

Food quality and egg laying patterns in the Colorado potato beetle, *Leptinotarsa
decemlineata*

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Abstract

In Colorado potato beetles, *Leptinotarsa decemlineata*, neonates begin consuming unhatched eggs in as little as one hour after hatching, and cannibals are overall more fit than non-cannibals. I hypothesized that in order to maximize the fitness of the clutch, when subjected to less than ideal nutritional circumstances females will lay their eggs in such a way that facilitates cannibalism. Cannibalism facilitation was measured by the degree of asynchronous hatching of offspring in the same clutch, and an increased ratio of sterile eggs to fertile eggs. Females from three different populations (Maine, Ohio, and a laboratory standard population) were subjected to three qualities of plant ages: 4 weeks (young; high concentration of plant toxins), 5 weeks (control; optimal quality), and 6 weeks (beginning to senesce; minimal quality). There was a significant effect of plant age on hatching synchrony, but differently than I predicted—clutches hatched more asynchronously as plant age increased, instead of hatching most synchronously on 5 week old plants. There was no effect of plant age on the proportion of sterile eggs.

Introduction

Species are adapted to compensate for offspring's inability to provide adequately for themselves, whether because of their altricial nature at birth, or a depletion in the amount or quality of resources available for consumption once they hatch. Parental care for offspring is represented by a variety of behavioral tendencies and strategies across taxa. Parental care, or the investment of a parent, or parents, in the successful development and maturation of offspring, can vary from a complete absence of care following the birth or oviposition of offspring, to active parental involvement until the offspring reach sexual maturity.

Not only does parental care vary in duration, but also in the degree to which each of the parents is involved. In many bird species, both the male and female in a breeding pair will participate in some form of parental care. In monogamous bird species, such as the snow bunting, *Plectrophenax nivalis*, male parental care is necessary for providing sufficient amounts of food for the young, particularly in periods of low food availability (Lyon, et al., 1987). However, in most species the female is the primary, if not the only, caregiver, if parental care is provided at all. This is especially the case in polygamous species in which the males invest heavily in sexual competition (Patris and Baudoin, 2000).

This gender-biased trend in parental care is also true in the vast majority of insects (Tallamy, 1984). Insects display a variety of forms of parental care, from defending eggs against potential predators to adjusting the pattern of oviposition in time and space

(Tallany, 1984) by, for example, dispersing the egg batch in order to reduce the likelihood that a predator will find and consume the eggs. The tactics employed by the parent(s) depends on the adaptive pressure specific to their environment. For example, in the lace bug, *Gargaphia solani*, mothers will actively protect and defend their eggs until they hatch—this form of parental care evolved in response to heavy predation on eggs and nymphs (Tallany, 1984). Without the presence of these predators and no maternal investment, there was an 80% survival rate among offspring, but with predation and no parental protection, survivorship is negligible (Tallany, 1984).

Insect parental care and investment in the fitness of the clutch is limited not only to active protection of the eggs, but can be manifested in such tactics as adjusting egg laying techniques (Tallany, 1984). For example, because the ratio of the number of fertile to infertile eggs varies naturally in each clutch, it is possible that insect mothers manipulate their egg laying to provide trophic eggs, eggs that are infertile for the purpose of providing a source of food for their offspring (Perry & Roitberg, 2006). Mothers may also employ such tactics as adjusting locations of oviposition or manipulating hatch synchrony to ensure a food supply for the offspring when they hatch. This is particularly common in cannibalistic insect species, in which consumption of a conspecific can significantly increase the fitness of the cannibal. In the ladybird beetle, *Harmonia axyridis*, conspecifics can serve as a valuable source of nutrition for larvae, increasing survivorship and decreasing the developmental time of the cannibal (Piiroinen et al., 2011). Cannibalism can be of particular importance if the food supply is diminished or limited, whether as a consequence of an increase in the number of individuals in a given area, or a reduction in the amount of resources. In the case of the aphidophagus ladybird

species *Propylea dissecta* and *Coccinella transversalis*, when food supply for larvae was low, the frequency of cannibalism increased significantly under laboratory conditions (Pervez et al., 2006).

A decrease in the quality of food available for offspring can also result in an increased benefit from the consumption of a conspecific. In the ladybird species *Harmonia axyridis*, Snyder and colleagues found that when larvae were fed a combination of either one or both of two aphid quality types (low or intermediate quality), and were either permitted to or prevented from cannibalizing, they were able to complete development only if they were fed intermediate quality aphids in combination with the consumption of conspecifics, but on no other diet (2000). For species in these circumstances that have specific food requirements, cannibalism is not only beneficial, but a necessary adaptation for the continuation and success of the population.

Another species in which cannibalism is a common behavioral tendency, particularly in neonates, is the Colorado potato beetle, *Leptinotarsa decemlineata*, an introduced pest of the potato plant, *Solanum tuberosum* (Hare, 1990). This species has been the single most economically important crop pest in the American potato industry for over 130 years, as attempts to control them using pesticides, though successful for about 80 years (Casagrande, 1987), have ultimately proven to be unsustainable. From the time they were recognized as a crop pest in the middle of the 19th century (Edgerton, 1861), a variety of chemical control agents, such as Paris Green, arsenicals, and DDT, were utilized (Casagrande, 1987). However, due to issues of environmental chemical pollution and pesticide resistance, the use of many of these chemicals had to be

terminated. Because of the pest management difficulties unique to Colorado Potato Beetles, this species is of particular interest in scientific research.

L. decemlineata, native to Mexico, is in the Chrysomelid family, which classifies all leaf eating beetles. Adult potato beetles overwinter by burying themselves in the soil, cued, in part, by the cessation of the host plant leaves, and beetles in colder climates tend to, on average, bury themselves deeper into the soil than do beetles in warmer climates (Hiisaar, 2006). They remain in diapause until spring, when they emerge and immediately disperse to find food. Females will begin laying clutches of 20-60 eggs on the underside of potato leaves between 5-7 days after emergence from the soil, and will mate repeatedly (Hare, 1990). After hatching, offspring progress through 4 instars before reaching pupation, following which is the adult stage (Hare, 1990).

After hatching from their eggs, the neonates begin to consume unhatched eggs in as little as one hour (Collie et al., 2013). Cannibalism in this species, which occurs primarily between larvae and eggs, provides an important source of protein to newly hatched individuals of this primarily herbivorous species (Polis, 1981). Collie and colleagues found that individuals that consumed a conspecific egg spent significantly less time in the first instar than did individuals who did not cannibalize (2013). Because of the shortened first instar, the cannibalistic individuals emerged as adults earlier than did non-cannibals (Collie et al., 2013). Early emergence from pupation is advantageous for the individual because it allows them access to food more quickly than their non-cannibal siblings, as well as reduces the amount of time they spend in stages that are more vulnerable to predation. Cannibalism has an increased benefit when there is a shortage in

food availability or a reduction in food quality, as eating a conspecific would result in a decrease in competition as well as increased fitness for the cannibal.

However, because cannibalism usually takes place among siblings, there is an overall fitness cost. Cannibalistic offspring are eliminating shared genes, on average 50% for full siblings and 25% for half siblings, by consuming an individual that could have otherwise developed to reach sexual maturity. Cannibalism will only be favored, then, if the consumption of a sibling increases the fitness of the cannibal enough to compensate for the loss of the sibling (Perry & Roitberg, 2005). For the consumption of a sibling to be favorable, the fitness of the cannibal must increase by at least 50% when the victim is a full sibling and by at least 25% if the victim is a half sibling. In such cases when the food available to offspring is reduced in quantity or quality, and cannibalism increases the overall fitness of the clutch, it can be adaptive for the mothers to employ tactics to facilitate cannibalism.

In a study done by Izzo et al., *L. decemlineata* were shown to be able to assess the quality of potato plants (2014). The beetles were provided with plants that were being grown either on a long day cycle, as is typical of plants during the summer, or on a short day cycle, as is typical of plants as fall and winter approach. As the photoperiod decreased, the nitrogen levels in the plants decreased; 65% of beetles being fed short-day plants initiated diapause in response to this chemical cue (Izzo et al., 2014). This suggests that the potato beetle is capable of assessing and responding to the quality of the host plants in order to, in this case, process the plant's cue that winter is approaching and proceed to enter diapause. There is, therefore, a possibility that potato beetles may take these assessments into consideration when laying their eggs. Since *L. decemlineata*

mothers have expressed discretion in their egg laying techniques (Collie, 2013), and may, therefore, manipulate oviposition through the adjustment of hatching synchrony, dividing of batches, and altering the ratio of viable to inviable eggs, will they do so in response to the quality of the plant provided?

I hypothesized that in response to plant quality, mothers will adjust their oviposition strategies to maximize the overall fitness of the clutch; as food quality decreases, the females may increase the ratio of trophic/infertile eggs to fertile eggs, may decrease the number of eggs per clutch, and may increase hatching asynchrony. To test this hypothesis gravid females were subjected to optimum quality, medium quality, or low quality potato plants, and the females' oviposition patterns and neonate hatching patterns were monitored for each clutch. Female age was also taken into consideration, as insect fecundity tends to decline as the female senesces (Medeiros et. al, 2000). A *post hoc* analysis of the effect of female age was conducted.

Methods

Sample

The sample included several populations of Colorado potato beetles. One sample originated from a population collected in a field site in Maine and had been reared in the laboratory for one year, one sample originated from a population that had been lab-reared in Michigan for 20 years, and the third sample was collected directly from a wild Ohio population as eggs, neonates, and adults. We received the first two populations as eggs and reared them to sexual maturity. The second generation of offspring from the lab-

reared eggs were the individuals used for this experiment, and the wild individuals were used directly.

The grandparents and parents of the individuals in our lab-reared samples were strategically outbred in order to prevent confounding variables that may stem from inbreeding. The individual populations were kept separate in order to evaluate any behavior differences that may exist as a result of the number of generations that each of the three populations spent in a laboratory environment.

Enclosures

The eggs were incubated in 250 mL plastic cylindrical containers lined with paper towel on the bottom and covered with mesh for ventilation. They remained in these enclosures on a potato leaf, the base of which was submerged in water held within a floral pick, until they hatched. Once the individuals reached the fourth instar, which was indicated by the presence of a single dark band behind the head, 3 inches of soil was added to their enclosures to facilitate pupation. Five days after emerging from pupation, all of the lab-reared individuals were sexed and 30 females (15 from each lab population) were immediately paired with a designated male for mating. For eggs collected from the wild Ohio population, the same methods applied. For adults collected from the Ohio population, females were paired with a male 5 days after collection to insure sexual receptivity. The breeding enclosures were identical to the rearing enclosures. Each female was paired with a male for at least 24 hours and allowed to lay three clutches of eggs before being placed into their respective experimental conditions.

The dimensions of the experimental enclosures were 1'L x 1'W x 2'H and had one of three different plant qualities present at a time. Each enclosure contained a full potted Yukon Gold potato plant and one gravid female.

Plants

Yukon Gold potato plants were used for all feeding throughout rearing, breeding, and experimentation. For the rearing and breeding enclosures, the beetles were fed potato leaves from a water pick *ad libitum*.

Experimental conditions

For the experimental enclosures there were 3 kinds of conditions: 4 week old plants (young); 5 weeks old plants (optimal), and 6 week old plants (old). At 5 weeks the plant is considered to be of optimal nutritional quality, at 4 weeks it considered to be is of lesser quality because of the concentration of toxins in the younger plant, and at 6 weeks it is considered to be of minimal quality because the plant begins to senesce and nitrogen levels begin to drop.

Experimental Data

After each of the females laid eggs in their respective experimental conditions, the clutches were assessed for the following data at the 24 and 48 hour mark: presence or

absence of eggs, number of eggs total, number of eggs per clutch, number of fertile eggs (indicated by the presence of pigmentation 24 hours before hatching), number of sterile eggs (indicated by a lack of pigmentation 24 hours prior to projected hatch date), ratio of sterile eggs to fertile eggs in each clutch, hatching synchrony, and the number of days since the female's initial pairing with the pre-selected male.

Statistical analysis

The effect of the variables on number of eggs total, number of eggs per clutch, and ratio of sterile eggs to fertile eggs (analyzed as the number of sterile eggs standardized to number of fertile eggs), was analyzed using a mixed model, employing restricted maximum likelihood (REML) to provide unbiased estimates of variance among results. The ratio of sterile eggs to fertile eggs and all measures of hatching synchrony were transformed in order to improve the normality of the distributions. For the ratio of sterile eggs to fertile eggs, the number of sterile eggs laid per day was logarithmically transformed, number of sterile eggs' = $\ln(\text{number of sterile eggs}+1)$. The total number of eggs per day and the number of eggs per clutch were not transformed.

Three different measures were used to analyze hatching synchrony: (1) calculating total hatch time as standardized to mean batch size, using the equation: standardized hatch time = mean batch size x (total hatch time)/(number of eggs); (2) calculating the average interval between 2 sequential hatching events; (3) calculating the

proportion of eggs per clutch that were vulnerable to cannibalism, analyzed as the number of delayed-hatch eggs standardized to clutch size (Perry and Roitberg, 2005). The time threshold for determining the proportion of delayed hatch eggs was 7 hours (Collie, 2013). The first measure, standardized hatch time for each clutch, was logarithmically transformed, $\text{standardized hatch time}' = \ln(\text{standardized hatch time} + 1)$. For the second measure, the average interval between 2 sequential hatching events for each clutch was reciprocally transformed, $\text{average interval}' = 1/(\text{average interval})$. For the third measure, proportion of delayed hatch eggs for each clutch was logarithmically transformed, $\text{proportion of delayed-hatch eggs}' = \ln(\text{proportion of delayed-hatch eggs} + 1)$. All statistical tests and transformations were assessed using JMP v.8.0.2 (SAS Institute, Inc., Cary, NC, U.S.A.).

Results

Number of eggs total and number of eggs per clutch

The number of total eggs laid per day was negatively correlated with the age of the female ($P = 0.015$; Fig. 1, Table 1). The number of eggs per clutch was also negatively correlated with the age of the female ($P = 0.016$; Table 1). There was no significant effect of plant age on number of number of eggs laid per day ($P=0.99$; Table 1) or per clutch ($P=0.97$; Table 1).

Ratio of sterile eggs to fertile eggs

There was no effect of plant age on the number of sterile eggs standardized to number of fertile eggs ($P=0.37$; Table 2).

Average interval between hatching events

Plant age had a significant positive effect on the average interval between two sequential hatching events ($P=0.005$; Fig. 2, Table 3). There was a positive correlation between the age of the plant and the asynchrony of the clutches; as plant age increased, the average interval between hatchlings increased. The day had a marginal effect on this measure of synchrony ($P=0.068$; Table 3). Clutches tended to hatch more synchronously on day 2 than on day 1.

Total hatch time standardized to mean batch size

Plant age had a marginal effect on the standardized hatch time of each clutch ($P=0.094$; Table 4); there was a trend toward asynchrony as plant age increased.

Proportion of delayed-hatch eggs

For the proportion of delayed-hatch eggs in each clutch, there was a significant effect of population ($P=0.045$; Table 5), female age ($P=0.030$; Table 5), plant age ($P=0.025$; Table 5), day ($P=0.012$; Table), number of eggs per clutch ($P=0.0003$; Table 5), and interaction between female age and plant age ($P=0.01$; Table 5). The wild-caught

Ohio population had a significantly higher proportion of delayed-hatch eggs overall, compared to the Maine or lab-standard populations, with the greatest difference in proportion being between the Ohio population and the lab standard population. As female age increased there was an increase in the proportion of delayed-hatch eggs in each clutch. As plant age increased, there was an increase in the proportion of delayed-hatch eggs, and most significantly so between 4 and 5 week old plants. On day 2 there was a decrease in the proportion of delayed hatch eggs per clutch compared to day 1. Finally, younger females had a significantly higher proportion of delayed-hatch eggs on 4 week old plants than did older females, a significantly lower proportion on 5 week old plants than did older females, and both young and old females had similar proportions on 6 week old plants.

Discussion

This experiment yielded both predicted and unpredicted results, especially as it relates to the effect of plant age. For number of eggs laid, a decrease in the number of eggs per clutch was expected with decreasing plant quality, but there was no effect of plant age on this measure.

Plant age did have some of the predicted effects on hatching synchrony. For the measures of synchrony that calculated average interval between sequential hatching events, total hatch time standardized to mean batch size, and the proportion of delayed-hatch eggs, as expected, clutches laid on 6 week old plants had the highest level of asynchrony. While not providing sterile or trophic eggs, asynchronous hatching on senescing plants would provide early hatching individuals a larger window of time to

consume unhatched siblings, providing the survivors with a high-protein nutritional supplement to the senescing plant on which they hatched. Given that hatchlings have increased fitness when they consume an unhatched sibling (Collie et al., 2013), it follows that the female would manipulate this measure in order to increase survivorship of successful hatchlings. However, instead being most synchronous on 5 week old plants, synchrony steadily decreased as plant age increased. Clutches laid on 5 week old plants were expected to have the most synchronous hatching patterns, as sibling cannibalism would not be necessary to maximize the fitness of the clutch on an optimally nutritious plant; the optimally nutritious plant would provide the necessary nutrients for each individual in the clutch.

One possible explanation for this trend is that 4 week old plants may actually be the most nutritionally optimal of the 3 plant ages, rather than the 5 week old plants. Using a younger plant, possibly 2 to 3 weeks old, may yield results that better illustrate the effect of concentrated toxins in younger plants. However, even if this is the case, the hypothesis concerning egg laying patterns of females on aging plants, specifically as it relates to offspring hatching synchrony, is supported. Ecologically, being able to detect plant age, through nitrogen levels for example, is adaptive not only for maximizing overall clutch fitness in response to whatever nutritional value remains in a plant as it begins to age, but for cueing circannual rhythms in the adults (Izzo et al., 2014).

Fecundity in female insects is negatively affected by female age once they begin to senesce (Medeiros et. Al, 2000), and the results from my analysis reflect this relationship. As females aged, the number of eggs laid per clutch and in total each day decreased significantly; this relationship corresponds to the pattern observed in *Podisus*

nigisprius (Medeiros et. al, 2000). These findings confirm that the activity of the reproductive system decreases. Female age also had an effect on the proportion of delayed hatch eggs. Offspring of older female Coleopterans tend to develop more slowly (Fox, 1993), which corresponds to the increased proportion of delayed-hatch eggs seen in this analysis.

Concerning the effect of population on the results, there was a significant effect on the proportion of delayed-hatch eggs. It is possible that these behavior differences result from the varying degrees of removal from the wild stock population that exists between the 3 sample groups, as is implied by the trend of increased proportion of delayed-hatch eggs as the time spent in lab conditions increases (lab standard population > Maine > Ohio). The lack of natural selection pressure that comes from exposure to naturally varying qualities of food may explain the increased response of the lab populations.

The effect of day on the hatching synchrony measured by the proportion of delayed-hatch eggs was significant in that the clutches tended to hatch more synchronously on the second day, regardless of the plant age. It may be possible that as a female has more time to assess the plant that she is consuming and on which she is laying her eggs, she adjusts the synchrony with which her eggs hatch. The effect of the interaction between plant age and female age indicated that there is a significant difference between the degree of hatching synchrony between young and old females on 4 week and 5 week old plants. On 4 week old plants, younger females laid their highest proportion of delayed-hatch eggs and older females laid their lowest proportion of delayed hatch eggs. On 5 week old plants the pattern switched; younger females laid their

lowest proportion of delayed-hatch eggs, older females laid their highest. Though neither young nor old females exhibited the trend of increased proportion of delayed hatch eggs with the increase of plant age, this difference may reflect the deterioration of the reproductive competence of the older females due to senescence.

The results of this study illuminate future questions to be answered concerning the reproductive behavior patterns of Colorado potato beetles, potential subsequent pest management strategies, and assessments of current management strategies used most commonly by potato farmers. For example, at what plant age is hatching synchrony, on average, maximized? Similarly, what is the trend of increasing hatching asynchrony throughout the average growing season of a potato plant? What is the difference in survivorship and fitness between individuals raised on low quality plants versus those raised on optimum quality plants? How are potato farmers currently distributing pesticides throughout the growing season? Although the data from this study reflects behavior trends in response to 4, 5, and 6 week old plants, a study of egg laying behavior trends as it relates to increasing hatching synchrony throughout the entire growing season of a potato plant could provide more useable information to pest managers.

Given the history of the potato beetle's developed resistance to a variety of pesticides, future research along these lines would potentially allow farmers to vary the concentration of pesticides used throughout the growing season. If, overall, the fitness and number of surviving offspring vary throughout the growing season in response to plant age, farmers may be able to decrease the concentration of pesticides during certain times in the season. This would not only begin to minimize the ecological impacts of

pesticide use, but would potentially increase the amount of time it takes for a population of potato beetles to develop resistance to the pesticide being used.

Understanding the behavioral ecology of a pest species, especially one that has impacts on so grand a scale as the Colorado potato beetle, is central to constructing natural, environmentally sensitive forms of control. Because historic control efforts have so heavily impacted the health of the environment due to the toxicity of the pesticides, we are forced to think ecologically in our future management attempts. When we pursue knowledge about the mechanisms of reproduction, feeding behaviors, circannual rhythms, symbiosis, genetics, and other details about the natural histories of pest species, we contribute to a future of sustainable, maximally effective forms of pest control. Because of biological research, we are able to consider such specialized management alternatives as genetically modifying microorganisms that live within the gut of certain insect pest species (Taracena et al., 2014). The field of biological science and research played a significant role our in nation's past, known for a widespread lack of accountability and proper research in the use of dangerous chemicals. However, out of what became a looming ecological crisis was birthed an awakening in the conscience of the scientific community, prompting us to engage in humble inquisition of the natural world.

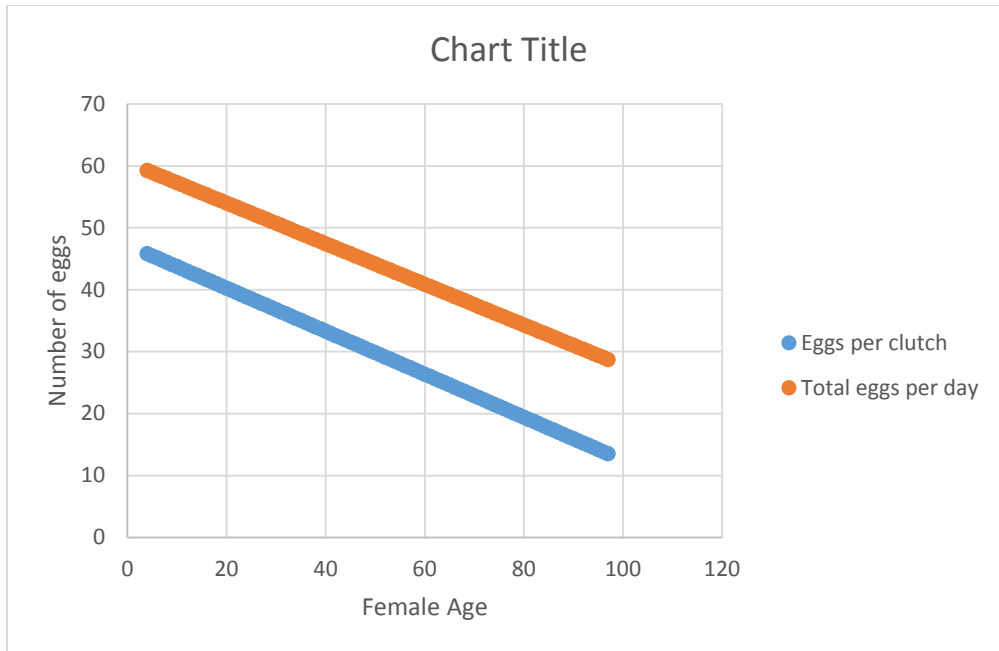


Figure 1. Effect of female age on number of eggs laid per clutch and total eggs per day. The slope of the line indicates that as female age increases, the total number of eggs laid per clutch and day decreases.

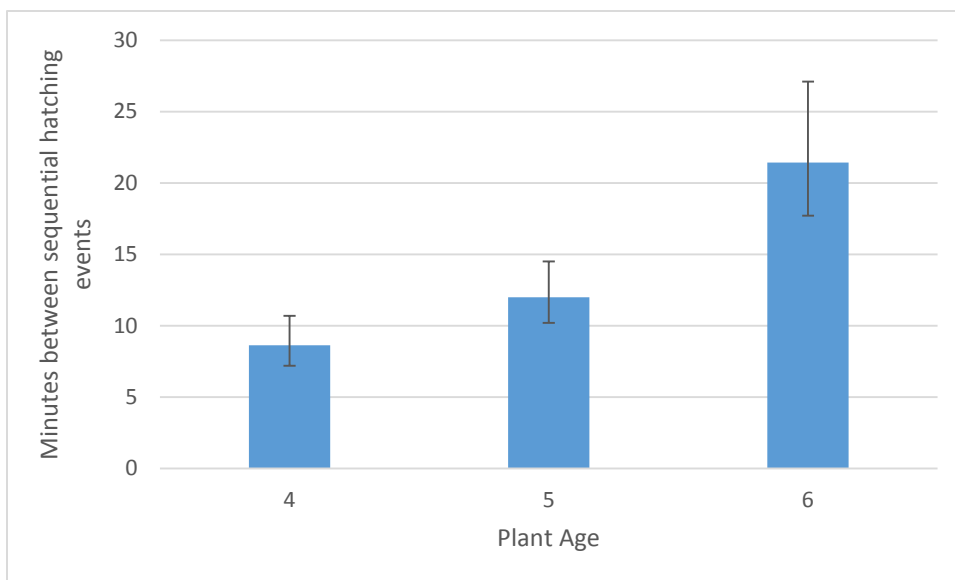


Figure 2. Effect of plant age on the average interval between 2 sequential hatching events. As plant age increased the amount of time between 2 sequential hatching events increased. The error bars indicate 95% confidence interval. Average interval values are reverse transformed.

Table 1. The effect of female age and plant age on the number of eggs total per day and number of eggs per clutch.

<i>Number of eggs total (per day)</i>				
R ²		0.511		
<i>Female age</i>	F value	DF	P value	
	6.7	1, 30.4	0.015	
<i>Plant age effect</i>	F value	DF	P value	
	.009	2, 31.4	0.99	
		Plant age	LS means	Confidence interval
		4	46.4	33.4-59.4
		5	47.4	36.4-58.4
		6	46.9	38.5-55.3
<i>Number of eggs per clutch (per day)</i>				
R ²		0.774		
<i>Female age</i>	F value	DF	P value	
	6.4	1,36.0	0.016	
<i>Plant age effect</i>	F value	DF	P value	
	.03	2, 36.3	0.97	
		Plant age	LS means	Confidence interval
		4	33.1	18.7-47.6
		5	31.7	19.3-44.1
		6	31.1	22.1-40.2

Table2. The effect of plant age on ratio of sterile eggs to fertile eggs, analyzed as the number of sterile eggs standardized to the number of fertile eggs.

<i>Ratio of sterile eggs to fertile eggs</i>				
R ²		0.816		
	F value	1.04		
<i>Plant Age Effect</i>	DF	2, 34.2		
	P value	0.37		
	Plant age	LS means	Confidence interval	
	4	1.6	0.4-3.6	
	5	0.6	0.0010-1.6	
	6	0.8	1.6-0.3	

LS means and confidence interval are reversed transformed

Table 3. The effect of population, plant age, and day on the average interval between 2 sequential hatching events.

<i>Average interval between 2 sequential hatching events</i>			
R ²		0.783	
<i>Population Effect</i>	F value	DF	P value
	5.4	1, 4.5	0.073
	Population	LS mean	Confidence interval
	MI	10.86484	9.7-12.3
	OH	13.90627	11.6-17.4
<i>Plant Age Effect</i>	F value	DF	P value
	20.9	2, 4.6	0.005
	Plant age	LS means	Confidence interval
	4	8.623788	A 7.2-10.7
	5	12.00194	B 10.2-14.5
	6	21.43347	C 17.7-27.1
<i>Day Effect</i>	F value	4.02	
	DF	1, 11.7	
	P value	.068	
	Day	LS means	Confidence interval
	1	13.73061	11.5-17.1
	2	10.97333	9.8-12.5

LS means and confidence intervals reverse transformed. Different letters indicate significant differences between confidence intervals.

Table 4. The effect of plant age on total hatch time as standardized to mean batch size.

<i>Total hatch time as standardized to mean batch size</i>			
R ²		0.783	
<i>Plant Age Effect</i>	F value	6.0	
	DF	2, 2.9	
	P value	0.095	
	Plant age	LS means	Confidence interval
	4	296.7182	97.5-898.3
	5	598.3191	255.6-1398.8
	6	1859.384	1152.2-3000.3

LS means and confidence intervals reverse transformed

Table 5. The effects of population, female age, plant age, day, and the interaction between female age and plant age on proportion of delayed-hatch eggs.

<i>Proportion of delayed hatch eggs</i>				
R²		0.881		
<i>Population Effect</i>	F value	4.6		
	DF	2, 8.2		
	P value	0.045		
	Population	LS mean	Confidence interval	
	ME	1.445292	A, B 0*-6.02	
	MI	0.241428	A 0*-1.9	
	OH	3.046797	B 0.4-10.3	
<i>Female Age Effect</i>	F value	5.9		
	DF	1, 13.1		
	P value	0.03		
<i>Plant Age Effect</i>	F value	4.9		
	DF	2, 13.2		
	P value	0.025		
	Plant age	LS mean	Confidence interval	
	4	0*	A 0*-.8	
	5	8.49838	B, C 3.4-19.4	
	6	9.899295	C, B 6.7-14.4	
<i>Day Effect</i>	F value	8.06		
	DF	1, 15.9		
	P value	0.012		
	Day	LS mean	Confidence interval	
	1	2.481969	A 0.5-7.2	
	2	0.529036	B 0*-3.0	
<i>Interaction between female age and plant age</i>	F value	.0112		
	DF	2, 13.3		
	P value	0.011		

*Negative values rounded to 0. LS means and confidence intervals reverse transformed. Different letters indicate significant differences between confidence intervals.

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