) of wild *Cucurbita pepo* from three populations (a, Onward; b, Eagle; c, Vaiden) subjected to three virus treatments: No, uninoculated plants; CMV, plants inoculated with *Cucumber mosaic virus*; and ZYMV, plants inoculated with *Zucchini yellow mosaic virus*. Different 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.

transitions and life-history traits most 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 population

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 & Mihail, 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 non-transgenic 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.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 Population growth rates of wild *Cucurbita pepo* estimated from the literature.

Fig. S2 Contributions of life-history traits to λ for each virus treatment, each wild *Cucurbita pepo* population, and each population × virus treatment combination.

Fig. S3 Stable stage distribution indicates the proportion of wild *Cucurbita pepo* population in each stage with each virus treatment.

Fig. S4 Sensitivity values for each matrix element by each wild *Cucurbita pepo* population and virus treatment.

Fig. S5 Sensitivity values for each life-history trait by wild *Cucurbita pepo* population and virus treatment.

Fig. S6 Elasticity values of demographic transitions for each wild *Cucurbita pepo* population and virus treatment combination.

Fig. 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.

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