5-2018

CONDITION-DEPENDENT LIFE HISTORY STRATEGIES

Shivani V. Jadeja
University of Nebraska-Lincoln, shivanivj@gmail.com

Jadeja, Shivani V., "CONDITION-DEPENDENT LIFE HISTORY STRATEGIES" (2018). *Dissertations and Theses in Biological Sciences*. 100.
https://digitalcommons.unl.edu/bioscidiss/100

CONDITION-DEPENDENT LIFE HISTORY STRATEGIES

by

Shivani Vijayraj Jadeja

A DISSERTATION

Presented to the Faculty of

The Graduate College at the University of Nebraska

In Partial Fulfilment of Requirements

For the Degree of Doctor of Philosophy

Major: Biological Sciences

(Ecology, Evolution and Behavior)

Under the Supervision of Professor Brigitte Tenhumberg

Lincoln, Nebraska

May, 2018

CONDITION-DEPENDENT LIFE HISTORY STRATEGIES

Shivani Vijayraj Jadeja, Ph.D.

University of Nebraska, 2018

Adviser: Brigitte Tenhumberg

Many organisms evolve condition-dependent life history strategies to maximize their lifetime fitness in response to intrinsic and extrinsic processes. I investigated a sequentially flowering plant's strategy to allocate resources to retain flowers versus grow existing basal fruits to a larger size, using the plant *Yucca glauca*. The sink strength hypothesis suggests basal fruits are nutrient sinks depriving distal flowers of resources and reducing their probability of retention. A low probability of retention of distal flowers can also be explained by the architectural effects hypothesis. This hypothesis posits inherent positional differences in structures along an inflorescence such as flower size and amount of vascular tissues decrease flower retention with increasing flower position, independent of the number of basal fruits. I experimentally showed that the presence of basal fruits decreased the probability of retention of distal flowers, which supports the sink strength hypothesis. Further, in the absence of fruits, plants retained distal flowers at a probability similar to that of basal flowers, which is inconsistent with the architectural effects hypothesis. Next, I developed a stochastic dynamic programming model to examine the conditions under which decreasing flower retention in response to existing basal fruits is optimal for sequentially flowering plants. The model predicts that plants should decrease flower retention with increasing number of basal fruits when large fruits produce more viable seeds than small fruits (fruit size-dependent viability benefit).

Finally, I tested if a higher probability of flower abortion in the presence of basal fruits affects the life history strategy of insects that lay eggs in flowers. *Yucca glauca* flowers are egg-laying sites for seed-eating insect *Tegeticula yuccasella*. Flowers that have a high probability of being aborted are low quality egg-laying sites for *T. yuccasella* because all eggs in aborted flowers die. I experimentally showed that when basal fruits were present, *T. yuccasella* were less likely to lay eggs in flowers. These investigations help identify mechanisms underlying condition-dependent plant and animal life history strategies that contribute to intra-population variation in life history strategies.

ACKNOWLEDGEMENTS

I thank my advisor, Brigitte Tenhumberg, for her continuous guidance and support, and constructive criticism that have been critical to the success and completion of my dissertation. I thank my committee, Diana Pilson, Drew Tyre, Daizaburo Shizuka, and Jeffrey Stevens, for their support and feedback at all stages of my research that has greatly helped improve my work. I thank the Cedar Point Biological Station staff, especially Jon Garbisch, for logistical support at the field site, and Nate Nielson for permission to access the Kingsley dam surface for field work. I thank, the Holland Computing Center of the University of Nebraska, which receives support from the Nebraska Research Initiative, for use of their supercomputing facilities to run my mathematical models. I thank field assistants Tiffany Riffle, Masiel Maza and Pushpraj Chauhan, and laboratory assistants Andrew Gebara, Laura Smuskiewicz and Megan Bjorkman, for their enthusiasm for my research projects and untiring help. I am grateful to the faculty at SBS with whom I interacted during my program for interactions that have helped me become a better researcher, especially Sabrina Russo, from whom I have learned many research skills. I am grateful to my peers who have helped me in so many ways from refining my research ideas and writing to providing much needed support, especially Sarah Cowles, Colton Watts, Alissa Anderson, Jean Philippe Gibert, Marie-Claire Chelini, Yi Yang, Abigail Neyer, Benjamin Reed, Anna Tatarko, Maria Goller, Melissa Whitman, and Chih-Chung Lee. I am also grateful to the SBS administrative staff who have helped with processes necessary for the successful completion of my dissertation, especially Mindy Peck, Kristin Patrick, Julie McManamey, Tammy Kortum, and Linda Trouba. Lastly, I thank my family for their help, unconditional support, and encouragement at every step of my doctoral research.

GRANT INFORMATION

This research was supported by funds from the J. Ve. Srb Memorial Fund, Sigma Xi Grants-in-Aid of Research Award, Center for Great Plains Studies, and Warren and Edith Day Dissertation Travel Award to Shivani Jadeja. Field research assistants were supported by funds from the J. Ve. Srb Memorial Fund to Tiffany Riffle, and Janovy Fund Award to Masiel Maza.

TABLE OF CONTENTS

Introduction 1

Chapter 1: Presence of fruits decrease probability of retaining flowers in a sequentially flowering plant 15

- ABSTRACT 15
- INTRODUCTION 16
- MATERIALS AND METHODS 20
- RESULTS 29
- DISCUSSION 31
- CONCLUSIONS 35
- ACKNOWLEDGEMENTS 36
- REFERENCES 36
- FIGURES 41
- SUPPLEMENTARY MATERIAL 44

Chapter 2: Optimal flower retention and fruit growth strategies in sequentially flowering plants 55

- INTRODUCTION 55
- THE MODEL 59
- CASE STUDY OF YUCCA GLAUCA 69
- RESULTS 73
- DISCUSSION 77
- REFERENCES 80

TABLES AND FIGURES 85

SUPPLEMENTARY MATERIAL..92

Chapter 3: Phytophagous insect oviposition shifts in response to probability of flower abortion owing to the presence of basal fruits 106

ABSTRACT...106

INTRODUCTION 107

MATERIALS AND METHODS...110

RESULTS 122

DISCUSSION...124

ACKNOWLEDGEMENTS...131

REFERENCES 131

FIGURES...137

SUPPLEMENTARY MATERIAL...142

Introduction

Life history strategies are constrained by limited time and resources resulting in trade-offs between life history components such as trade-offs between current and future fitness, and size and number of offspring (Stearns 1989). Consider the trade off between size and number of offspring; an organism with limited resources can either have a life history strategy to allocate resources to produce many small offpsring or a strategy to produce few large offspring. Under these constraints, life history theory predicts that organisms will evolve strategies that maximize their fitness.

In some cases, life history strategies may have evolved that are plastic with respect to heterogeneous conditions such as both low and high predation levels. Species may have evolved condition-dependent strategies that shift with changes in the conditions of the organisms (Gisel 1976, Nylin and Gotthard 1998, Nussey et al. 2007). Such plasticity may allow organisms to maximize their fitness depending on their environment or condition, which is likely a mechanism for buffering species from extinction in the face of changing environmental conditions including anthropogenic habitat degradation (González-Suárez and Revilla 2013). Conditions that organisms may plasticly respond to (i.e. the underlying mechanisms) may be intrinsic or extrinsic to the organism (McNamara and Houston 1996).

Intrinsic conditions that may influence a plant’s life history strategy include its physiology and size (Lloyd 1980, Stephenson 1981, Diggle 1995). For example, larger plants may produce a larger number of fruits than smaller plants (Stephenson 1981).

Extrinsic conditions that organisms may respond to may arise from the abiotic environment. For example, plant species in open-habitats where seedlings are not light-limited produce many small seeds (offspring) with low nutritional provisioning from the parent plant (Salisbury 1974). In contrast, shade-tolerant plant species produce few large seeds with high nutritional provisioning from the parent plant to increase the chances of sustaining seedlings until they can access light to produce their own food (Salisbury 1974).

A major component of biotic interactions that may influence a plant's life history strategies are plant-animal interactions. These interactions may be beneficial (animals pollinate plants) or detrimental (animals herbivorize plants). For example, pollinator behavior may influence which flowers are pollinated, and the extent of seed damage by herbivores can influence which flowers can produce viable seeds, respectively.

Similarly, in animals both intrinsic and extrinsic processes may influence an animal's life history strategy. Intrinsic processes may include age and size of an organism. For example, for an individual with high life expectancy, it may be better to be choosy about egg-laying sites, but for an individual that is nearing the end of its life, it may be better to deposit all its eggs in available sites without concern for their quality instead of wasting time trying to find better egg-laying sites (Roitberg et al. 1993, 1999). Extrinsic processes may be environmental conditions, or an animal's interactions with plants. Interactions with plants can be beneficial or detrimental to the animal. An example of beneficial interactions are pollinators that depends on plants for food such as nectar. Pollinators shift their life history strategy in response to nectar availability. An example of detrimental interactions for herbivores are plant defenses such as toxins that may decrease an animal’s feeding rate. Herbivores may have evolved strategies to minimize the effect of plant toxins, for instance by preferentially feeding on plant tissue with a low toxin concentration.

Figure 1 shows a schematic diagram of processes and strategies that may influence the life history strategies of plants and animals that interact with each other. These factors are likely the driving forces underlying the diversity of life history strategies within and among species. Moreover, elucidating mechanisms underlying condition-dependent life history strategies is an important component for explaining the high intra-species variation in life history traits. Solid-lined arrows indicate mechanisms investigated in this dissertation.

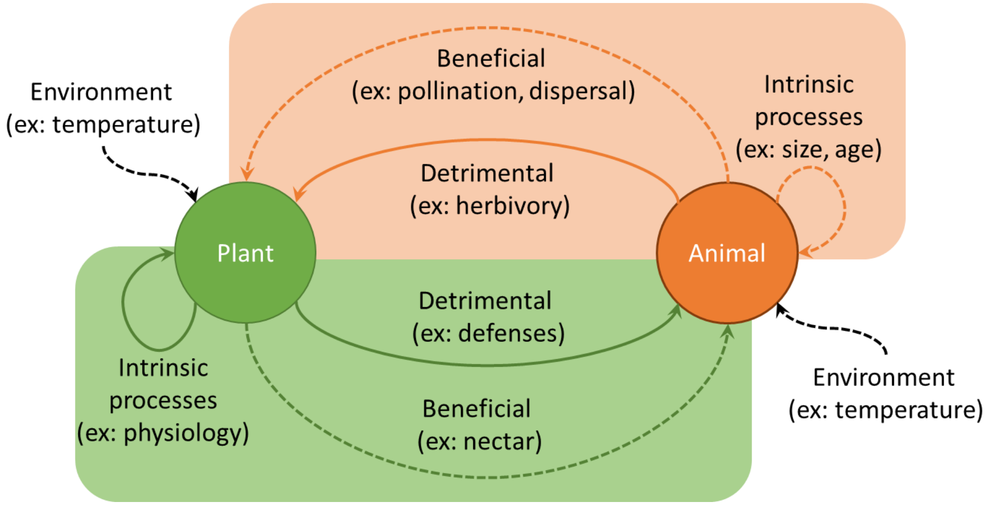


Figure 1. Schematic diagram showing processes/strategies (arrows) that may influence life history strategies of plants (green circle) and animals (orange circle) interacting with each other. Solid-lined arrows are processes/strategies investigated in this dissertation.

Among plants, sequentially flowering plants offer an interesting case for testing hypotheses on context-dependent life history strategies because the presence and abundance of reproductive structures (fruits) may provide information on the allocation of resources among other plant organs. Allocating resources to develop fruits from flowers (henceforth, retain flowers) varies in sequentially flowering plants owing to uncertainty in flower pollination and fruit survival (Stephenson 1981). As a consequence, the number and size of fruits a plant develops early in the flowering period may influence the probability of retaining flowers later in the flowering period. The sink strength hypothesis posits that plants allocate resources to grow fruits that deprive distal flowers of resources (Lloyd 1980, Marcelis et al. 2004), but in the absence of basal fruits, the plant may allocate resources to distal flowers. The sink strength of basal fruits likely results in the widely observed pattern of flower retention along an inflorescence where retention probability of basal flowers is higher compared to distal flowers (Lloyd 1980, Stephenson 1981, Diggle 1995).

For a number of plant species there is evidence that basal flowers have an inherently higher probability of being retained than distal flowers independent of the number of basal fruits (architectural effects hypothesis, reviewed by Diggle 1995, 2003). If over many reprodutive seasons plants are unlikely to retain distal flowers owing to low resource or pollen availability, plants may evolve to reduce allocation to ovaries/ovules of distal flowers, making distal flowers less likely to successfully mature compared to basal flowers. Plants may do this by decreasing amount of vascular tissues, flower size, and/or ovule:pollen ratio with increasing flower position (Diggle 2003). As a consequence, distal flowers become functionally more males.

Which intrinsic process (sink strength or architectural effects) influences a plant's flower retention strategy is important for a better understanding of general patterns of life history strategies in plants. In the first chapter, I explored whether the sink strength or the architectural effects hypothesis explains the flower retention probability in the wild, sequentially flowering plant species *Yucca glauca*. The sink-strength hypothesis has been previously tested mostly with laboratory-based experiments using cultivated plants (Tamas et al. 1979, Stephenson 1980, Susko and Lovett-Doust 1999, Marcelis et al. 2004). In these experiments, cultivated plants are genetically homogeneous and experience a controlled environment. Findings from cultivated plants may not necessarily extend to flower retention in wild plants that have large genetic variation and that grow in heterogeneous environments.

I conducted experimental and observational studies under natural conditions in the field. My results suggest that the presence of basal fruits decreased the retention probability of distal flowers and provide support for the sink-strength hypothesis, but not the architectural effects hypotheses (Chapter 1). These results are consistent with studies on other *Yucca* spp. (Huth and Pellmyr 1997).

A plant's strategy to abort flowers may also be influenced by extrinsic processes of seed herbivory. Seed herbivory damages seeds, which decreases a plant's fitness. Plants may use early cues to assess the potential extent of seed herbivory to selectively retain productive flowers (Burd 1998). Ovules damaged during oviposition by the seed herbivore *Tegeticula yuccasella* has been shown to serve as an early cue for aborting flowers in the congeneric species, *Y. filamentosa* (Marr and Pellmyr 2003).

As part of the first chapter, I investigated whether the condition dependent probability of flower abortion in *Y. glauca* is influenced by early cues of future seed damage by *T. yuccasella*. Following the procedure of Marr and Pellmyr (2003) I mechanically damaged ovules to mimic oviposition by *T. yuccasella*. Contrary to previous work on *Yucca* spp., I did not see an effect of the number of artificial ovipositions on flower retention in *Y. glauca* (Chapter 1) likely because my oviposition treatments were not successful.

Ecologists generally assume that natural selection selects for life history strategies that maximize the fitness of organisms and hence the observed strategies are considered "optimal". The fitness of plants is influenced by the number and size of fruits which determine the number of seedlings a plant produces in the next season. In many plant species larger sized fruits produce larger seeds (Primack 1987) that are more viable (i.e. more likely to transition to a seedling) (Sork 1993, Westoby et al. 1996, Moles et al. 2006). But, larger fruits require more resources to grow and maintain than smaller fruits. A plant's strategy to maximize fitness involves trading-off the number and size of fruits.

The size number trade-off in offspring production has been well-studied theoretically. To my knowledge, all but one study assume offspring are produced at one time during each reproductive period (i.e. not sequentially) (Smith and Fretwell 1974, Lloyd 1987, reviewd by Dani and Kodandaramaiah 2017, except Sakai and Harada 2004). These studies suggest that an organism's optimal number-size strategy depends on the shape of the fitness function (Smith and Fretwell 1974, Lloyd 1987, Dani and Kodandaramaiah 2017), resource availability, and density-dependent offspring mortality (Venable 1992).

The one exception is a model examining whether it is optimal for organisms to produce offspring sequentially within one reproductive period (Sakai and Harada 2004). The model predicts that sequential offspring production is rare, and yet, sequentially flowering plants are common in nature. One possible reason for this contradiction is that the model does not consider the role of mutualists in fertilization success. Many plant species may have evolved to flower sequentially to increase the chances that flowers are open when pollinators are present (Rathcke and Lacey 1985).

If it is most beneficial to grow few large fruits, then we expect sequentially flowering plants to abort distal flowers in response to the presence of basal fruits (Tamas et al. 1979, Stephenson 1980, Susko and Lovett-Doust 1999, Marcelis et al. 2004, Chapter 1) A plant may benefit from growing fruits to a larger size if there is a size-dependent fitness benefit including (1) larger fruits produce larger seeds (Primack 1987) that are more viable (i.e. a size-dependent viability benefit) (Sork 1993, Westoby et al. 1996, Moles et al. 2006), and (2) larger fruits have a higher survival probability owing to a lower risk of damage from herbivory or storms owing to their stronger tissues than smaller fruits (i.e. a size-dependent survival benefit) (Stephenson 1981).

In the second chapter, I investigated the role of size-dependent viability and size-dependent survival in the optimal reproductive strategy (number and size of fruits) of sequentially flowering plants. I developed a stochastic dynamic programming (SDP) model (Clark and Mangel 2000) predicting how plants should allocate resources between retaining pollinated flowers (initiating new fruits) and growing existing fruits. The model incorporates stochastic processes of flower pollination and fruit survival. Further, the model prediction teases apart flower abortion owing to lack of pollination versus a plant’s strategy to abort pollinated flowers, which is difficult to observe empirically. To create a stronger link between theory and empirical work, I parametrized my model for *Y. glauca* for which I have empirically observed fruiting patterns (Chapter 1).

My model predicts that in the presence of a size-dependent viability benefit, it is optimal for plants to grow few large fruits and to decrease the proportion of flowers retained with increasing number of basal fruits. One proximate process that produces the same pattern of flower retention is the strong sink strength of basal fruits depriving distal flowers of resources (Lloyd 1980, Marcelis et al. 2004). But, when a plant's efficiency to convert photosynthate to fruit tissue (fruit growth efficiency) is either too high or too low, flower retention is independent of the number of basal fruits. Plants with a very high fruit growth efficiency tend to retain more flowers with the same amount of resources than plants with lower fruit growth efficiencies. A high fruit growth efficiency might be expected in plants producing green, photosynthesizing fruits. Plants with a very low fruit growth efficiency also tend to retain more flowers because available resources are insufficient to grow fruits to the largest size.

When plants have a size-dependent survival benefit and no size-dependent viability benefit, my model predicts plants grow existing fruits to a larger size. However, plants do not grow any fruits to the largest possible size. Instead, plants retain all pollinated flowers, independent of the number of basal fruits. It is likely that plants get the maximum fitness from growing fruits to intermediate sizes to escape very low survival probabilities at small fruit sizes, and utilizing remaining resources to retain flowers.

In the third chapter, I studied the detrimental process of flower abortion by the plant (extrinsic process) on the egg-laying strategy of a seed-eating insect that lays eggs in flowers. Optimal oviposition theory (Resetarits 1996) and the preference performance hypothesis for plant-eating insects (Thompson 1988, Mayhew 1997) suggest that females will lay eggs in high quality sites that maximize the survival of their offspring. Much of the studies have focused on how plant-eating insects shift their strategy to lay eggs in response to differences in quality among host plant species (Renwick and Chew 1994, Gripenberg et al. 2010). Less attention has been given to how strategies to lay eggs shift in response to potential differences in the quality of egg-laying sites within a single plant species. These are equally important in determining mechanisms underlying condition-dependent life history strategies.

Female egg-laying site quality may vary considerably within a host plant species. I studied the insect species, *Tegeticula yuccasella*, that lays eggs in and pollinates host plant flowers, and their developing larvae herbivorize host plant seeds. A host plant may abort flowers with eggs for multiple reasons including a high number of eggs (Pellmyr and Huth 1994) and high sink strength of basal fruits (Chapter 1). If a flower is aborted, all eggs within die. Hence, flowers that are more likely to be aborted are poor egg-laying sites. I hypothesized, that insects avoid oviposition in flowers that are more likely to be aborted owing to the presence of basal fruits. It is possible that egg-laying strategies of mutualistic seed herbivores like *T. yuccasella* may differ from those of antagonistic seed herbivores. Testing my hypothesis using seed herbivore *T. yuccasella* that is in a mutualistic relationship with *Y. glauca* is a conservative test of life history strategies of seed herbivores in response to the quality of egg-laying sites on host plants.

Consistent with my hypothesis, I show that *T. yuccasella* avoids laying eggs in distal flowers that are more likely to be aborted due to the presence of basal fruits (Chapter 3), which to my knowledge has not been previously reported for seed herbivores. What possible cues would enable this life history strategy to evolve needs further investigation and may include chemical or tactile cues in distal flowers or basal fruits. This study contributes to the growing body of evidence showing that females prefer laying eggs in sites that increase the survival chances of their offspring (Gripenberg et al. 2010).

References

Burd, M. 1998. “Excess” flower production and selective fruit abortion: a model of potential benefits. - Ecology 79: 2123–2132.

Clark, C. and Mangel, M. 2000. Dynamic state variable models in ecology: Methods and applications. - Oxford University Press.

Dani, K. G. S. and Kodandaramaiah, U. 2017. Plant and animal reproductive strategies: lessons from offspring size and number tradeoffs. - Front. Ecol. Evol. 5: 38.

Diggle, P. K. 1995. Architectural effects and the interpretation of patterns of fruit and seed development. - Annu. Rev. Ecol. Syst. 26: 531–552.

Diggle, P. K. 2003. Architectural effects on floral form and function: a review. - In: Stuessy, T. F. et al. (eds), Deep morphology: toward a renaissance of morphology in plant systematics. ARG Gantner Verlag: Ruggell, Lichtenstein, pp. 63–80.

Gisel, J. T. 1976. Reproductive strategies as adaptations to life in temporally heterogeneous environments. - Annu. Rev. Ecol. Syst. 7: 57–79.

González-Suárez, M. and Revilla, E. 2013. Variability in life-history and ecological traits is a buffer against extinction in mammals. - Ecol. Lett. 16: 242–251.

Gripenberg, S. et al. 2010. A meta-analysis of preference-performance relationships in phytophagous insects. - Ecol. Lett. 13: 383–393.

Huth, C. J. and Pellmyr, O. 1997. Non-random fruit retention in *Yucca filamentosa*: consequences for an obligate mutualism. - Oikos 78: 576–584.

Lloyd, D. G. 1980. Sexual strategies in plants. I. An hypothesis of serial adjustement of maternal investment during one reproductive session. - New Phytol. 86: 69–79.

Lloyd, D. G. 1987. Selection of offspring size at independence and other size- versus-number strategies. - Am. Nat. 129: 800–817.

Marcelis, L. F. M. et al. 2004. Flower and fruit abortion in sweet pepper in relation to source and sink strength. - J. Exp. Bot. 55: 2261–2268.

Marr, D. L. and Pellmyr, O. 2003. Effect of pollinator-inflicted ovule damage on floral abscission in the yucca-yucca moth mutualism: the role of mechanical and chemical factors. - Oecologia 136: 236–243.

Mayhew, P. J. 1997. Adaptive patterns of host-plant selection by phytophagous insects. - Oikos 79: 417–428.

McNamara, J. and Houston, A. I. 1996. State-dependent life histories. - Nature 380: 215–221.

Moles, A. T. et al. 2006. Seed size and plant strategy across the whole life cycle. - Oikos 113: 91–105.

Nussey, D. H. et al. 2007. The evolutionary ecology of individual phenotypic plasticity in wild populations. - J. Evol. Biol. 20: 831–844.

Nylin, S. and Gotthard, K. 1998. Plasticity in life-history traits. - Annu. Rev. Entomol. 43: 63–83.

Pellmyr, O. and Huth, C. J. 1994. Evolutionary stability of mutualism between yuccas and yucca moths. - Nature 372: 257–260.

Primack, R. B. 1987. Relationships among flowers, fruits, and seeds. - Ann. Rev. Ecol. Syst. 18: 430.

Rathcke, B. and Lacey, E. P. 1985. Phenological patterns of terrestrial plants. - Annu. Rev. Ecol. Syst. 16: 179–214.

Renwick, J. A. A. and Chew, F. S. 1994. Oviposition behavior in Lepidoptera. - Annu. Rev. Entomol. 39: 377–400.

Resetarits, W. J. 1996. Oviposition site choice and life history evolution. - Integr. Comp. Biol. 36: 205–215.

Roitberg, B. et al. 1993. Life expectancy and reproduction. - Nature 364: 108–108.

Roitberg, B. D. et al. 1999. Vive la variance: A functional oviposition theory for insect herbivores. - Entomol. Exp. Appl. 91: 187–194.

Sakai, S. and Harada, Y. 2004. Size-number trade-off and optimal offspring size for offspring produced sequentially using a fixed amount of reserves. - J. Theor. Biol.

226: 253–264.

Salisbury, E. 1974. Seed size and mass in relation to environment. - Proc. R. Soc. Lond. B 186: 83–88.

Smith, C. C. and Fretwell, S. D. 1974. The optimal balance between size and number of offspring. - Am. Nat. 108: 499–506.

Sork, V. L. 1993. Evolutionary ecology of mast-seeding in temperate and tropical oaks (*Quercus* spp.). - Vegetatio 107/108: 133–147.

Stearns, S. C. 1989. Trade-offs in life-history evolution. - Funct. Ecol. 3: 259–268.

Stephenson, A. G. 1980. Fruit set, herbivory, fruit reduction, and the fruiting strategy of *Catalpa speciosa* (Bignoniaceae). - Ecology 61: 57–64.

Stephenson, A. G. 1981. Flower and fruit abortion: proximate causes and ultimate functions. - Annu. Rev. Ecol. Syst. 12: 253–279.

Susko, D. J. and Lovett-Doust, L. 1999. Effects of resource availability, and fruit and ovule position on components of fecundity in *Alliaria petiolata* (Brassicaceae). - New Phytol. 144: 295–306.

Tamas, I. A. et al. 1979. Effect of older fruits on abortion and abscisic acid concentration of younger fruits in *Phaseolus vulgaris* L. - Plant Physiol. 64: 620–622.

Thompson, J. N. 1988. Evolutionary ecology of the relationship between oviposition preference and performance of offprings in phytophagous insects. - Entomol. Exp. Appl. 47: 3–14.

Venable, D. L. 1992. Size-number trade-offs and the variation of seed size with plant resource status. - Am. Nat. 140: 287–304.

Westoby, M. et al. 1996. Comparative ecology of seed size and dispersal. - Philos. Trans. R. Soc. London B 351: 1309–1318.

Chapter 1

PRESENCE OF FRUITS DECREASE PROBABILITY OF RETAINING FLOWERS IN A SEQUENTIALLY FLOWERING PLANT

ABSTRACT

Both intrinsic and extrinsic plant processes affect the fate of flowers along an inflorescence in sequentially flowering plants. We investigated whether the intrinsic process of sink strength of basal fruits, or architectural effects due to positional differences in the probability of retaining flowers, explains a lower probability of retaining distal flowers in the sequentially flowering plant *Yucca glauca*. Further, we investigated how the extrinsic process of seed herbivory interacts with the plant's intrinsic processes of flower retention. We carried out a field experiment to compare flower retention among nine combinations of three inflorescence treatments (basal flowers only, distal flowers only, distal flowers with presence of basal fruits) and three ovule damage treatments (no, low, and high) that serve as a cue for potential future seed herbivory. Also, we quantified flower retention in naturally-pollinated inflorescences. Experimental results showed that the probabilities of retaining basal and distal flowers in the absence of basal fruits were similar, thus, rejecting the architectural effects hypothesis. Further, in the presence of basal fruits, the probability of retaining distal flowers decreased, which supports the sink strength hypothesis. We did not see an effect of ovule damage. In naturally-pollinated inflorescences, the probability of retaining distal flowers decreased with increasing number of basal fruits. Results suggest that basal fruits constitute strong resource sinks reducing the probability of retaining distal flowers.

Previous studies have tested this mechanism in cultivated plants. Our study shows evidence for this mechanism in a wild flower population.

INTRODUCTION

Both intrinsic and extrinsic plant processes affect the fate of flowers along an inflorescence in sequentially flowering plants (Lloyd 1980, Stephenson 1981, Diggle 1995). Intrinsic processes relate to a plant's physiology and the position of flowers along an inflorescence, and extrinsic plant processes relate to environmental variables including resource and pollen availability and herbivory. One widely tested hypothesis for intrinsic plant processes that affect the fate of flowers is the sink strength hypothesis (Stephenson 1981). When early-opening basal flowers are pollinated and develop into fruits, they constitute strong resource sinks. These basal fruits may compete with distal late-opening flowers for resources. Consequently, distal flowers may abort in the presence of basal fruits. As a result, inflorescences produce more fruits from basal flowers. However, this pattern of selective development of fruits on basal positions on an inflorescence may also be explained by the architectural effects hypothesis, according to which flowers at distal positions have an inherently lower probability of developing into fruits owing to quantitative architectural or positional differences along the inflorescence, independent of fruits developed from basal flowers (Diggle 1995). The proximate mechanisms of architectural effects are not yet known, but may involve decreasing quantity of vascular tissue from basal to distal flower positions or differences in the size of floral organs along the inflorescence (Diggle 2014). We tested whether the resource sink hypothesis or the architectural effects hypothesis explains the probability of basal and distal flowers developing into fruits in sequentially flowering plants using *Yucca glauca* Nutt., (soapweed yucca, Family: Agavaceae) as a case study. For brevity, we refer to flowers developing into mature fruits as flower retention following previous studies (Humphries and Addicott 2000, 2004).

We only know of a single study that has tested the architectural effects hypothesis in a *Yucca* sp. (Huth and Pellmyr 1997). In this study researchers manipulated *Yucca filamentosa* inflorescences to obtain only early and only late opening flowers by removing all other flowers and compared flower retentions with un-manipulated inflorescences. All flowers were hand-pollinated to remove the effect of pollen limitation. Manipulated inflorescences retained a similar proportion of flowers to unmanipulated inflorescences (Huth and Pellmyr 1997). This suggests that the probability of retaining fruits is plastic along an inflorescence, and unlikely due to architectural differences. In this manuscript, we tested the generality of the architectural effects with *Y. glauca* inflorescences.

Intrinsic plant processes may interact with extrinsic processes such as herbivory, pollen limitation, nutrient availability and weather. *Yucca* spp. are pollinated by obligate nursery pollinators that lay eggs in the flowers they pollinate, and their larvae feed on the produced seeds (Riley 1892, Pellmyr 2003). While the survival of nursery pollinator larvae is essential for host plants to produce fruits in future flowering seasons, plants may not benefit from producing fruits with relatively high number of pollinator larvae. It is well-established that *Yucca* spp. host plants selectively abort flowers with a high number of pollinator eggs and selectively retain fruits with the potential of producing a high proportion of intact viable seeds (Pellmyr and Huth 1994, Humphries and Addicott 2000, Pellmyr 2003, Shapiro and Addicott 2004). Pollinator offspring cannot develop in aborted flowers. There is evidence that host plants respond to excessive seed damage and abort flowers before investing a significant amount of resources (Marr and Pellmyr 2003).

The goal of this manuscript is to use a combination of field experiments and observations to unravel the causes underlying lower flower retention and its interaction with ovule damage. Damage to a high number of ovules likely reduces the resource sink strength of flowers (Marr and Pellmyr 2003, Shapiro and Addicott 2004), which may trigger host plants to abort those flowers. One possible consequence of that may be that flowers with high pollinator oviposition have a consistently low probability of retention, irrespective of the plant's intrinsic process of flower retention. Alternatively, it is possible that flower retention in response to nursery pollinator oviposition interacts with the plant's intrinsic processes, i.e., basal and distal flowers may differ in their response to high pollinator ovipositions. If sink strength determines retention of flowers with few ovipositions, we expect both basal flowers and distal flowers without basal fruits to have a high probability of flower retention. However, in the presence of basal fruits, we expect distal flowers with few ovipositions to show a low probability of retention. In contrast, if architectural effects determine flower retention, we expect basal flowers with few ovipositions to have a higher probability of retention, and distal flowers to have a low probability of retention, independent of the presence of basal fruits. Further, when flowers receive high ovipositions, we expect a decrease in these probabilities of retaining flowers. So, high and low probabilities of retaining flowers when ovipositions are few will decrease to medium and very low probabilities of retaining flowers, respectively, when ovipositions are many.

Flower retention in the field is more complicated because of possible pollen limitation, and uncertainty in herbivory over the flowering season. We studied flower retention in naturally pollinated inflorescences to gain insights into how the host plant's intrinsic processes work in tandem with extrinsic processes to affect flower retention. In contrast to our experiment, naturally pollinated inflorescences likely varied in pollen availability, and the number of basal fruits that could divert resources from distal flowers. We predicted that, if sink strength of basal fruits decreases the chances of retaining distal flowers, the probability of retaining naturally-pollinated distal flowers will decrease with increase in the number of basal fruits. The naturally-pollinated inflorescences could not be used to test the architectural effects hypothesis that requires a comparison between the probabilities of retaining basal and distal flowers in the absence of pollen limitation and herbivory. Hence, we relied solely on our experiment to test the architectural effects hypothesis.

Finally, the magnitude of sink strength of fruits might be related to fruit size because larger fruits require more resources. Fruit size is likely influenced by the number of pollinated and damaged ovules. We used indices quantifying the extent of pollination and oviposition to gain insights into their role in influencing fruit size along the inflorescence.

MATERIALS AND METHODS

Study system

We used the *Y. glauca* and *Tegeticula yuccasella* (Riley) (yucca moth) mutualism as our study system. Both species are unprotected and abundant in their habitat. *Yucca* spp. and their obligate pollinator moths are native to arid habitats across North and South America. *Yucca* spp. are pollinated only by *Tegeticula* spp. that lay their eggs in the flower's ovary, and, the *Tegeticula* spp. larvae feed on the *Yucca* spp. seeds (Riley 1892).

Yucca spp. also reproduce asexually through ramets and lateral buds that give rise to new rosettes that are genetic clones of the parent plants. Clones can remain connected underground through rhizomes and may share resources. Typically each rosette grows for multiple years before it is capable of sexual reproduction (Kingsolver 1986). For sexual reproduction, a *Yucca* spp. rosette gives rise to an inflorescence during the summer. The inflorescence of *Y. glauca* is a raceme that may produce 17 to 140 buds (Kingsolver 1986, Svensson et al. 2011, Jadeja personal observation). The *Y. glauca* flowering period is usually 15-30 days long during which flowers open from the bottom of the raceme to the top (Kingsolver 1984). After flowering, the rosette dies (Kingsolver 1986). Old rosettes are replaced by one or more new rosettes, allowing yucca clumps to expand and persist for years.

Yucca spp. flowers are most receptive for 1-2 days after opening during which a female *Tegeticula* spp. may lay eggs in the flower's ovary and actively pollinate it (Dodd and Linhart 1994, Huth and Pellmyr 1997, Humphries and Addicott 2000). Within 7-10 days after oviposition, pollinator eggs hatch and feed on the developing seeds within the maturing host plant ovary (Huth and Pellmyr 1999). The number of ovules damaged increases with increasing number of *Tegeticula* spp. ovipositions (Marr and Pellmyr 2003, Shapiro and Addicott 2003). Ovules are not only damaged by female *Tegeticula* spp. during oviposition but also by *Carpophilus* sp. (florivorous beetles) and their larvae (Huth and Pellmyr 1997). The damage to ovules serves as a cue for flower abortion (Marr and Pellmyr 2003). Ninety-five percent of the flowers that the plant aborts are aborted within seven days after they open (Pellmyr and Huth 1994). On average, *Yucca* spp. set fruit from less than 15% of their flowers (Udovic and Aker 1981, Pellmyr 1997, Addicott 1998), primarily due to limited resources (Huth and Pellmyr 1997).

Hand pollination

To obtain donor flowers for hand-pollination, we protected donor *Y. glauca* inflorescences using a mesh sleeve made of fine tulle fabric that prevented pollen collection by *T. yuccasella* and reduced damage by *Carpophilus* sp. We collected fresh donor flowers, usually the topmost herbivore-free flowers on an inflorescence, from donor plants at least 25m away from the recipient plant (except one recipient for which donor flowers were collected from 10m away). We placed collected flowers away from direct sunlight in a plastic container lined with paper towels. We utilized pollen from donor flowers within three hours of collection. *Yucca glauca* pollen is known to be viable for 4 days (Dodd and Linhart 1994).

To hand pollinate flowers, we used a toothpick to collect pollen from one anther lobe of a donor flower and placed it on the stigmatic opening of the recipient flower. One anther lobe produces a few thousand pollen grains (Jadeja, personal observation) which is more than sufficient pollen to pollinate all ovules within a single *Y. glauca* ovary that contains on average nearly 300 ovules (Addicott 1986). Next, we used a size 0 brush to push the pollen well inside the stylar canal. We pollinated all experimental flowers within an inflorescence with pollen from the same pollen donor to control for the effect of differences in pollen quality on flower retention. Further, we thoroughly cleaned both the toothpick and the brush between pollen donors to prevent the transfer of mixed pollen genotypes.

Artificial oviposition

We used artificial wounding to mimic different levels of ovipositions following Marr and Pellmyr (2003). We constructed an artificial ovipositor by attaching a microneedle (minutien insect pin) to a matchstick, as done by Marr and Pellmyr (2003). The artificial ovipositor's thickness is similar to that of the *T. yuccasella* ovipositor (Marr and Pellmyr 2003). We quantified the thickness of the ovary wall from the groove within the ovary along the middle of the ovary for 55 flowers (2-5 flowers from 19 inflorescences) from the study site. This is where we have observed *T. yuccasella* insert their ovipositor. The thickness of the ovary wall was 1.96±0.04 mm (mean±SE) based on which we constructed 2.5 mm long artificial ovipositors such that they were long enough to damage ovules.

Following Marr and Pellmyr (2003), we applied 0, 6, or 24 artificial ovipositions to mimic no, low, and high number of ovipositions, respectively, to each experimental flower on an inflorescence. Twenty-four ovipositions is close to the maximum of 30 ovipositions observed at this study site in 2014 (Jadeja, personal observation). We applied the artificial oviposition treatment at the groove in the middle of the ovary as done by the natural pollinator. We distributed all artificial ovipositions equally across the six compartments of the ovary (locules) that are clearly differentiated by the anther filaments.

Flower manipulation experiment

We carried out a field experiment from early May to early July 2015 at the Cedar Point Biological Station (CPBS), Keith County, Nebraska, USA. We selected 114 undamaged *Y. glauca* inflorescences that had yet to begin flowering and protected them from deer herbivory using tomato cages with sides wrapped with 2.54 cm hex netting. Among inflorescences from visibly identifiable clones, we haphazardly selected only one focal inflorescence for our experiment. In addition, we removed buds from the remaining clonal inflorescences to minimize fruit abortion on focal inflorescences due to division of resources among clonal inflorescences.

To prevent *T. yuccasella* from visiting flowers on the selected inflorescences, we covered inflorescences with long mesh sleeves similar to donor inflorescences. If an inflorescence had already opened a few early flowers, we broke the flowers off before placing the sleeve. We also removed all visible florivorous beetles (*Carpophilus* sp.) from the inflorescence.

It was not possible to remove all florivorous beetles from all protected inflorescences, and some excluded florivorous beetles were able to damage flowers that abutted the mesh sleeve. Since, *Yucca* spp. abort flowers damaged by florivorous beetles (Huth and Pellmyr 1997), we discarded visibly beetle-infested and damaged inflorescences from the experiment. Overall, we discarded 30 inflorescences, leaving 84 inflorescences for the experiment. All flowers used in the experiment were less than two nights old, after which they may no longer be receptive.

We randomly assigned inflorescences to one of three treatments of flower position and presence of fruits, (1) early-opening flowers with buds above, (2) late-opening flowers without existing fruits on the inflorescence, and (3) late-opening flowers with basal fruits on the inflorescence (Appendix S1, see Supplemental Material). We manipulated inflorescences to obtain their assigned treatments (henceforth, inflorescence treatments). For the inflorescence treatment – late-opening flowers with already-existing fruits, we used one to three basal fruits formed by hand-pollinating flowers. On each manipulated inflorescence, we used three experimental flowers. Out of the 18 inflorescences with basal fruits, eight inflorescences had three fruits, five inflorescences had two fruits, and the remaining five inflorescences had one fruit. The small number of flowers and fruits used in the experiment reduced the chance of flower abortion due to limited resources. The maximum number of fruits that could be produced on a focal inflorescence was four to six fruits (three fruits developing from experimental flowers + one to three already-existing fruits). *Y. glauca* can retain more than six fruits (Kingsolver 1986).

We randomly assigned each manipulated inflorescence to an oviposition treatment – no, low, or high oviposition. This yielded nine treatments, which we distributed as evenly as possible across early and late flowering inflorescences. Discarding inflorescences with beetle damage and inflorescences that did not form basal fruits required for the treatment with prior fruiting resulted in unequal sample sizes between treatments (Appendix S1).

Ten days after applying the oviposition treatment and hand-pollinating, we recorded the number of retained flowers. Since 95% of flower abortions take place within seven days (Pellmyr and Huth 1994), we considered any fruit remaining after ten days as retained flowers. We collected fruits developed from experimental flowers 25 days after hand-pollination. We weighed fruits immediately after collection to determine whether our treatments affected fruit mass.

We used collected fruits to check whether our artificial oviposition treatment damaged ovules as intended. Ovules damaged during oviposition are white and unfertilized, whereas fertilized ovules are black. We quantified the number of infertile white seeds from a haphazardly selected subset of collected fruits. Fruits were from inflorescences without basal fruits, and with no and high artificial oviposition treatments (n = 10 and 12, respectively). We expected the artificial oviposition treatment to increase the number of white seeds.

Flower retention and fruit size in natural population

We used naturally pollinated *Y. glauca* inflorescences to determine the effect of the number of already-initiated basal fruits, on the probability of flower retention. At the end of the *Y. glauca* flowering season in July 2015, we sampled a 55 x 25 m patch of *Y. glauca* on the northeast slope of the Kingsley dam at Lake McConaughy, Keith County, Nebraska. The patch consisted of 106 visibly distinct *Y. glauca* clumps representing one or more clonal rosettes. This patch is 5 km from CPBS where we carried out the field experiment. The patch had 90 inflorescences of which 15 inflorescences were either damaged or used for another study. Of the remaining 75 inflorescences, we only used inflorescences with at least one fruit (57 inflorescences) for our analyses.

We quantified the number of buds produced on each inflorescence by counting the number of persistent pedicels (remnant flower stalks) and fruits. For each inflorescence, we recorded the position of each flower and whether the flower was retained. For example, we gave the fruits formed from the 1st and 10th flowers from the bottom of the inflorescence positions 1 and 10, respectively. In some cases, one axil could produce two fruits in which case we haphazardly gave fruits consecutive position values. In addition, for each distal flower i.e. each flower from the top third flowers of the inflorescence we recorded the number of fruits formed at flower positions below it (basal fruits).

Out of the 57 fruiting inflorescences at the *Y. glauca* patch, we collected fruits from 30 inflorescences in late July 2015. Collected fruits were from inflorescences distributed across the patch and across the range of the total number of fruits produced by inflorescences. We labelled each collected fruit with the identity of the inflorescence and flower position, and transported them to our laboratory at the University of Nebraska-Lincoln. We allowed fruits to air dry at room temperature. Approximately four months after the fruits were collected we determined indices of their size – fruit mass and length, which in turn are indices of the plant's resource allocation to the retained flower. We weighed the fruits, and measured their length from the base to the tip of the remnant style. The mass of fruits was strongly correlated with its length ($r = 0.78$, $n = 229$, $P < 0.0001$). Since fruit mass decreases with increasing seed consumption by pollinator larvae and non-pollinating seed predators, we used fruit length as a proxy for the fruit size in our analysis. Further, we recorded the following indices of pollinator oviposition and pollination in the collected fruits: (1) The number of locules with constrictions (out of six locules) on each fruit, which occur when many ovules are damaged at the site of pollinator oviposition (Riley 1892, Shapiro and Addicott 2003). (2) The number of fruits tapered or rounded at the base indicating that ovules were not fertilized most likely due to insufficient pollen grains (Humphries and Addicott 2000).

Statistical analysis

Flower manipulation experiment

We analyzed the probability of flower retention from our experimental data using a generalized linear mixed-effects model (GLMM) with binomial errors. The proportion of flowers retained was the response variable, and inflorescence identity was an observation-level random-effect to account for overdispersion (Harrison 2015). Predictor variables were inflorescence treatment (early flowers with buds above, late flowers with no fruits, and late flowers with basal fruits), level of artificial ovipositions (no, low, and high), and their interaction. We analyzed the average mass of fruits from experimental flowers that retained at least one flower using a linear model (LM) with the same predictor variables as those for fruit retention. We used backward model selection to eliminate predictors that did not significantly influence flower retention and fruit mass, respectively. Further, we carried out post-hoc analysis of all categorical variables in the final model using Tukey’s pairwise comparisons.

To determine whether our artificial oviposition treatment was effective, we used a generalized linear model (GLM), with quasipoisson family of errors to account for overdispersion. The number of white seeds was the response variable and oviposition treatment (high or no level of oviposition) was the predictor variable.

Flower retention and fruit size in natural population

Similar to our field experiment, we expected the probability of retaining distal flowers to decease with increasing number of basal fruits in naturally-pollinated inflorescences. To test our expectation, we constructed a GLMM with binomial error distribution where the response variable was whether or not a top flower was retained, and the predictor variable was the number of basal fruits. Each inflorescence had multiple top flowers. We accounted for the repeated measures by using inflorescence identity as a random effect.

Fruit length is an index of fruit size and the resources plants allocate to fruits. We analyzed predictors of fruit length from the naturally-pollinated *Y. glauca* inflorescences using separate linear mixed-effects models (LMMs) for fruits from the bottom, middle and top flowers. In each LMM, the random effect was inflorescence identity to account for repeated measures within inflorescences. The predictor variables were basal diameter of the inflorescence's rosette, whether the fruit had a tapering base, and the number of locules with constrictions.

We carried out all statistical analyses in R version 3.2.4 (R Core Development Team 2014), and packages lme4 (Bates et al. 2015), gamm4 (Wood and Scheipl 2015), and nlme (Pinheiro et al. 2016).

RESULTS

Flower manipulation experiment

Overall, 45.6% of the 252 hand-pollinated experimental flowers were retained during the experiment. Across inflorescences, nearly 30% of the inflorescences did not retain any flowers while nearly 23% of the inflorescences retained all three experimental flowers.

The artificial oviposition treatment did not significantly affect flower retention (Appendix S2). Hence, we only present the results of the effect of presence of basal fruits on retaining distal flowers. Inflorescences with late-opening distal flowers with already-existing basal fruits retained a significantly lower number of flowers than inflorescences without fruits (P = 0.003, Fig. 1, Appendices S3-S4). On average, inflorescences with already-existing basal fruits retained less than one out of three distal flowers while inflorescences without already-existing basal fruits retained 1 to 2 distal flowers. Further, neither inflorescence treatment nor oviposition level significantly affected the average mass of fruits from experimental flowers retained in the experiment (Appendix S5).

We counted the number of infertile white seeds produced by a subset of the fruits collected from the no and high oviposition treatments of the experiment to determine the effectiveness of our oviposition treatment. These fruits produced 351±14 seeds (mean±SE), of which 38.8±0.1% (mean±SE) were infertile white seeds. The number of white seeds did not differ significantly between fruits with no and high artificial oviposition treatments (Appendix S6).

Flower retention and fruit size in natural population

At the naturally-pollinated *Y. glauca* patch, each inflorescence retained 7±0.7 % of its flowers (mean±SE, n = 75 inflorescences), where each fruiting inflorescence produced 6.8±0.7 fruits (mean±SE) (n = 57 inflorescences). *Y. glauca* rosettes with basal diameters within the range of rosettes used in the experiment produced on average 5.8±0.6 (mean±SE) fruits with a maximum of 18 fruits in the same year as our experiment (see Fig. 2 for frequency distribution of number of fruits set). Of these, 34% inflorescences produced more than 6 fruits i.e. the maximum fruits that could be produced in our experiment.

Overall, the number of fruits produced varied along naturally-pollinated inflorescences. Fruiting inflorescences produced on average significantly more fruits from the middle flowers than the top or bottom flowers, with the bottom flowers producing the least number of fruits ($P < 0.05$, Appendix S7). Overall, the length of fruits, an index of fruit size, at the naturally-pollinated *Y. glauca* patch was 57.61±0.66 mm (mean±SE). A fruit with a tapered base, as opposed to a rounded base indicates partial fertilization of ovules due to limited pollen. And, increase in locules with constrictions on a fruit indicates increasing level of pollinator oviposition. There was no significant difference in fruit length, the number of fruits with tapered bases, and number of locules per fruit with constrictions among bottom, middle, and top fruits (Appendix S7).

The probability of retaining top flowers significantly decreased with increasing number of basal fruits ($P = 0.046$, Fig. 3, Appendix S8). In the absence of basal fruits, the probability of retaining top flowers was 0.09, which decreased to 0.07 in the presence of five basal fruits.

All three LMMs for the length of fruits, an index of fruit size, from the bottom, middle and top flowers showed no significant effect of the basal diameter of the rosette and the number of locules with constrictions on fruit size (Appendices S9-S11). Also, LMMs for the length of fruits from middle and top flowers showed no significant effect of tapered fruit bases on fruit size (Appendices S10 and S11). However, fruits with tapered bases from the bottom third flowers were 23% smaller than fruits without tapering bases (P = 0.01, Appendix S9).

DISCUSSION

Flower manipulation experiment

We carried out a field experiment to determine whether flower retention in sequentially flowering *Y. glauca* is driven by intrinsic processes related to presence of fruits as resource sinks or architectural effects and whether these interact with ovule damage due to pollinator oviposition (extrinsic process). We found that, in the absence of basal fruits, the probability of retaining basal and distal flowers was similar. These findings are in line with studies on *Y. glauca* congeners. (Huth and Pellmyr 1997, Humphries and Addicott 2000, 2004), and reject the hypothesis that architectural effects decrease the probability of retaining distal flowers. We also found that that the presence of basal fruits decreases the probability of retaining late-opening distal flowers, which supports the hypothesis that strong sink strength of basal fruits decreases the probability of retaining distal flowers in *Y. glauca*.

Experimental evidence for the sink strength hypothesis has been documented for cultivated plants (Tamas et al. 1979, Stephenson 1980, Marcelis et al. 2004). These studies were carried out in a controlled laboratory environment with genetically homogenous plants. However, findings from cultivated plants may not necessarily extend to intrinsic processes underlying flower retention in natural plant populations with larger genetic variation that grow in a heterogenous environment. Hence, our field study strengthens the empirical support for the role of sink strength of basal fruits in reducing the probability of retaining distal flowers in sequentially flowering plants.

The artificial oviposition treatment did not significantly affect flower retention in our experiment. Wounding during ovipositions damages ovules (Marr and Pellmyr 2003). Hence, one way to test the success of our artificial treatment is by comparing the number of damaged ovules in fruits from flowers with and without the artificial oviposition treatment. The number of damaged ovules that are visible as infertile white seeds was similar between fruits from experimental flowers with and without the artificial oviposition treatment. This suggests that our artificial oviposition treatment was unsuccessful. However, we cannot completely reject the possibility that the artificial oviposition treatment succeeded, but plants did not abort flowers likely to allow some pollinator larvae to survive to ensure pollinators in future flowering seasons.

Three hypotheses may explain why plants have evolved mechanisms such as resource sinks that ensure a lower probability of flower retention when basal fruits are already initiated. One is the resource conservation hypothesis, according to which organs that are further in development are preferred by plants for resource investment because they require lower resources in the future to complete development (Nakamura 1986).

Conserved resources may be stored for future flowering seasons (Primack and Hall 1990). Another hypothesis is the asset protection principle (Clark 1994). According to this, fruits are more valuable assets to plants than flowers. When fruits are small they are more vulnerable to risks of damage due to herbivory and storms. Plants can protect their fruits (valuable assets) from such risks by enlarging them quickly to a size that is less vulnerable. Therefore, plants are more likely to allocate resources to enlarge fruits to protect these more valuable assets than retain new flowers that are less valuable assets. A fruiting strategy following the asset protection principle (Clark 1994) may be adaptive for *Yucca* spp. because the risk of losing smaller fruits is high due to herbivory by *Carpophilus* sp. (florivorous beetles) and aphids (Dodd and Linhart 1994, Pellmyr 1995, Huth and Pellmyr 1997), and storms (Jadeja, personal observation). The third hypothesis is that resource limited plants allocate resources to develop a small number of large fruits in favor of many small fruits because large fruits have larger, more viable seeds (Stanton 1984, Venable 1992, Sakai and Sakai 1995).

Flower retention and fruit size in natural population

We quantified the probability of retaining naturally-pollinated distal flowers with increasing number of basal fruits. Consistent with experimental results, the probability of retaining top flowers on naturally-pollinated inflorescences decreased with increasing number of basal fruits, which also supports the sink strength hypothesis. To our knowledge, the effect of basal fruits on the probability of retaining distal flowers of naturally-pollinated *Yucca* spp. has not been studied previously.

In our experiment, the retention probability of basal *Y. glauca* flowers was similar to or higher than distal flowers. However, in naturally-pollinated inflorescences basal flowers produced fewer fruits than top flowers, and middle flowers produced the most number of fruits. Higher fruit-set from middle fruits is consistent with other studies (Stephenson 1981), including *Y. kanabensis* (Humphries and Addicott 2000, 2004) and *Myrosmodes cochleare* (Berry and Calvo 1991). Our experimental flowers were herbivory-free while the naturally-pollinated flowers were not. Hence, one possible reason for the discrepancy between our experimental results and field observations could be high herbivory early in the flowering season. For example, a study with a *Y. filamentosa* population showed higher floral herbivory by *Carpophilus* sp. early in the flowering season contributed to low fruit-set of early-opening basal flowers (Huth and Pellmyr 1997).

Our experimental flowers were hand-pollinated. Hence, another likely reason for lower fruit-set from early-opening bottom flowers was poor pollination. It is possible that early in the flowering season when bottom flowers open the abundance of nursery pollinators was low. This was observed in *Y. kanabensis* where nursery pollinator *T. altiplanella* visitation peaked in the middle of the flowering season when many inflorescences likely opened their middle flowers (Addicott 1998). Alternatively, bottom flowers may be poorly pollinated due to the nursery pollinator’s preferences for flowers at higher positions on the inflorescence. For example, nursery pollinator *T. altiplanella* prefers ovipositing and pollinating higher flowers (Wilson and Addicott 1998) because flowers higher on the inflorescence are more likely to be receptive and virgin (not-visited by other conspecific nursery pollinators).

We detected that the extent of pollination affected the size of bottom fruits. Fruits with tapered bases indicate partial pollination due to unfertilized ovules from low pollen availability (Humphries and Addicott 2000). The bottom fruits were significantly smaller when they were partially fertilized. We did not detect a similar significant relationship in fruits from middle or top flowers. This suggests that bottom flowers were much more pollen limited than middle and top flowers. This raises the question, why do plants retain partially fertilized early-opening flowers with fewer viable seeds when they could abort those flowers, and retain flowers that open later in the flowering season? Perhaps, if plants abort basal flowers early in the flowering season, their future flowers may not receive pollen, or may be damaged due to herbivory. Therefore, it may not be adaptive for the plants to abort early-opening pollinated flowers, even if they are smaller owing to poor fertilization of ovules.

CONCLUSIONS

Our experimental and observational results from a field population of *Y. glauca* provide support for the hypothesis that strong sink strength of basal fruits reduces the probability of retaining distal flowers but not the architectural effects hypothesis. We discussed three hypotheses that may explain why this strategy has evolved; resource conservation hypothesis, asset protection principle, and production of larger more viable seeds. Further, we found evidence of pollen limitation during the early part of the flowering season resulting in significantly smaller fruits from bottom flowers.

ACKNOWLEDGEMENTS

The authors thank the Cedar Point Biological Station staff for logistical support at the field site, Nate Nielson for permission to access the Kingsley dam surface, Drew Tyre for discussions related to our experimental set-up and statistical analysis, Pushpraj Chauhan for help with fieldwork, Andrew Gebara and Megan Bjorkman for help with lab work, and Drew Tyre, Daizaburo Shizuka, Diana Pilson and Jeffrey Stevens for helpful comments on earlier versions of the manuscript. This research was supported by funds from the J. Ve. Srb Memorial Fund to SJ.

REFERENCES

Addicott, J. 1986. Variation in the costs and benefits of mutualism: the interaction between yuccas and yucca moths. *Oecologia* 70: 486–494.

Addicott, J. F. 1998. Regulation of mutualism between yuccas and yucca moths: population level processes. *Oikos* 81: 119–129.

Bates, D., M. Mächler, B. M. Bolker, and S. C. Walker. 2015. Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* 67: 1–51.

Berry, P. E., and R. N. Calvo. 1991. Pollinator limitation and position dependent fruit-set in the high Andean orchid *Myrosmodes cochleare* (Orchidaceae). *Plant Systematics and Evolution* 174: 93–101.

Clark, C. W. 1994. Antipredator behavior and the asset-protection principle. *Behavioral Ecology* 5: 159–170.

Diggle, P. K. 1995. Architectural effects and the interpretation of patterns of fruit and seed development. *Annual Review of Ecology and Systematics* 26: 531–552.

Diggle, P. K. 2014. Modularity and intra-floral integration in metameric organisms : plants are more than the sum of their parts. *Philosophical transactions of the Royal Society of London, B, Biological Sciences* 369: 20130253.

Dodd, R. J., and Y. B. Linhart. 1994. Reproductive consequences of interactions between *Yucca glauca* (Agavaceae) and *Tegeticula yuccasella* (Lepidoptera) in Colorado. *American Journal of Botany* 81: 815–825.

Harrison, X. A. 2015. A comparison of observation-level random effect and Beta-Binomial models for modelling overdispersion in Binomial data in ecology & evolution. *PeerJ* 3: e1114.

Humphries, S. A., and J. F. Addicott. 2004. Regulation of the mutualism between yuccas and yucca moths: intrinsic and extrinsic patterns of fruit set. *Canadian Journal of Botany* 82: 573–581.

Humphries, S., and J. Addicott. 2000. Regulation of the mutualism between yuccas and yucca moths: intrinsic and extrinsic factors affecting flower retention. *Oikos* 89: 329–339.

Huth, C. J., and O. Pellmyr. 1997. Non-random fruit retention in *Yucca filamentosa*: consequences for an obligate mutualism. *Oikos* 78: 576–584.

Huth, C. J., and O. Pellmyr. 1999. Yucca moth oviposition and pollination behavior is affected by past flower visitors: evidence for a host-marking pheromone. *Oecologia* 119: 593–599.

Kingsolver, R. 1984. Population biology of a mutualistic association: *Yucca glauca* and *Tegeticula yuccasella*. PhD Thesis. University of Kansas, Lawrence, Kansas, USA.

Kingsolver, R. 1986. Vegetative reproduction as a stabilizing feature of the population dynamics of *Yucca glauca*. *Oecologia* 69: 380–387.

Lloyd, D. G. 1980. Sexual strategies in plants. I. An hypothesis of serial adjustement of maternal investment during one reproductive session. *New phytologist* 86: 69–79.

Marcelis, L. F. M., E. Heuvelink, L. R. Baan Hofman-Eijer, J. Den Bakker, and L. B. Xue. 2004. Flower and fruit abortion in sweet pepper in relation to source and sink strength. *Journal of Experimental Botany* 55: 2261–2268.

Marr, D. L., and O. Pellmyr. 2003. Effect of pollinator-inflicted ovule damage on floral abscission in the yucca-yucca moth mutualism: the role of mechanical and chemical factors. *Oecologia* 136: 236–243.

Nakamura, R. R. 1986. Maternal investment and fruit abortion in *Phaseolus vulgaris*. *American Journal of Botany* 73: 1049–1057.

Pellmyr, O. 1995. Windows of fruit production in yuccas? No. A critique of James et al. *Oikos* 72: 145–147.

Pellmyr, O. 1997. Pollinating seed eaters: why is active pollination so rare? *Ecology* 78: 1655–1660.

Pellmyr, O. 2003. Yuccas, yucca moths, and coevolution: a review. *Annals of the Missouri Botanical Garden* 90: 35–55.

Pellmyr, O., and C. J. Huth. 1994. Evolutionary stability of mutualism between yuccas and yucca moths. *Nature* 372: 257–260.

Pinheiro, J., D. Bates, S. DebRoy, D. Sarkar, EISPACK, S. Heisterkamp, B. Van Willigen, and R-core. 2016. nlme: linear and nonlinear mixed effects models. 3.1-126.

Primack, R. B., and P. Hall. 1990. Costs of reproduction in the pink lady’s slipper orchid:

a four-year experimental study. *American Naturalist* 136: 638–656.

R Core Team. 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Riley, C. V. 1892. The yucca moth and yucca pollination. *Missouri Botanical Garden Annual Report* 3: 99–158.

Sakai, S., and A. Sakai. 1995. Flower size-dependent variation in seed size: theory and a test. *American Naturalist* 145: 918–934.

Shapiro, J., and J. F. Addicott. 2004. Re-evaluating the role of selective abscission in moth/yucca mutualisms. *Oikos* 105: 449–460.

Shapiro, J. M., and J. F. Addicott. 2003. Regulation of moth–yucca mutualisms: mortality of eggs in oviposition-induced "damage zones." *Ecology Letters* 6: 440–447.

Stanton, M. L. 1984. Seed variation in wild radish: effect of seed size on components of seedling and adult fitness. *Ecology* 65: 1105–1112.

Stephenson, A. G. 1980. Fruit set, herbivory, fruit reduction, and the fruiting strategy of *Catalpa speciosa* (Bignoniaceae). *Ecology* 61: 57–64.

Stephenson, A. G. 1981. Flower and fruit abortion: proximate causes and ultimate functions. *Annual Review of Ecology and Systematics* 12: 253–279.

Svensson, G. P., O. Pellmyr, and R. A. Raguso. 2011. Pollinator attraction to volatiles from virgin and pollinated host flowers in a yucca/moth obligate mutualism. *Oikos* 120: 1577–1583.

Tamas, I. A., D. H. Wallace, P. M. Luford, and J. L. Ozbun. 1979. Effect of older fruits on abortion and abscisic acid concentration of younger fruits in *Phaseolus vulgaris*

L. *Plant Physiology* 64: 620–622.

Udovic, D., and C. Aker. 1981. Fruit abortion and the regulation of fruit number in *Yucca whipplei*. *Oecologia* 49: 245–248.

Venable, D. 1992. Size-number trade-offs and the variation of seed size with plant resource status. *American Naturalist* 140: 287–304.

Wilson, R. D., and J. F. Addicott. 1998. Regulation of mutualism between yuccas and yucca moths: is oviposition behavior responsive to selective abscission of flowers? *Oikos* 81:109–118.

Wood, S., and F. Scheipl. 2015. gamm4: generalized additive mixed models using mgcv and lme4. 0.2-3.

FIGURES

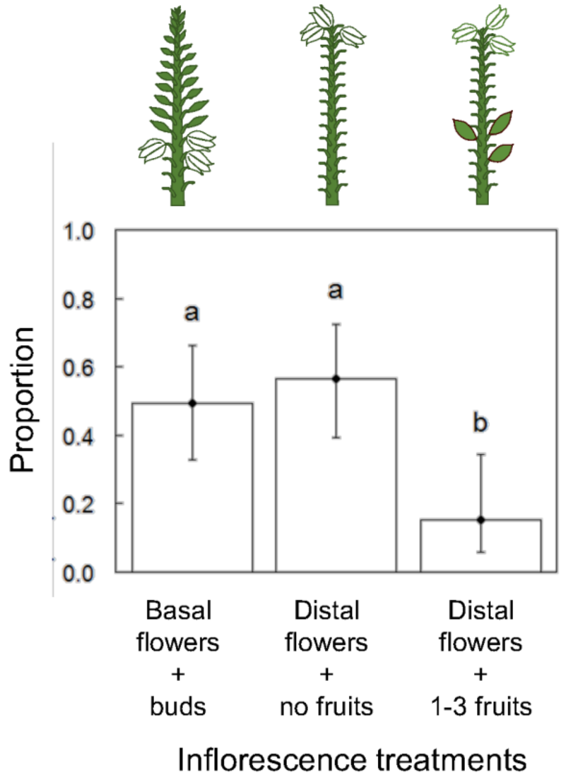


Figure 1. The proportion of experimental flowers retained out of three flowers under each inflorescence treatment. Inflorescence treatments (basal flowers with buds above, distal flowers with no fruits, and distal flowers with already-existing basal fruits) are shown above each bar using schematic diagram of inflorescences. The probability of flower retention decreased significantly when basal fruits were present. Differing letters above bars show significant differences based on post-hoc Tukey's test for all pairwise comparisons of the inflorescence treatments. The error bars show profile likelihood-based 95% confidence intervals. n = 33, 33, and 18.

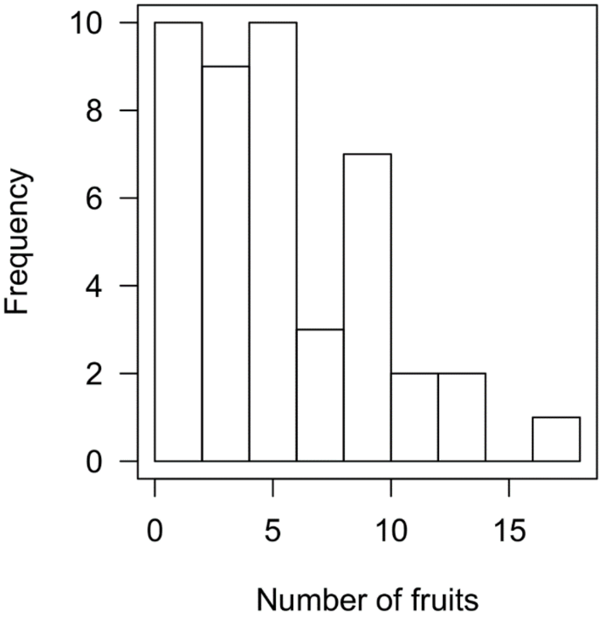


Figure 2. Frequency distribution of number of fruits on naturally-pollinated inflorescences with at least one fruit, on rosettes with basal diameters within the range rosettes used in the experiment. n = 44 inflorescences.

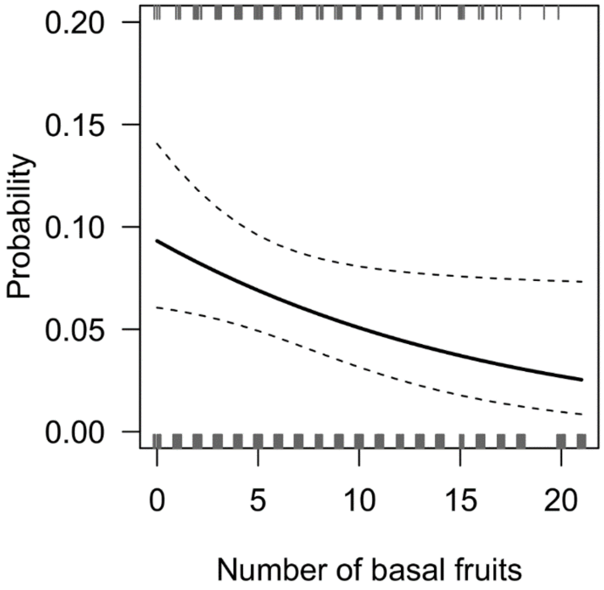
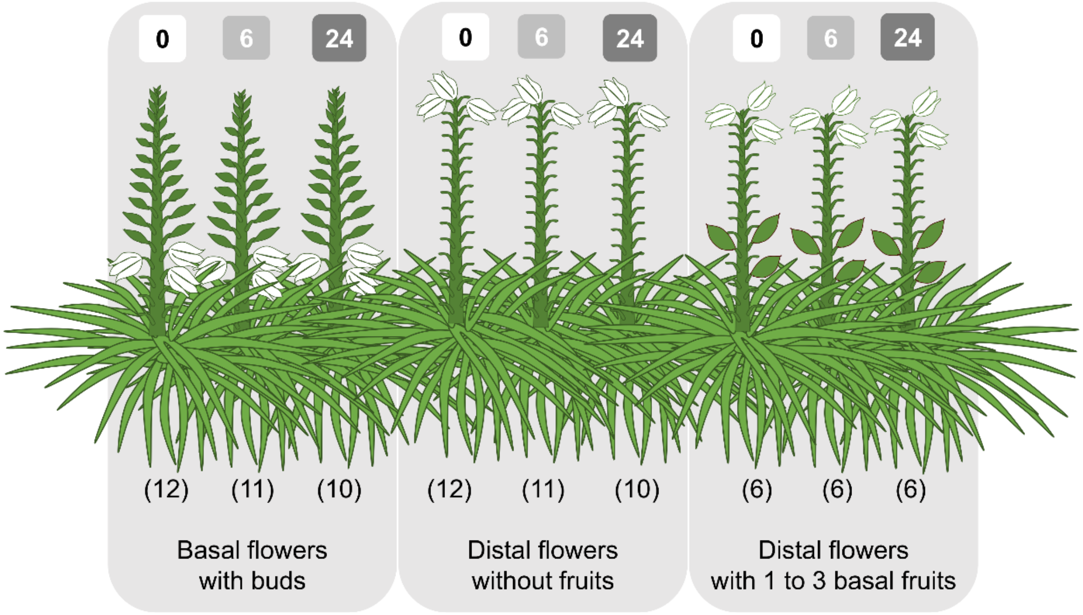


Figure 3. The probability of retaining top flowers decreased with increasing number of basal fruits. Lines are model predicted mean (solid) and 95% CIs (dashed). Rugs show observed successes (top rugs) and failures (bottom rugs) of retaining top flowers. n = 57 inflorescences.

SUPPLEMENTARY MATERIAL



Appendix S1. Experimental set-up for determining flower retention under different inflorescence and oviposition treatments. Schematic diagram shows *Yucca glauca* inflorescences in each treatment with experimental flowers (white), buds (light green), and basal fruits (dark green). Numbers above inflorescences indicate the number of artificial ovipositions applied to each experimental flower. The numbers in parentheses below inflorescences indicate samples sizes after discarding inflorescences with beetle damage and inflorescences that did not form at least one fruit for the inflorescence treatment with one to three basal fruits.

Appendix S2. Results of the full generalized linear mixed-effects model for the proportion of flowers retained with inflorescence identity as random-effect and binomial distribution. We display the untransformed means of the estimated fixed variables (Estimate) for the effect of inflorescence treatments, artificial oviposition treatments (O) and their interaction on the proportion of flowers retained. SE indicates standard errors, and n is the sample size (number of inflorescences). Variance of the random effect was 1.79.

Parameter	n	Estimate	SE	z value	Pr(>|z|)
Intercept (distal flowers - basal fruits**:**high O)	10	-0.00	0.61	0	1
basal flowers + buds**:**high O	10	-0.98	0.89	-1.11	0.27
distal flowers + basal fruits**:**high O	6	-1.67	1.06	-1.57	0.12
distal flowers – basal fruits**:**low O	11	-0.43	0.85	-0.50	0.62
distal flowers – basal fruits**:**no O	12	1.12	0.84	1.32	0.19
basal flowers + buds**:**low O	11	2.15	1.23	1.75	0.08
distal flowers + basal fruits**:**low O	6	0.5	1.48	0.34	0.74
basal flowers + buds**:**no O	12	-0.14	1.19	-0.11	0.91
distal flowers + basal fruits**:**no O	6	-1.19	1.49	-0.8	0.43

Appendix S3. Results of the final generalized linear mixed-effects model for the proportion of flowers retained with inflorescence identity as random-effect and binomial distribution. We display the untransformed means of the estimated fixed variables (Estimate) for the effect of inflorescence treatment on the proportion of flowers retained. SE indicates standard errors, and n is the sample size (number of inflorescences). Variance of the random effect was 2.11. Significant p-values (Pr<0.05) are in bold.

Parameter	n	Estimate	SE	z value	Pr(>|z|)
Intercept (distal flowers – basal fruits)	33	0.27	0.36	0.74	0.46
basal flowers + buds	33	-0.29	0.5	-0.58	0.56
distal flowers + fruits	18	-1.99	0.66	-3.02	**0.003**

Appendix S4. Untransformed mean parameter estimates (Estimate) from Tukey's all pairwise comparisons of proportion of flowers retained among inflorescence treatments from the final model. SE indicates standard errors. Significant p-values (Pr<0.05) are in bold.

Pairwise comparisons (using final model)	Estimate	SE	z value	Pr(>|z|)
basal flowers + buds / distal flowers – basal fruits	-0.29	0.50	-0.58	0.83
distal flowers – basal fruits / distal flowers + basal fruits	-1.99	0.66	-3.02	**0.007**
distal flowers + basal fruits / basal flowers + buds	-1.69	0.65	-2.6	**0.024**

Appendix S5. Results of the full linear model for the average mass of fruits retained on inflorescences with at least one fruit formed from experimental flowers. We display means of the parameter estimates (Estimate) for the effect of inflorescence treatment and artificial oviposition treatments (O) and their interaction on the average mass of fruits retained from experimental flowers. SE indicates standard errors, and n is the sample size (number of inflorescences). Significant p-values (Pr<0.05) are in bold.

Parameter	n	Estimate	SE	z value	Pr(>|z|)
Intercept (distal flowers - basal fruits**:**high O)	8	58.38	6.26	9.32	**<0.0001**
basal flowers + buds**:**high O	5	-6.31	10.1	-0.63	0.54
distal flowers + basal fruits**:**high O	2	2.16	14.01	0.15	0.88
distal flowers – basal fruits**:**low O	7	-11.49	9.17	-1.25	0.22
distal flowers – basal fruits**:**no O	10	-8.34	8.40	-0.99	0.33
basal flowers + buds**:**low O	10	17.33	13.35	1.3	0.20
distal flowers + basal fruits**:**low O	4	-16.41	17.87	-0.92	0.36
basal flowers + buds**:**no O	7	17.19	13.35	1.29	0.20
distal flowers + basal fruits**:**no O	2	-3.23	19.61	-0.17	0.87

Appendix S6. Results of the generalized linear model with quasipoisson distribution to determine effectiveness of the artificial oviposition treatment. We display the untransformed means of the estimated variables (Estimate) for the effect of high and no artificial oviposition treatment on the number of infertile white seeds in experimental fruits. SE indicates standard errors, and n is the sample size (number of fruits). Significant p-values (Pr<0.05) are in bold.

Parameter	n	Estimate	SE	t value	Pr(>|t|)
Intercept (high oviposition)	12	4.72	0.20	23.50	**<0.0001**
no oviposition	10	0.28	0.28	1.00	0.33

Appendix S7. The mean±SE number of fruits, length of fruits (mm), number of fruits with tapering bases (an index of partial fertilization of ovules), and number of locules with constrictions per fruit, from the bottom, middle and top one-third flowers on fruiting inflorescences in the naturally-pollinated *Yucca glauca* patch. Numbers in parentheses show number of inflorescences. Length of fruits, number of fruits with tapered bases and number of locules with constrictions were based on fruits collected from 30 inflorescences. Not all inflorescences had fruits in each third of the flowers. Different superscripts within each variable shows significantly different means ($p<0.05$), based on pairwise tests with p-values adjusted using the Holm-Bonferroni method. Pairwise t-tests were used for parametric variables and pairwise Wilcoxon rank sum tests were used for non-parametric variables.

Variables	Bottom	Middle	Top
number of fruits	1.2 ± 0.2^{a} (57)	3.2 ± 0.4^{b} (57)	2.4 ± 0.4^{c} (57)
length of fruits	55.12 ± 2.97^{d} (16)	60.47 ± 1.93^{d} (28)	57.74 ± 1.63^{d} (19)
number of fruits with tapered bases	0.4 ± 0.3^{e} (16)	0.4 ± 0.1^{e} (28)	0.3 ± 0.1^{e} (19)
number of locules with constrictions	1.3 ± 0.4^{f} (16)	1.0 ± 0.2^{f} (28)	1.1 ± 0.3^{f} (19)

Appendix S8. Results of the generalized linear mixed effects model with binomial distribution for the probability of retaining top flowers with inflorescence identity as a random effect. We display the untransformed mean of parameter estimates (Estimate) for the effect of the number of basal fruits. SE indicates standard errors. n = 1552 flowers across 57 inflorescences. Significant p-values (Pr<0.05) are in bold. Variance of the random effect was 0.83.

Parameter	Estimate	SE	z value	Pr(>|z|)
Intercept	-2.28	0.24	-9.58	**<0.001**
number of basal fruits	-0.07	0.03	-1.99	**0.046**

Appendix S9. Results of the linear mixed-effects model with inflorescence identity as random effect for the length of fruits (mm) from the bottom third flowers. We show the means of estimate values (Estimate) for the effect of basal diameter of inflorescence's rosette, whether the base of the fruit is tapered or not, and the number of locules with constrictions. SE indicates standard errors, and n is the sample size in number of inflorescences. Significant p-values (Pr<0.05) are in bold. Variance of the random effect was 2.86. n = 33 fruits on 16 inflorescences.

Parameter	n	Estimate	SE	t value	Pr(>|t|)
Intercept (fruit without tapered base)	26	65.27	7.14	9.14	**<0.0001**
basal diameter of rosette	33	-0.07	0.06	-1.08	0.3
fruit with tapered base	7	-15.95	5.04	-2.97	**0.011**
number of locules with constrictions	33	-0.65	1.34	-0.49	0.63

Appendix S10. Results of the linear mixed-effects model with inflorescence identity as random effect for the length of fruits (mm) from the middle third flowers. We show the means of estimate values (Estimate) for the effect of basal diameter of inflorescence's rosette, whether the base of the fruit is tapered or not, and the number of locules with constrictions. SE indicates standard errors, and n is the sample size in number of inflorescences. Variance of the random effect was 8.24. n = 114 fruits on 28 inflorescences.

Parameter	n	Estimate	SE	t value	Pr(>|t|)
Intercept (fruit without tapered base)	104	66.78	5.75	11.61	<0.0001
basal diameter of rosette	114	-0.07	0.057	-1.25	0.22
fruit with tapered base	10	-2.18	3.29	-0.66	0.51
number of locules with constrictions	114	0.61	0.51	1.21	0.23

Appendix S11. Results of the linear mixed-effects model with inflorescence identity as random effect for the length of fruits (mm) from the top third flowers. We show the means of estimate values (Estimate) for the effect of basal diameter of inflorescence's rosette, whether the base of the fruit is tapered or not, and the number of locules with constrictions. SE indicates standard errors, and n is the sample size in number of inflorescences. Variance of the random effect was 6.1. n = 82 fruits on 19 inflorescences.

Parameter	n	Estimate	SE	t value	Pr(>|t|)
Intercept (fruit without tapered base)	76	57.59	6.91	8.34	<0.0001
basal diameter of rosette	82	0.01	0.06	0.16	0.88
fruit with tapered base	6	-5.78	3.26	-1.77	0.08
number of locules with constrictions	82	0.23	0.59	0.39	0.7

Chapter 2

OPTIMAL FLOWER RETENTION AND FRUIT GROWTH STRATEGIES IN SEQUENTIALLY FLOWERING PLANTS

INTRODUCTION

Many plant species retain only a subset of produced flowers as fruits because of incomplete or lack of pollination (Stephenson 1981, Burd 1994), limited resource availability (Lloyd 1980, Stephenson 1981, Guitián et al. 2001), and herbivory (Stephenson 1981, Louda and Potvin 1995). If fruiting resources are limited, not all sufficiently pollinated flowers can be retained (Huth and Pellmyr 1997, Medrano et al. 2000, Guitián et al. 2001, Humphries and Addicott 2004). A plant's strategy to allocate limited resources to maximize fitness likely involves trading off the number of fruits initiated with the size of fruits (Stephenson 1981, Stephenson and Winsor 1986, Dani and Kodandaramaiah 2017). In general, larger fruits produce larger, more viable seeds compared to smaller fruits (i.e., a size-dependent viability benefit) (Primack 1987) because larger seeds have a higher probability of germinating and surviving to become a seedling (Stanton 1984, Moles et al. 2006). Large seeds are particularly beneficial for plants native to drought-prone or arid habitats (Stanton 1984, Westoby et al. 1996, Jakobsson and Eriksson 2000, Moles et al. 2006). Growing fruits quickly to a larger size may increase fruit survival (i.e., a size-dependent survival benefit). This has been observed in plants where smaller fruits with weaker tissues have a lower probability of survival because they are more likely to be damaged by herbivores and storms than larger fruits (Stephenson 1981).

Many plants produce fruit (offspring) sequentially within one flowering season likely to increase the chances of synchrony between flowering and pollinator availability (Rathcke and Lacey 1985). This may be particularly important for plants that depend on a small set of pollinators. Processes including uncertainty in pollination of flowers and survival of fruits may result in different physiological conditions for allocating resources to retain flowers and grow existing fruits. For instance, the number and size of fruits a plant develops early in the flowering period may influence the probability of retaining flowers later in the flowering period. Changing physiological condition owing to prior fruiting in sequentially flowering plants makes an ideal case for testing hypotheses on condition-dependent life history strategies, particularly strategies to allocate resources to fruiting in response to the presence and abundance of reproductive structures (fruits).

There is ample evidence in the literature that for many sequentially flowering plant species basal fruits are nutrient sinks depriving distal flowers of resources (sink-strength hypothesis) (Lloyd 1980, Stephenson 1981, Diggle 1995). Growing fruits (strong resource sinks) from basal flowers divert resources away from distal flowers (Tamas et al. 1979, Stephenson 1980, Susko and Lovett-Doust 1999, Marcelis et al. 2004, Chapter 1). The strong sink strength of basal fruits may be a mechanism for plants to maximize fitness by trading-off the number and size off fruits and producing few large fruits instead of producing many small fruits.

Evolution of optimal life histories under the offspring number-size trade-off has been examined by many theoretical studies (reviewed by Dani and Kodandaramaiah 2017). Studies on reproductive systems that produce offspring at one time within a reproductive period (i.e., non-sequentially) show optimal offspring size depends on the shape of the fitness function (Smith and Fretwell 1974, Lloyd 1987, Dani and Kodandaramaiah 2017), resource availability and density-dependence of offspring survival (Venable 1992), and fertilization/pollen limitation (Huang et al. 2017). These models cannot assess the condition-dependent fruiting strategies of sequentially flowering plants.

To our knowledge, only one model has investigated optimal number-size strategies in a system that produces offspring sequentially within a reproductive period (see Sakai and Harada 2004). Sakai and Harada (2004) investigated under what circumstances it is optimal for organisms to produce offspring in sequential cohorts within a reproductive season. Their model suggests that producing offspring sequentially is rare and not optimal unless there is a constraint on the number of offspring an organism can produce in each cohort. In contrast to the model predictions, a wide range of plants produce flowers sequentially. Many plants depend on mutualists to fertilize their eggs. Hence, these plants may have evolved to flower sequentially to increase synchrony between flowering and pollinator availability (Rathcke and Lacey 1985), which Sakai and Harada's (2004) model does not consider.

To predict a sequentially flowering plant’s optimal strategy to allocate limited resources to (1) develop pollinated flowers into fruits (henceforth, retain flowers) and (2) grow existing fruits larger, we developed a state-dependent stochastic dynamic optimization model (Bellman 1957, Clark and Mangel 2000). We chose a state-dependent model because a plant’s strategy to retain a flower or grow fruits likely depends on the plant’s states – the number of fruits and their sizes and the time in the flowering period. For example, early in the flowering period when a plant may not have many fruits, a strategy to retain flowers may give a high fitness benefit. The same strategy may also give a high fitness benefit later in the flowering period for a plant that was not successful in producing fruits earlier. However, a plant that already initiated many fruits early in the flowering period may benefit more from growing the existing fruits to produce larger seeds with higher viability than from allocating resources to initiate more fruits later in the flowering period. We parametrized the model using data from *Y. glauca* as a case study.

We further explored the effect of ecologically important parameters on a plant's optimal resource allocation strategy. First, we examined if the presence of a size-dependent fitness benefit selects for plants that are more likely to abort distal flowers when basal fruits are present and allocate resources to grow existing fruits to a larger size. Fitness benefit may be size-dependent when larger fruits produce larger seeds with higher viability (henceforth "size-dependent fruit viability") and when fruit survival increases with fruit size (henceforth "size-dependent fruit survival"). We expected both size-dependent viability and size-dependent survival to make it adaptive for plants to grow existing fruits larger at the cost of retaining distal flowers.

Second, we examined the effect of the efficiency of a plant to convert photosynthate into fruit tissue (fruit growth efficiency) on the optimal strategy to allocate resources to fruiting. A high fruit growth efficiency may be seen in plants producing green, photosynthesizing fruits (Bazzaz et al. 1979, Obeso 2002) and in plants producing simple fruits that lack complex accessory parts either to aid dispersal or protect seeds from herbivores (Sutherland 1986, Lord and Westoby 2006). We expected plants with high fruit growth efficiency to produce more fruits than plants with low fruit growth efficiency, as has been observed empirically (Sutherland 1986). Finally, we examined how pollen limitation influences a plant's resource allocation strategy. Decreasing pollination probability decreases the number of flowers available to a plant. Hence, we expected that with decreasing pollination probability plants will mature fewer fruits and grow them to a larger size.

THE MODEL

We explored the effect of time in the flowering period, and the number of existing fruits and their sizes on a plant's optimal resource allocation strategy. To do so, we developed a state-dependent stochastic dynamic programming (SDP) model (Bellman 1957, Clark and Mangel 2000) using the programming language R version 3.4 (R Core Team 2017). The states in our model were the number of fruits of each size and time in the flowering period. Our model assumed that plants maximize the expected number of seedlings from all the fruits that a plant produces at the end of its fruiting period, which depends on the number of fruits and viability of seeds inside the fruits. For each time step, the model predicted (1) which existing fruits to grow to a larger size and (2) how many pollinated flowers to retain to maximize fitness. We considered this prediction the plant's optimal life history strategy. Obviously, a plant does not consciously make those decisions. It is fair to assume that over evolutionary time genes associated with strategies providing the highest fitness persist and spread in the population, while those following suboptimal strategies die out (Kokko 2007).

In our model, a plant's life history strategy was constrained by the availability of resources, *R*, which a plant obtained via photosynthesis. We made the simplifying assumption that R was the same each time step because plant size and associated photosynthetic tissue does not change much during the short flowering period. This is likely true for slow growing plants such as *Y. glauca* (Kingsolver 1986) and *Catalpa speciosa* (Stephenson 1980). We set $R = 1$, which represented 100% of photosynthate produced by a plant at time step t. Each time step, the amount of resources needed to maintain existing fruits increased with increasing number and size of fruits, which in turn decreased the resources available to grow existing fruits to a larger size and retain flowers.

Flowering period, time steps, and fruit size classes

In our model, a plant's flowering period started from the day its first flower opened ($d = 1$) and ended after D days when the last flower stopped being receptive. We discretized the flowering period into, T time steps. Each time step, t, in the flowering period was $(D - 1)/T$ days long.

Each time step, a number of flowers opened on an inflorescence, starting from the bottom of the inflorescence. Each flower was pollinated with probability, p, which was the average pollination probability. In the field, the pollination probability likely varies over the flowering season. Since it would be challenging for plants to predict the exact value each day, they have likely evolved to respond to the expected average pollination probability.

We used the following sigmoidal curve to model fruit growth over time, which is consistent with the growth of many fruits and plant organs (Gustafson 1926; Nitsch 1953; Stephenson 1981, Appendix S1).

$$W_d = \frac{\emptyset_1}{\left(1 + e^{-\left(\frac{d - \emptyset_2}{\emptyset_3}\right)}\right)}, \qquad (1)$$

where $\emptyset_1$, $\emptyset_2$, and $\emptyset_3$ were the maximum fruit size at the end of the flowering period, the day at which the growth curve had an inflexion point, and a scaling parameter specifying how quickly the fruit reached the maximum size, respectively. We scaled the maximum fruit size $\emptyset_1$ to 1, which was equivalent to 100% of the maximum fruit size.

We discretized the continuous size distribution of fruits (equation 1) during the flowering period into discrete size classes. Discrete size classes are commonly used in matrix models to examine changes in population dynamics in a range of organisms including plants (Keeler and Tenhumberg 2011, Rees et al. 2014, Haridas et al. 2015). We denoted a size class by τ and assumed that fruits could grow from one size class (τ) to the next $(\tau + 1)$ in a single time step, t. This implied that the total number of time steps, T, equaled the number of size classes. For a flower τ equaled 0. If a plant retained a flower, the flower's ovary grew for one time step and became a fruit in the first size class $\tau = 1$. If this fruit was initiated in the first size class at the first time step ($t = 1$), it could have grown to the largest size class by the end of the flowering period.

We assumed that the flowering period was six time steps long and that at each time step two flowers opened (a=2). This implied that there could only be 0, 1, or 2 number of fruits in the largest size class, and 0-4 number of fruits in the second largest size class. Going from smallest to largest size class the maximum number of fruits possible in each size class was 12, 10, 8, 6, 4, and 2. Then, the number of possible state combinations (i.e., the state space) of the model was $(13\times11\times9\times7\times5\times3)\times$

$6 \text{ time steps} = 810{,}810$. We used the supercomputing facilities at the Holland Computing Center, University of Nebraska-Lincoln, for which access was limited to 168 h for a single run (one parameter combination). The model with the baseline parameter combinations where two flowers opened each time step took 4 h to run while the model where three flowers opened each time step took 118 h to run. Increasing either the number of size classes, time steps or the number of flowers opening each time step enlarged the state space of the model and the computational time considerably. The exponential increase in computational time with increasing number of states has been termed “curse of dimensionality”, and renders SDP models with a large number of states infeasible (Bellman 1957).

Since our model was limited to six size classes, we assumed that fruits continued to grow until the end of the fruiting period following a species-specific average growth trajectory. We considered two different post-flowering growth scenarios. First, fruit growth slowed down after the flowering season such that smaller fruits at the end of the flowering season remained smaller at the end of the fruiting season, similar to fruit growth in *Y. glauca* (Fig. S1.1). In many plant species, larger fruits produce larger seeds that are more viable than smaller fruits (Primack 1987). This is likely the case for plants that are adapted to habitats where larger seeds increase the probability of transitioning to seedlings (Stanton 1984, Westoby et al. 1996, Jakobsson and Eriksson 2000, Moles et al. 2006). In this first post-flowering growth scenario, we assumed that the relationship between fruit size and number of seedlings in the following season was sigmoidal. This shape is widely used to model the fitness associated with offspring size (Lloyd 1987, Dani and Kodandaramaiah 2017). We refer to this scenario as the “size-dependent viability scenario”.

In the second, “size-independent viability scenario”, we assumed that after the flowering season smaller fruits grew at a faster rate than larger fruits resulting in similar fruit sizes at the end of the fruiting season. In this case, the plant received similar fitness from fruits, independent of their sizes at the end of the flowering season. Alternatively, seed viability was independent of seed size, which has been shown for plants growing in favorable environments such as high light and moisture environments (Gross 1984).

Terminal Fitness Function, Z

Assuming the number of fruits in each size class at the end of the flowering period predicted the final size class distribution at the end of the fruiting period, we used the following sigmoidal function to determine a plant’s total fitness.

$$Z = \sum_{\tau=1}^{\tau_{max}} n_\tau \frac{1}{1-\lambda e^{-\left(\frac{\tau_{max}}{\tau_{max}-\tau}\right)}}, \qquad (2)$$

where n_τ was the number of fruits of size class τ, and λ was the fitness coefficient.

As λ increased, the difference in viability of fruits in small and large size classes increased. λ = 100 represented the first post-flowering growth scenario, and λ = 0 represented the second post-flowering growth scenario (Fig. 1).

Growth and maintenance costs

While larger fruits provided a higher fitness benefit to a plant, larger fruits costed more resources to grow and maintain than smaller ones.

The cost of maintaining a fruit, m, was proportional to its size class with a scale exponent of 0.75 (Kooijman 2000). The following equation showed the cost of maintaining a fruit in each size class. The maintenance coefficient, ω, was much smaller than one because the cost of maintaining fruit tissue is very small (Thornley 1970, Grossman and DeJong 1994, Marcelis and Hofman-Eijer 1995).

$$m_\tau = \omega {S_\tau}^{0.75} \qquad (3)$$

where, S_τ was the size of a fruit in size class τ. The cost of growing a fruit from one size class to the next was much greater than maintaining a fruit in the same size class. We assumed that a plant growing a fruit from one size class to the next allocated resources proportional to the change in fruit size (Thornley 1970, Marcelis and Hofman-Eijer 1995) using the following equation.

$$r_\tau = \beta(S_\tau - S_{(\tau-1)}), \qquad (4)$$

where the growth coefficient, β>1 because the conversion of resources to fruit tissue is not 100% efficient (Thornley 1970, Marcelis and Hofman-Eijer 1995).

Survival probability

Fruits may not survive from one time step to the next. We examined two survival scenarios. In the first scenario, we assumed that smaller fruits had a smaller survival probability σ_τ compared to larger fruits (i.e., size-dependent survival). This is observed in plants where smaller fruits have weaker tissues, which makes them more prone to injury by herbivores and damage by storms (Stephenson 1981). In the second scenario, we assumed fruit survival was independent of fruit size (i.e. size-independent survival).

Dynamic programming equation

We solved the SDP using backward iteration (Clark and Mangel 2000). The model stepped backward in time, starting at the last time step of the flowering season, T. To initialize the model, at the last time step the model determined the fitness associated with the final fruit size distribution using the terminal fitness function, Z (equation 2). At each time step, the model calculated the fitness associated with all possible resource allocation options, *i*, and picked the one with the highest expected fitness benefit. A resource allocation option specified the sequence $\{g_0, g_1, \dots, g_5\}$, where g_0 was the proportion of flowers retained and $g_1, \dots., g_5$ were the proportion of fruits that grew from size class τ to $\tau + 1$. For any given *i*, the number of fruits in time step *t*+1 was calculated as

$$n(t+1) = G(i)n(t) + aA(i), \qquad (5)$$

and the expected future fitness associated with *i* in the next time step was

$F(G(i)n(t) + aA(i), t + 1)$.

$n(t)$ was a vector indicating the number of fruits in each size class τ.

$$n(t) = \begin{bmatrix} n_1 \\ n_2 \\ \vdots \\ n_6 \end{bmatrix} \qquad (6)$$

G was a matrix specifying the proportion of fruits transitioning between size classes.

$$G(i) = \begin{bmatrix} 1-g_1 & 0 & 0 & 0 & 0 & 0 \\ g_1 & 1-g_2 & 0 & 0 & 0 & 0 \\ 0 & g_2 & 1-g_3 & 0 & 0 & 0 \\ 0 & 0 & g_3 & 1-g_4 & 0 & 0 \\ 0 & 0 & 0 & g_4 & 1-g_5 & 0 \\ 0 & 0 & 0 & 0 & g_5 & 1 \end{bmatrix} \qquad (7)$$

A was a vector specifying the proportion of flowers retained as new fruits in the first size class.

$$A(i) = g_0 \begin{bmatrix} 1 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \end{bmatrix} \qquad (8)$$

For i, only sequences were permissible that produced integer values for $n(t+1)$ because individual inflorescences cannot initiate or grow partial fruits. Further, all choices were constrained by the resources available each time step to initiate new fruits, maintain existing fruits, and grow fruits to the next size class. The resources required to implement strategy *i* was given by the following equation.

$$R_i = \left(g_0 a m_1 r_1 + \sum_{\tau=1}^{\tau_{max}-1} (1-g_\tau) n_\tau m_\tau + n_{\tau_{max}} m_\tau + \sum_{\tau=1}^{\tau_{max}-1} g_\tau n_\tau m_{\tau+1} r_{\tau+1}\right) \qquad (9)$$

Our formulation thus far ignored that not all fruits survived to the next size class and that not all flowers were pollinated. To include survival and pollination, the model weighed the fitness associated with an outcome of strategy *i* by its survival and pollination probability. Let P_k be the probability that *k* number of flowers were pollinated with probability *p*, and let S_j be the probability that $n'(t+1)$ fruits survived. $n'(t+1)$ was a vector specifying the number of surviving fruits in the different size classes and S_j was the multinomial probability of $n'(t+1)$ fruits surviving and the probability of fruit survival in each size class was σ_τ. Then the programming equation was,

$$F(n(t),t) = \max_{i|R_i \le R}\left(\sum_k P_k \sum_j S_j F(G(i,j)n(t) + aA(i,j,k), t+1)\right) \qquad (10)$$

The max implied that for any given time step during the flowering period the model considered the strategy associated with the highest expected fitness $F(n(t),t)$ as the optimal life history strategy.

Forward simulation

The SDP calculated the expected fitness and optimal life history strategies under all possible combinations of number of fruits in each size class. Hence, the model produced a multidimensional output that was difficult to interpret. One effective way to understand the model predictions is to simulate a population of plants following the optimal strategy forward through time. We simulated the fate of a population of $N = 10^6$ plants through a flowering period (t=1 to T) using the same parameter values and fruit size classes as the SDP. The simulation model predicted how the distribution of size classes changed on an inflorescence over one flowering period. The simulation started at the first times step t = 1 when a flowers opened and were pollinated with the pollination probability p. Next, the plant used the output of the SDP as a "look-up" table and picked the optimal strategy. The plant retained the number of flowers specified in the optimal strategy, up to the number of flowers that were pollinated. Then, fruits survived to the next size class with the survival probability σ. Based on the number of fruits surviving in each size class, the simulation updated the plant's states in the next time step.

To examine a plant's number-size strategy, we calculated the population averages for the number of fruits produced and the mean fruit size, and 95% CIs based on the 0.025th and 0.975th quantiles of the simulated distributions. To visualize the mean flower retention strategy of a plant at each time step for all possible numbers of basal fruits, we used the mean prediction from a generalized linear model with a binomial family of errors (lines in right hand panels of Figs. 3-4 and S2.1-S2.3). The response variable in each model was the proportion of pollinated flowers that plants retained and that survived, and the predictor variable was time.

CASE STUDY OF *YUCCA GLAUCA*

Study system

I parametrized the model using empirical data from the sequentially flowering *Yucca glauca* Nutt., (soapweed yucca, Family: Agavaceae) at Cedar Point Biological Station, Ogallala, Keith County, NE. *Yucca glauca* produce rosettes that may give rise to an inflorescence during the summer. Typically, each rosette grows for multiple years before it is capable of sexual reproduction (Kingsolver 1986). The *Yucca* spp. flowering period is about 15-30 days long (Powell 1992, Jadeja pers. obs.). Over the flowering period, plants open a subset of flowers from the bottom to the top of the flowering stalk. The total number of flowers produced by an inflorescence ranges from 17 to 140 buds (Kingsolver 1986, Svensson et al. 2011, Jadeja pers. obs.). After flowering, the rosette dies, and over time may be replaced by one or more new rosettes through vegetative reproduction. *Yucca glauca* flowers are receptive for 1-2 days after opening (Dodd and Linhart 1994). Retained fruits (both from basal and distal flowers) continue to simultaneously grow for a few weeks after the flowering period. Relative size differences between fruits at the end of the flowering period remain at the end of the fruiting period (this study).

Many *Y. glauca* fruits do not survive to maturation mainly due to frugivory by deer and cattle and excessive damage by the herbivorous insect *Carpophilus* sp. (florivorous beetle) (Jadeja pers. obs.). *Carpophilus* sp. and their larvae primarily damage flowers and young fruits (Huth and Pellmyr 1997, Jadeja pers. obs.). Hence, growing fruits quickly to a larger size likely gives fruits a survival benefit. Fruit set is low in

Yucca spp. with populations on average maturing fruits from less than 15% of their flowers (Udovic and Aker 1981, Pellmyr 1997, Addicott 1998), largely due to resource limitation (Huth and Pellmyr 1997).

Parameter estimation

The parameter estimates are listed in Table 1. In case parameter estimates for *Y. glauca* were not available, we used published parameter estimates for other plant species with a preference for congeneric species.

The flowering period of a *Y. glauca* inflorescence was 15 days (D ~ 15 days, Jadeja pers. obs.); hence, each time step was (15-1)/6 = $2.\overline{3}$ days. We assumed that at each time step two flowers opened and were receptive (a = 2). In the field, *Y. glauca* inflorescences opened on average 7.5 (SE = 0.5) flowers at a time (Chapter 3), but incorporating this large number of flowers into our model was not possible because it would have resulted in a too large state space. Opening 3 flowers each time step did not change our qualitative results (Appendix S2) but increased the duration of a single run from 4 h to 118 h. We assumed that a flower had a 60% chance of being pollinated (p = 0.6), which is consistent with the pollination probability in *Y. filamentosa* (Huth and Pellmyr 1997).

To estimate fruit growth and maintenance cost, we collected fruit volume data in summer 2016. On the first day of flowering we hand-pollinated 3-6 flowers of 15 *Y. glauca* inflorescences, and estimated the volume of initiated fruits every other day until they stopped growing, which was a median of 31 days after the flowers opened. Twenty fruits survived to maturity (Fig. S1.1).

We assumed the fruit growth curve of the largest fruit represented the maximum rate of fruit growth. In our study, the largest observed fruit had a volume of 148.08 cm^3, which was on an inflorescence with only one other fruit. We fitted equation 1 to the growth trajectory of this fruit using non-linear regression analysis, and considered this the maximum *Y. glauca* fruit growth trajectory. We scaled the growth equation so that the maximum fruit size was 1 ($\emptyset_1 = 1$, $\emptyset_2 = 13.77$, and $\emptyset_3 = 2.6$). See Appendix S1 for details on data collection and analysis. Next, we discretized the growth curve a fruit may follow during the flowering period into size classes. We determined how much a fruit increased in volume from one time step to the next, and used the mid-point of the time step as the representative volume for each size class. The volume of a flower was 0.013, and the volumes of fruits in the size classes from smallest to largest were 0.03, 0.07, 0.16, 0.35, 0.65, and 1, respectively.

Yucca glauca fruits increased in volume after the end of the flowering period (Fig. S1.1). Fruit volume at the end of the flowering period and fruit volume at the end of the fruiting period was significantly positively correlated (Spearman-rank correlation S = 310, $\rho = 0.77$, $p = 0.0001$). Therefore, *Y. glauca* fruit growth matches the first post-flowering growth scenario in our model ($\lambda = 100$, equation 2, Fig. 1).

We could not find cost estimates for fruit maintenance (ω) and growth (β) of *Yucca* spp. in the literature (equations 3, and 4). Hence, we used the large relative difference in carbon allocation estimates for growth and maintenance costs in fruits from the cultivated plants *Prunus persica* (Grossman and DeJong 1994) and *Cucumis sativus* (Marcelis and Hofman-Eijer 1995) to estimate values for maintenance and growth coefficients ($\omega = 0.05$ in equation 3, and $\beta = 2.5$ in equation 4). The cost of maintaining fruits in the six size classes $m_{\tau=1}, \dots, m_{\tau=6}$, was 0.004, 0.007, 0.013, 0.023, 0.036, and 0.05, respectively. The cost of growing flowers/fruits into the six size classes $r_{\tau=1}, \dots, r_{\tau=6}$, was 0.044, 0.104, 0.23, 0.459, 0.745, and 0.886, respectively.

The survival of fruits in *Yucca* spp. appears to be size-dependent with younger fruits being more likely to fail than older fruits owing to a higher tendency of attacks by frugivores and seed herbivores (Pellmyr and Huth 1994; Huth and Pellmyr 1997, Jadeja pers. obs.) and damage by storms (Jadeja pers. obs.). We set the survival probability of fruits in the first size class $\sigma_{\tau=1}$ to 0.903 so that approximately 54% of fruits in the smallest size class survived six time steps (0.903^6 = 0.542, Fig 2). For comparison, Udovic and Aker (1981) found that in *Y. whipplei* 51% of the initiated fruits survived to maturation. The corresponding survival probabilities of fruits in the six size classes, $\sigma_{\tau=1}, \dots, \sigma_{\tau=6}$, were 0.903, 0.907, 0.916, 0.934, 0.964, and 0.999, respectively.

Modeling scenarios

The main goal of this model was to explore the effect of fitness benefit of large fruit size on optimal life history strategies. For this we compared four scenarios: (1) small and large fruits had the same fitness (σ_τ = 0.903 and λ = 0); (2) large fruits had the same survival compared to small fruits but they produced more viable offspring (σ_τ = 0.903 and λ = 100); (3) large fruits had higher survival compared to small fruits but they produced the same number of viable offspring ($\sigma_{\tau=1}, \dots, \sigma_{\tau=6}$ = 0.903, …, 0.999 and λ = 0); We repeated this scenario by increasing the relative difference in survival of small and large fruits ($\sigma_{\tau=1}, \dots, \sigma_{\tau=6}$ = 0.709, 0.722, 0.749, 0.804, 0.893, 0.999 and λ = 100); (4)

Large fruits had higher survival and more viable offspring compared to small fruits (baseline model, $\sigma_{\tau=1}, \dots, \sigma_{\tau=6}$ = 0.903, …, 0.999 and λ = 100) (Figs. 3,4). Further, we explored the effect of floral display size (the number of flowers that opened each time step, a = 1, 2, and 3), fruit growth efficiency (β = 2.1, 2.3, 2.5, and 2.7), and pollination probability, (p = 0.3, 0.5, 0.6, 0.7, and 0.9) on optimal life history strategies.

RESULTS

Baseline model

The SDP model produced a multidimensional prediction specifying optimal life history strategies for each possible combination of states (i.e., number of fruits in each size class, and time step during the flowering period). The model predicted that plants should retain all pollinated flowers when zero basal fruits were present, independent of the time in the flowering period. In the presence of basal fruits, the optimal life history strategy depended on the number of fruits in each size class and the time in the flowering period.

The forward simulation predicted that by the end of the flowering period, (by the end of T = 6), 99.92% plants (N = 10^6) produced at least one fruit (Table S2.1). Since, we were interested in how plants allocate resources to fruit growth, we only show model predictions of plants that produced at least one fruit. In the remainder of the manuscript we report the lower and upper 95% confidence intervals in parenthesis. The model predicted that, by the end of the flowering period, a plant matured on average 4.57 (2, 7) fruits with a mean fruit size class of 2.6 (1.8, 3.7), The resulting average fitness per plant was 0.78 (0.08, 1.17) (Table S2.1).

In the baseline model, we examined the optimal life history strategy assuming both size-dependent viability and size-dependent survival. In this scenario, early in the flowering period when no or few basal fruits were present (time steps 1 to 3), plants had sufficient resources to retain all pollinated flowers and grew all existing fruits to the next size class (Fig. 3a). Later in the flowering period, plants could not grow all existing fruits to the next size class and as a result accumulated more fruits in the smaller size classes, while growing their largest fruits to the next size class. In addition, plants no longer had resources to retain all pollinated flowers and the likelihood of flower retention decreased with increasing number of fruits below (Fig 3b). This pattern was strongest at time step $t = 6$ but qualitatively similar during earlier time steps (4-5). However, when seven basal fruits were present at time step $t = 5$, flower retention was high and did not follow the general pattern of decreasing flower retention with increasing number of basal fruits (Fig. 3b). This and other similar exceptions might have been due to the small amount of resources remaining after allocating resources to maintain and grow existing fruits. While resources were sufficient to retain one or more pollinated flowers, they were not sufficient to grow existing fruits. This was likely an artifact of the model because resources could only be allocated in discrete amounts corresponding to the discretized fruit size classes in the model.

Effect of fruit size-dependency in viability and survival

In the absence of any fitness benefit from growing fruits to a larger size, a plant matured on average 5.07 (2.00, 8.00) fruits with a mean fruit size class of 1.6 (1.0, 2.4), The resulting average fitness per plant was 5.07 (2.00, 8.00) (Table S2.2). The optimal life history strategy was to invest resources mainly in retaining flowers and not in growing existing fruits to a larger size (Fig. 3g). Further, plants retained all pollinated flowers in each time step, independent of the number of basal fruits (Fig. 3h).

When plants had a size-dependent viability benefit but no size-dependent survival benefit, plants tended to mature fewer fruits of considerably larger average fruit size than when there was no size-dependent fitness benefit (Table S2.2). Plants did this by growing their largest fruits larger (compare Fig. 3c with Fig. 3g), and by decreasing flower retention with increasing number of basal fruits (compare Fig. 3d with Fig. 3h).

When plants had a size-dependent survival benefit but no size-dependent viability benefit, plants matured on average significantly larger fruits than when plants had no size-dependent fitness benefit (Table S2.2). Plants did this by growing fruits to larger size classes but not to the largest size class (compare Fig. 3e with Fig. 3g). This left resources for plants to retain all flowers independent of the number of basal fruits (Fig. 3f).

Increasing the relative difference in survival of small and large fruits, did not change the qualitative model predictions (Fig. S2.2a-h). When the relative difference in survival of small and large fruits was high, the average survival probability was low. At a lower average survival probability, plants retained on average fewer fruits resulting in a lower total fitness (Table S2.3).

Effect of fruit growth efficiency

With decreasing growth efficiency plants tended to invest less in growing fruits to the largest size, and, at the lowest growth efficiency values, available resources were insufficient to grow fruits to the largest size class (size class 6) (Table S2.4). However, the 95% CIs around the average number and size of fruits largely overlapped. On average, plants with very high or very low growth efficiency retained all pollinated flowers (Fig. 4a,g), independent of the number of basal fruits (Fig. 4b,h). At intermediate fruit growth efficiencies, plants on average decreased flower retention later in the flowering period (Fig. 4c,e), owing to increasing number of basal fruits (Fig. 4d,f). Hence, fruit growth efficiency had a non-linear effect on flower retention in response to the number of basal fruits.

Effect of pollination limitation

With increasing pollination probability a plant’s average number-size strategy shifted from few fruits of large average size to many fruits of small average size (Table S2.4). If pollination probability was high, plants still grew as many fruits to the largest size class as possible, but the overall higher number of fruits in the smaller size classes reduced the average size (Fig. S2.3a,c,e,g). Changing the pollination probability did not change the qualitative model predictions (Fig. S2.3a-h). Early in the flowering period plants retained every pollinated flower, but later they aborted flowers if the number of basal fruits was high. The model predicted that the time they started aborting flowers tended to shift to later time steps with increasing pollination probability (Fig. S2.3a,c,e,g). Functionally, increasing pollination probability was similar to increasing the number of flowers opening each time step as both increased the average number of flowers pollinated in a time step.

DISCUSSION

A plant's optimal fruit number-size strategy consists of the number of fruits a plant produces and the size of the fruits. Empirical work suggests that for some sequentially flowering plants it may be adaptive to produce only few, but large fruits, which may be achieved by retaining flowers conditional upon the number of existing basal flowers (Tamas et al. 1979, Stephenson 1980, Susko and Lovett-Doust 1999, Marcelis et al. 2004, Chapter 1). This is likely the case for plants where larger fruits contain large seeds with high viability and/or high probability of survival. Our model predicted the optimal fruit number-size strategies of sequentially flowering plants under different scenarios of fruit size-dependent fitness benefit.

Our model applies to a wide range of sequentially flowering plants. By scaling resource availability, maximum fruit size and associated maximum fitness to one, we reduced the number of parameters specific to *Y. glauca*. Our baseline model assumed that plants have a size-dependent viability benefit of growing fruits to a large size. The model predicted that under these conditions it is optimal for plants to allocate limited resources to produce few large fruits by decreasing flower retention with an increasing number of basal fruits and prioritizing the growth of their largest fruits. This prediction matches observed flower retention in *Y. glauca* (Chapter 1). A proximate mechanism that could produce such a pattern is the strong resource sink strength of basal fruits (Lloyd 1980, Stephenson 1981, Diggle 1995).

Our model suggested investing resources in growth is an evolutionary advantage when plants have a size-dependent viability or size-dependent survival benefit. In contrast, when plants had no size-dependent fitness benefit, our model predicted plants should produce many fruits of small average size. Our predictions match empirical evidence from small-seeded plants growing in favorable environments such as high light and moisture environments (Gross 1984) and theoretical predictions for plants with no size-dependent viability benefit of growing larger seeds (Venable and Brown 1988). Plants adapted to habitats where there is no viability advantage may have evolved proximate mechanisms to limit sink strength of fruits. Proximate mechanisms may include slower increase in the fruit stalk girth (peduncle) as fruit size increases to regulate influx of resources into the fruit (Gustafson 1926), and hormones to inhibit fruit growth and/or prevent abortion of flowers (Nitsch 1953).

In the absence of a size-dependent viability benefit our model predicted it is optimal for plants to retain many flowers by retaining flowers independent of the number of basal fruits, even when plants had a size-dependent survival benefit. Further, it is optimal for plants to maintain fruits at smaller size classes than when there is a size-dependent viability benefit. For the parameters we examined, our model suggested that size-dependent survival benefit is not sufficient for plants to grow basal fruits large enough to deprive distal flowers of resources.

In some plant species, fruit growth efficiency may be low because a large amount of resources is required to grow fruits. Plants with low fruit growth efficiency may either have fruits that are not photosynthetic and depend entirely on the plants for resources (Bazzaz et al. 1979, Obeso 2002) or fruits that produce complex structures for protection or dispersal that may require more resources to grow than simpler structures (Sutherland 1986, Lord and Westoby 2006). Empirical evidence shows that plants with a high fruit growth efficiency produce more fruits than plants with low fruit growth efficiency

(Sutherland 1986). Our predictions for the number and average size of fruits for plants with increasing fruit growth efficiency showed a large overlap in their 95% confidence intervals, which suggests fruit growth efficiency has no effect on a plant's fruit number-size strategy.

In many plants species, pollen limitation decreases the total number of fruits a plant produces (Burd 1994). Our model predicted that in sequentially flowering plants pollen limitation selects for plants producing few fruits of large size where large fruits produce seeds with high viability as would be the case with large seeds. This prediction is consistent with a previous model showing that with increasing pollen limitation plants produce larger seeds (Huang et al. 2017). So, when pollen limitation is low (i.e., pollination probability is high) we expected plants to retain many flowers. However, even at the highest level of pollination probability ($p = 0.9$) in our model, plants decreased flower retention with increasing number of basal fruits. This matches fruiting in *Yucca* spp. where the number of fruits a plant produces is limited by resources and not pollination (Huth and Pellmyr 1997, Humphries and Addicott 2004).

Compared to previous theoretical models examining optimal offspring number-size strategies that are general (reviewed by Dani and Kodandaramaiah 2017), our model is unique in being specific to sequentially flowering plants. This has allowed us to examine a plant's strategy to allocate resources to retain flowers (increase number of fruits) and grow existing fruits in response to size-dependent viability and survival benefit, fruit growth efficiency, and pollen limitation. Further, our case study using *Y. glauca* has allowed us to make meaningful comparisons between model predictions and empirical work (Chapter1).

REFERENCES

Addicott, J. F. 1998. Regulation of mutualism between yuccas and yucca moths: population level processes. - Oikos 81: 119–129.

Bazzaz, F. A. et al. 1979. Contribution to reproductive effort by photosynthesis of flowers and fruits. - Nature 279: 554–555.

Bellman, R. 1957. Dynamic programming. - Princeton University Press.

Burd, M. 1994. Bateman'a principle and plant reproduction: the role of pollen limitation in fruit and seed set. - Bot. Rev. 60: 83–139.

Clark, C. and Mangel, M. 2000. Dynamic state variable models in ecology: Methods and applications. - Oxford University Press.

Dani, K. G. S. and Kodandaramaiah, U. 2017. Plant and animal reproductive strategies: lessons from offspring size and number tradeoffs. - Front. Ecol. Evol. 5: 38.

Diggle, P. K. 1995. Architectural effects and the interpretation of patterns of fruit and seed development. - Annu. Rev. Ecol. Syst. 26: 531–552.

Dodd, R. J. and Linhart, Y. B. 1994. Reproductive consequences of interactions between *Yucca glauca* (Agavaceae) and *Tegeticula yuccasella* (Lepidoptera) in Colorado. - Am. J. Bot. 81: 815–825.

Gross, K. L. 1984. Effects of seed size and growth form on seedling establishment of six monocarpic perennial plants. - J. Ecol. 72: 369–387.

Grossman, Y. L. and DeJong, T. M. 1994. Peach: A simulation-model of reproductive and vegetative growth in peach trees. - Tree Physiol. 14: 329–345.

Guitián, J. et al. 2001. Causes of fruit set variation in *Polygonatum odoratum* (Liliaceae).

- Plant Biol. 3: 637–641.

Gustafson, F. G. 1926. Growth studies on fruits an explanation of the shape of the growth curve. - Plant Physiol. 2: 153–161.

Haridas, C. V. et al. 2015. Variation in the local population dynamics of the short-lived *Opuntia macrorhiza* (Cactaceae). - Ecology 96: 800–807.

Huang, Q. et al. 2017. Resource allocation and seed size selection in perennial plants under pollen limitation. - Am. Nat. 190: 430–441.

Humphries, S. A. and Addicott, J. F. 2004. Regulation of the mutualism between yuccas and yucca moths: intrinsic and extrinsic patterns of fruit set. - Can. J. Bot. 82: 573–581.

Huth, C. J. and Pellmyr, O. 1997. Non-random fruit retention in *Yucca filamentosa*: consequences for an obligate mutualism. - Oikos 78: 576–584.

Jakobsson, a and Eriksson, O. 2000. A comparative study of seed number, seed size, seedling size and recruitment in grassland plants. - Oikos 88: 494–502.

Keeler, K. H. and Tenhumberg, B. 2011. Population dynamics of the western prickly pear, *Opuntia macrorhiza* (Cactaceae). - Southwest. Nat. 56: 147–153.

Kingsolver, R. 1986. Vegetative reproduction as a stabilizing feature of the population dynamics of *Yucca glauca*. - Oecologia 69: 380–387.

Kokko, H. 2007. Modelling for field biologists and (other interesting people). - Cambridge University Press.

Kooijman, S. A. L. M. 2000. Chapter 1: Energetics and models. - In: Dynamic energy and mass budgets in biological systems. 2nd Edition. Cambridge University Press, in press.

Lloyd, D. G. 1980. Sexual strategies in plants. I. An hypothesis of serial adjustement of maternal investment during one reproductive session. - New Phytol. 86: 69–79.

Lloyd, D. G. 1987. Selection of offspring size at independence and other size- versus-number strategies. - Am. Nat. 129: 800–817.

Lord, J. M. and Westoby, M. 2006. Accessory costs of seed production. - Oecologia 150: 310–317.

Louda, S. M. and Potvin, M. A. 1995. Effect of inflorescence-feeding insects on the demography and lifetime fitness of a native plant. - Ecology 76: 229–245.

Marcelis, L. F. M. and Hofman-Eijer, L. R. B. 1995. Growth and maintenance respiratory costs of cucumber fruits as affected by temperature, and ontogeny and size of fruits. - Physiol. Plant. 93: 484–492.

Marcelis, L. F. M. et al. 2004. Flower and fruit abortion in sweet pepper in relation to source and sink strength. - J. Exp. Bot. 55: 2261–2268.

Medrano, M. et al. 2000. Patterns of fruit and seed set within inflorescences of *Pancratium maritimum* (Amaryllidaceae) : nonuniform pollination, resource limitation , or architectural effects? - Am. J. Bot. 87: 493–501.

Moles, A. T. et al. 2006. Seed size and plant strategy across the whole life cycle. - Oikos 113: 91–105.

Nitsch, J. P. 1953. The physiology of fruit growth. - Annu. Rev. Plant Physiol. 4: 199–236.

Obeso, J. R. 2002. The costs of reproduction in plants. - New Phytol. 155: 321–348.

Pellmyr, O. 1997. Pollinating seed eaters: why is active pollination so rare? - Ecology 78: 1655–1660.

Pellmyr, O. and Huth, C. J. 1994. Evolutionary stability of mutualism between yuccas and yucca moths. - Nature 372: 257–260.

Powell, J. A. 1992. Interrelationships of yuccas and yucca moths. - Trends Ecol. Evol. 7: 10–15.

Primack, R. B. 1987. Relationships among flowers, fruits, and seeds. - Ann. Rev. Ecol. Syst. 18: 430.

Rathcke, B. and Lacey, E. P. 1985. Phenological patterns of terrestrial plants. - Annu. Rev. Ecol. Syst. 16: 179–214.

R Core Team 2017. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Rees, M. et al. 2014. Building integral projection models: A user’s guide. - J. Anim. Ecol. 83: 528–545.

Sakai, S. and Harada, Y. 2004. Size-number trade-off and optimal offspring size for offspring produced sequentially using a fixed amount of reserves. - J. Theor. Biol. 226: 253–264.

Smith, C. C. and Fretwell, S. D. 1974. The optimal balance between size and number of offspring. - Am. Nat. 108: 499–506.

Stanton, M. L. 1984. Seed variation in wild radish: effect of seed size on components of seedling and adult fitness. - Ecology 65: 1105–1112.

Stephenson, A. G. 1980. Fruit set, herbivory, fruit reduction, and the fruiting strategy of *Catalpa speciosa* (Bignoniaceae). - Ecology 61: 57–64.

Stephenson, A. G. 1981. Flower and fruit abortion: proximate causes and ultimate functions. - Annu. Rev. Ecol. Syst. 12: 253–279.

Stephenson, A. G. and Winsor, J. A. 1986. *Lotus corniculatus* regulates offspring quality through selective fruit abortion. - Evolution (N. Y). 40: 453–458.

Susko, D. J. and Lovett-Doust, L. 1999. Effects of resource availability, and fruit and ovule position on components of fecundity in *Alliaria petiolata* (Brassicaceae). - New Phytol. 144: 295–306.

Sutherland, S. 1986. Patterns of fruit-set: what controls fruit-flower ratios in plants? - Evolution (N. Y). 40: 117–128.

Svensson, G. P. et al. 2011. Pollinator attraction to volatiles from virgin and pollinated host flowers in a yucca/moth obligate mutualism. - Oikos 120: 1577–1583.

Tamas, I. A. et al. 1979. Effect of older fruits on abortion and abscisic acid concentration of younger fruits in *Phaseolus vulgaris* L. - Plant Physiol. 64: 620–622.

Thornley, J. H. 1970. Respiration, growth and maintenance in plants. - Nature 227: 304–305.

Udovic, D. and Aker, C. 1981. Fruit abortion and the regulation of fruit number in *Yucca whipplei*. - Oecologia 49: 245–248.

Venable, D. L. 1992. Size-number trade-offs and the variation of seed size with plant resource status. - Am. Nat. 140: 287–304.

Venable, D. L. and Brown, J. S. 1988. The selective interactions of dispersal, dormancy, and seed size as adaptations for reducing risk in variable environments. - Am. Nat. 131: 360–384.

Westoby, M. et al. 1996. Comparative ecology of seed size and dispersal. - Philos. Trans. R. Soc. London B 351: 1309–1318.

TABLES AND FIGURES

Table 1. Parameters for *Yucca glauca* included in the baseline stochastic dynamic programming model.

Parameter	Description	Value	Source
D	Number of days in the flowering period	15	(Jadeja pers. obs.)
T	Number of time steps	6	
t	Time step of (D-1)/T in days	$2.\overline{3}$ days	
a	Number of flowers opening per time step	2	
p	Pollination probability	0.6	(Huth and Pellmyr 1997)
R	Resource availability per time step	1	
Growth curve parameters (Eq. 1):			
$\emptyset_1$	Maximum fruit size	1	
$\emptyset_2$	X value at inflection point	13.77	This study
$\emptyset_3$	Scaling parameter	2.63	This study
τ	Fruit size class, where 0 indicates flower	0-6	
λ	Coefficient for fitness function Z (Eq. 2)	100	
ω	Coefficient for maintenance cost m (Eq. 3)	0.05	
β	Coefficient for growth efficiency r (Eq. 4)	2.5	
σ_τ	Survival probability of a fruit in size class $\tau = 1, \dots, 6$	0.903, 0.907, 0.916, 0.934, 0.964, 0.999	

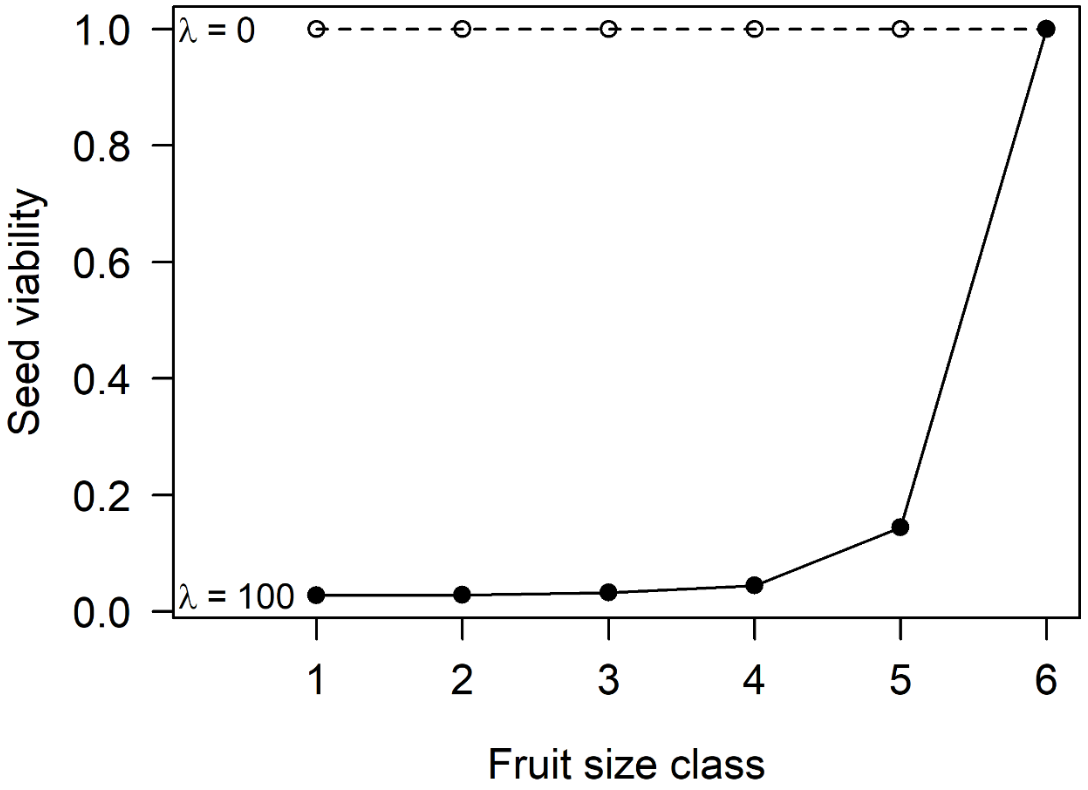


Figure 1. The examined viability benefit of growing fruits to larger size classes at the end of the flowering period under different scenarios of post-flowering growth. A high value of the fitness coefficient, λ = 100, (equation 2) implies a high size-dependent viability benefit (baseline model). In contrast, λ = 0, implies no size-dependent viability benefit.

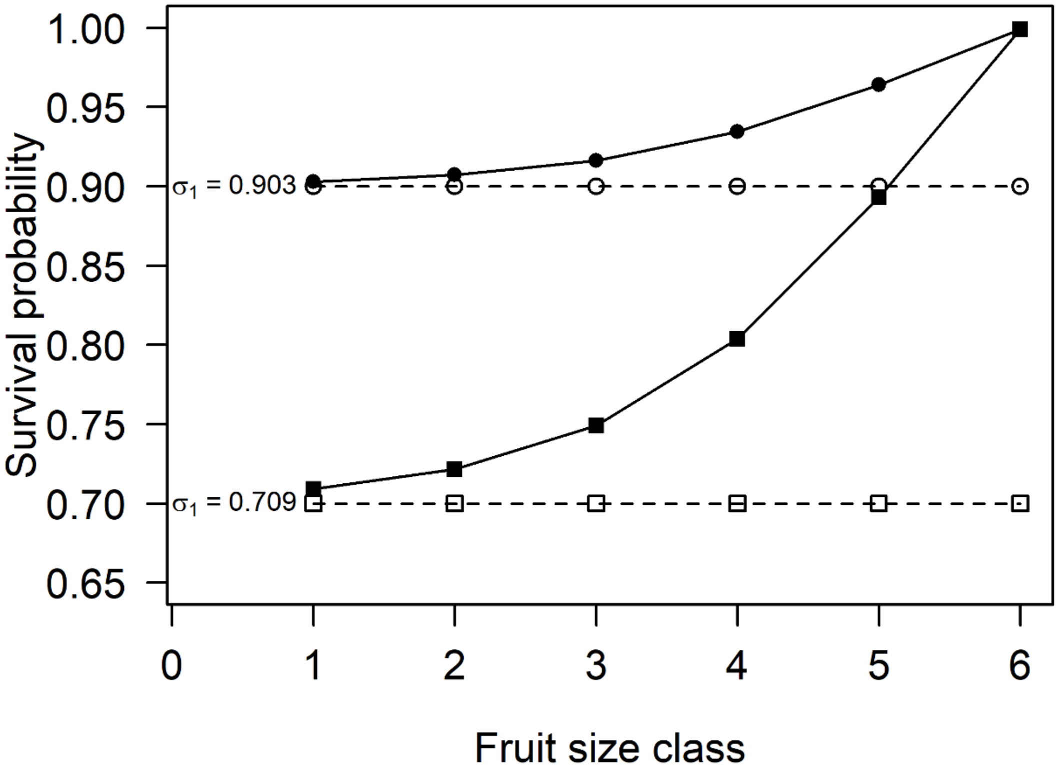
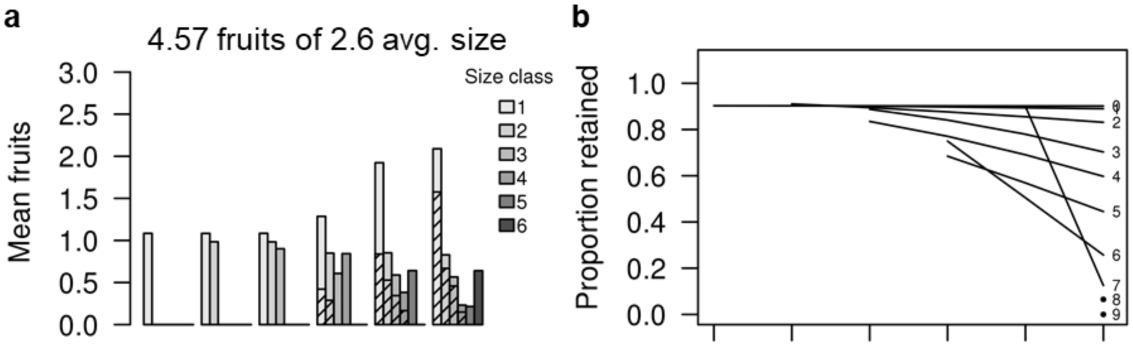
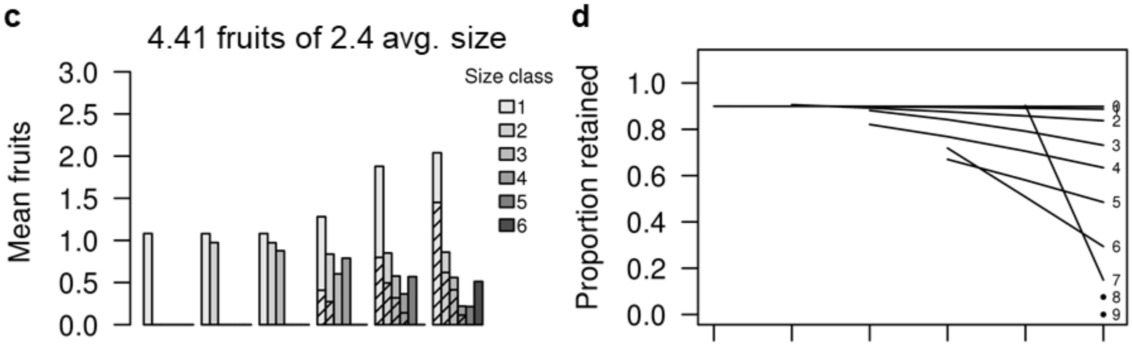


Figure 2. The examined survival benefit scenarios of growing fruits to larger size classes at high (circles) and low (squares) survival probability of the smallest size class ($\sigma_{\tau=1}$ = 0.903 and 0.709, respectively). Filled points and solid lines refer to a size-dependent survival benefit, and open points and dashed lines refer to a size-independent survival benefit.

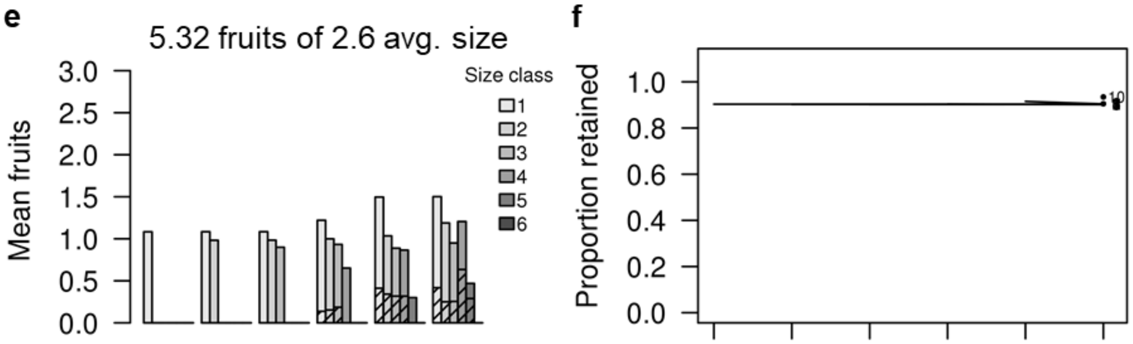
Baseline: Both size-dependent viability and size-dependent survival benefit



Only size-dependent viability benefit, no size-dependent survival benefit



Only size-dependent survival benefit, no size-dependent viability benefit



Neither size dependent viability nor size-dependent survival benefit

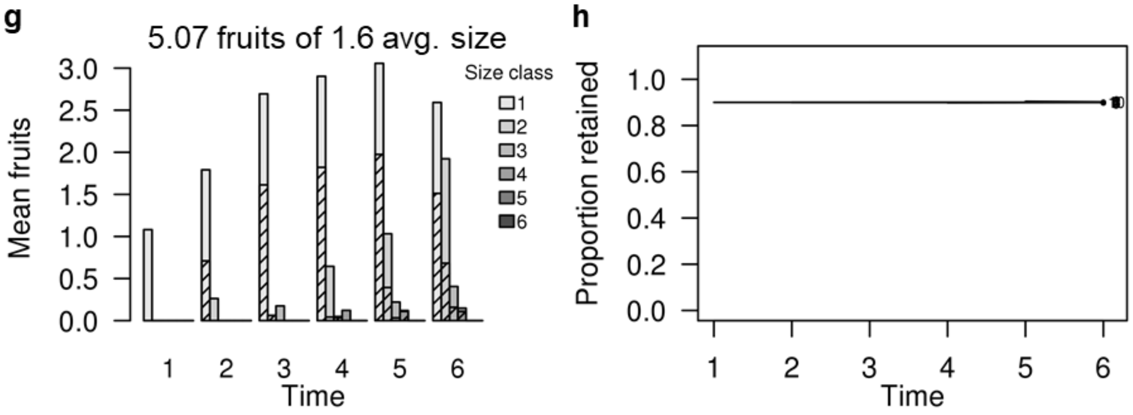
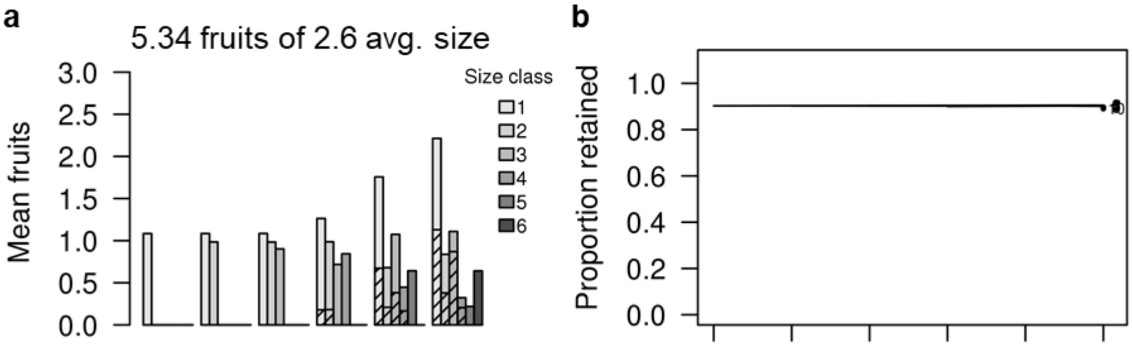
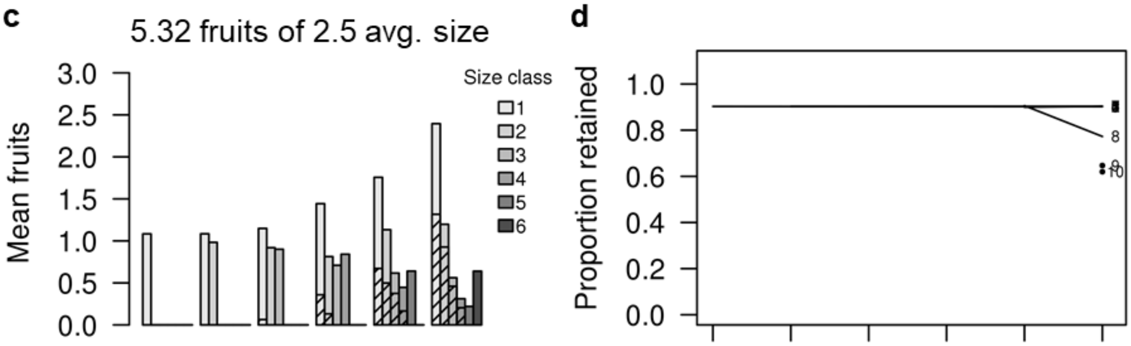


Figure 3. The effect of fruit size-dependency in seed viability (λ) and survival (σ_τ) on the plant's optimal life history strategy from simulated populations of 10^6 plants ($\sigma_{\tau=1}$ = 0.903). Fruits in each size class consist of "old" fruits that did not grow (slanted lines) and "new" fruits that were smaller the previous time step (no lines) or for size class 1 it indicates retained flowers. Sub-headings above panels show the average fruit number-size strategy at the end of the flowering period (table S2.2). The numbers following each line in the right had side panels indicate the number of basal fruits. Each line is the mean prediction for the proportion of pollinated flowers retained at each time step. See table 1 for the baseline parameter values. Other parameter values were σ_τ = 0.903 (c, d, g, h), and λ = 0 (e, f, g, h).

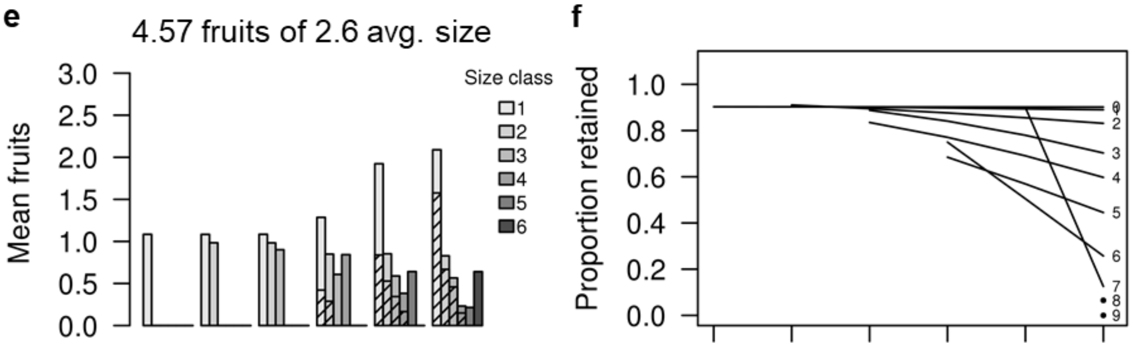
Very high growth efficiency (1.26 fruits possible in largest size class)



High growth efficiency (1.16 fruits possible in largest size class)



Baseline: Low growth efficiency (1.07 fruits possible in largest size class):



Very low growth efficiency (0.99 i.e. no fruits possible in largest size class)

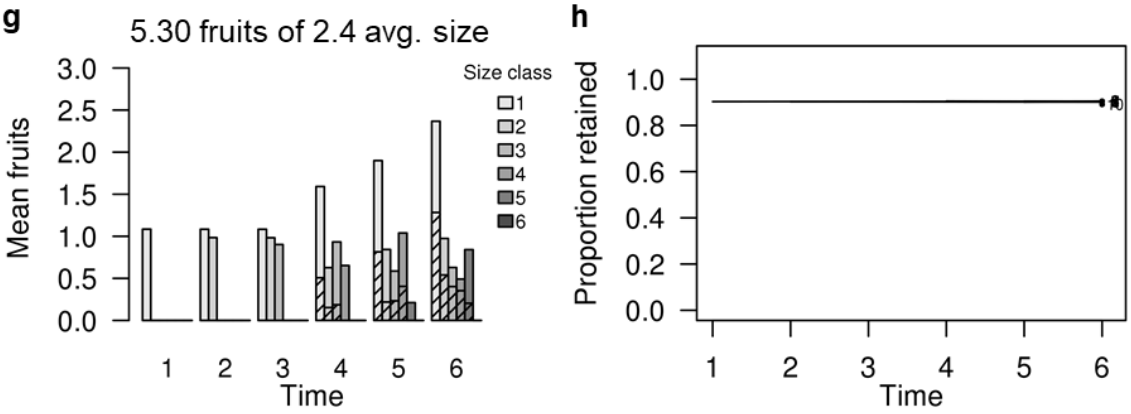


Figure 4. Effect of growth efficiency, β, on the plant's optimal life history strategy from simulated populations of 10^6 plants. Fruits in each size class consist of "old" fruits that did not grow (slanted lines) and "new" fruits that were smaller the previous time step (no lines) or for size class 1 it indicates retained flowers. Sub-headings above panels show the average fruit number-size strategy at the end of the flowering period (table S2.4). The numbers following each line in the right hand panels indicate the number of basal fruits. Each line is the mean prediction for the proportion of pollinated flowers retained at each time step. See table 1 for the baseline model parameter values. The other parameter values were β = 2.1 (a, b), 2.3 (c, d), and 2.7 (g, h).

SUPPLEMENTARY MATERIAL

Appendix S1

Parameter estimates for the maximum fruit growth curve from Yucca glauca

Data collection

We determined the growth of *Y. glauca* fruits in summer 2016 using 15 *Y. glauca* inflorescences at Cedar Point Biological Station, Ogallala, Keith County, NE. Before each inflorescence began flowering, we protected the inflorescences from pollinators and insect herbivores using fine tulle mesh sleeves, and from large herbivores like deer using tomato cages wrapped with hex-netting. The day the inflorescences started flowering, we hand-pollinated three to six early-opening basal flowers using pollen donors more than 25 m in distance away. Totally, we hand-pollinated 66 flowers across 15 inflorescences. For the detailed protocol on the procedure for protecting inflorescences and hand-pollinating flowers see Appendix S1 in the Supplementary Material section of Chapter 1. Every alternate day, starting from the day a flower was hand-pollinated, we measured the largest diameter of the ovary ($2r$) and its length from the base to the tip of the stigma (h). We used these two measurements to estimate the volume of the fruit assuming a cylindrical fruit shape using formula $\pi r^2 h$ where r is half the largest diameter of the ovary, and h is the length of the fruit. We measured fruits until they grew to their full sizes and showed no further growth. We only used data from fruits that reached their full size (n = 20). We discarded measurements of initiated fruits that the plants lost because their growth measurements may not be representative of the growth of fruits plants retain.

Summary of data

Overall, six inflorescences retained 30% of the hand-pollinated flowers. The number of fruits on each inflorescence ranged from 2 to 5 fruits. The largest fruit was on an inflorescence with only one other fruit (two fruits in total). All aborted flowers or young fruits were aborted within 6 days of pollination, except for one fruit that was aborted within 10 days of pollination. There was no significant difference between the size of the ovaries of the flowers that were retained versus aborted ($t = -0.91$, $df = 28.69$, $p = 0.37$). The median fruit volume stopped increasing 31 days after pollination, which we designated as end of the fruiting period. Further, *Y. glauca* flowering period was about 2 weeks long. The volume of fruits at the end of the flowering period (day 15) was significantly positively correlated with the volume of fruits at the end of the fruiting period (day 31) (Spearman-rank correlation $S = 310$, $\rho = 0.77$, $p = 0.0001$).

Estimating parameters for the maximum fruit growth curve

Like many plants, *Y. glauca* fruit growth from the day of pollination until the end of the fruiting period follows a sigmoidal pattern (Fig. S1.1). We considered the fruit growth curve of the largest fruit on inflorescences to represent the maximum rate at which fruits could grow from one time step to the next. Hence, we used the growth data of the largest fruit to estimate the parameters of equation 1 using non-linear least squares regression model. We used the programming language R version 3.4 (R Core Team 2017) for estimating the model parameters (SSlogis() function). Model parameters were $\emptyset_1 = 148.08$, $\emptyset_2 = 13.77$, and $\emptyset_3 = 2.64$ (Table S1.1). For the SDP model, we scaled the maximum fruit volume $\emptyset_1$ to 1, which was equivalent to 100% of the maximum fruit size.

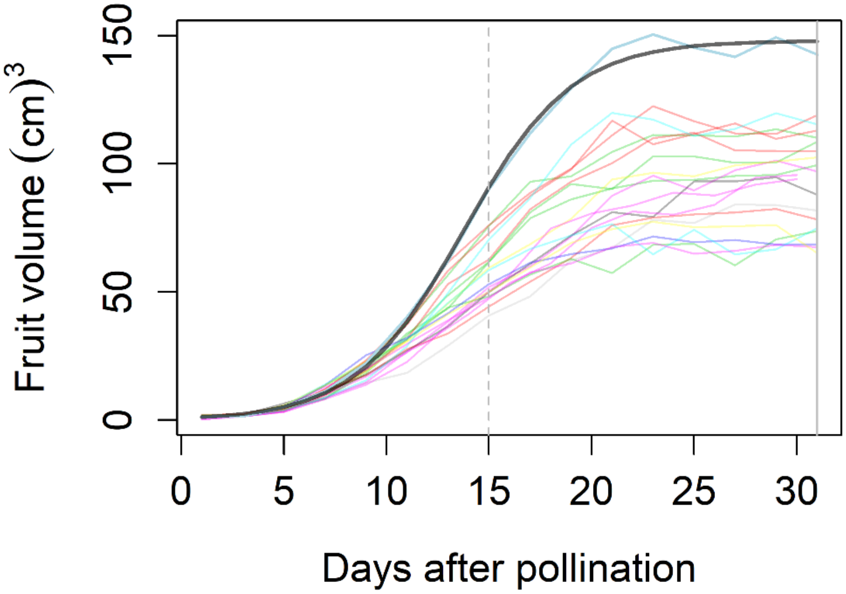


Figure S1.1. *Yucca glauca* fruit growth curves. Thin lines show the fruit growth curve of individual fruits. Thick line shows the model-predicted sigmoidal function for the largest fruit’s growth curve. Dashed vertical line indicates the end of the flowering period, and solid vertical line indicates the end of the fruiting period.

Table S1.1. Non-linear least squares regression model output for the logistic change in fruit volume of *Yucca glauca* plants over days for the largest fruit. Residual standard error was 3.453 with 13 degrees of freedom.

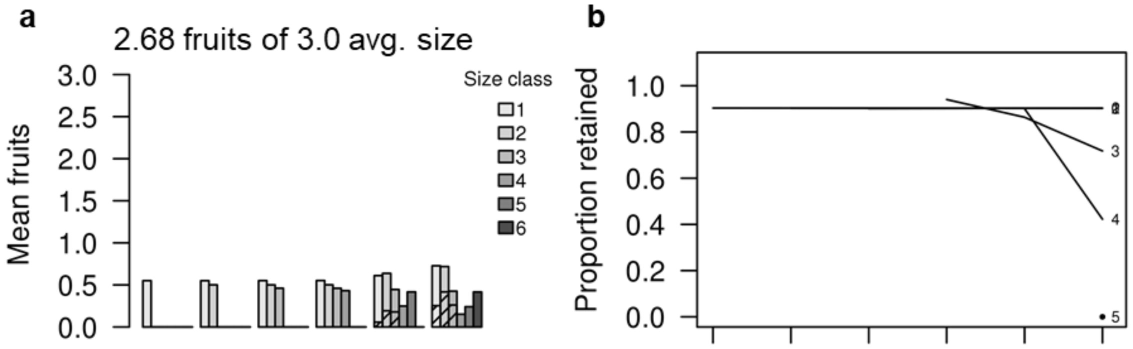
Parameter	**Estimated mean**	**SE**	**t**	**Pr(>|t|)**
Asymptote	148.08	1.66	89.30	<0.001
X value at the inflection point	13.77	0.16	87.05	<0.001
Scale parameter	2.64	0.13	19.68	<0.001

Appendix S2

Effect of floral display size

Changing the number of flowers opening each time step did not change the qualitative baseline model predictions (Fig. S2.1a-d). Plants opening three flowers each time step retained significantly more fruits than plants that opened one flower each time step (Table S2.1). A plant opening one flower each time step retained all pollinated flowers and grew all existing fruits until one time step later in the flowering period than the baseline model (Fig. S2.1a). This was because plants took more time steps before they faced a resource allocation trade-off. On the other hand, when three flowers opened each time step, plants faced a resource allocation trade-off one time step earlier in the flowering period than the baseline model (Fig. S2.1c).

One flower opening each time step



Three flowers opening each time step

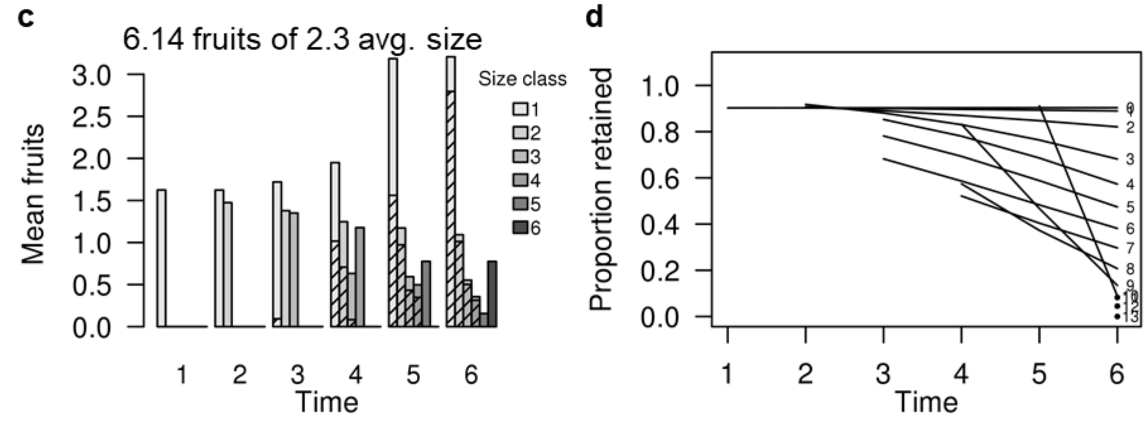
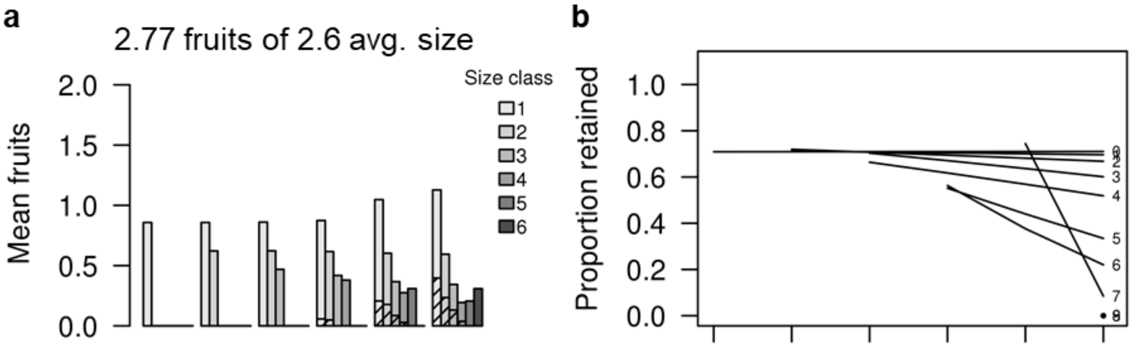
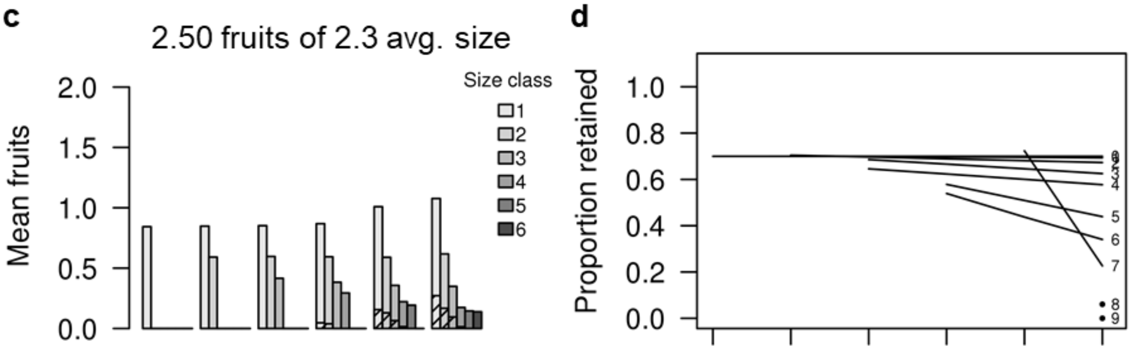


Figure S2.1. The effect of the number of flowers opening each time step, *a*, on the plant's optimal life history strategy from simulated populations of 10^6 plants. Fruits in each size class consist of "old" fruits that did not grow (slanted lines) and "new" fruits that were smaller the previous time step (no lines) or for size class 1 it indicates retained flowers. Sub-headings above panels show the average fruit number-size strategy at the end of the flowering period (table S2.1). The numbers following each line in the right hand panels indicate the number of basal fruits. Each line is the mean prediction for the proportion of pollinated flowers retained at each time step. See table 1 for the baseline model parameter values. The other parameter values were *a* = 1 (a, b) and 3 (c, d).

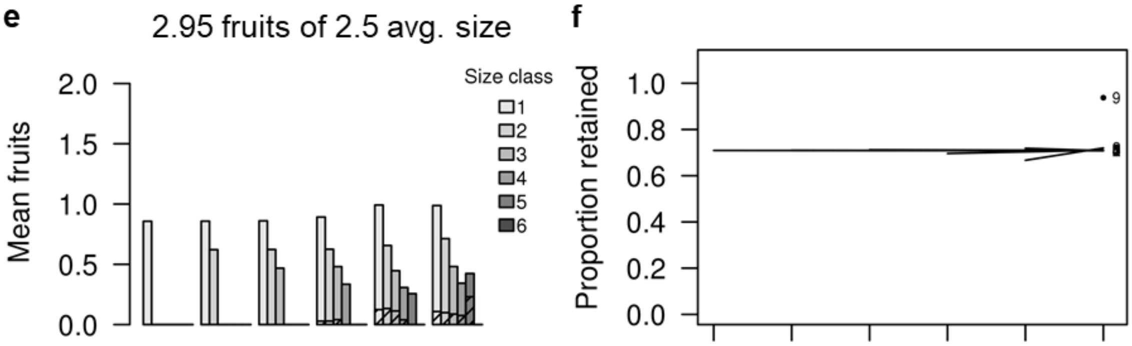
Both size-dependent viability and size-dependent survival benefit



Only size-dependent viability benefit, no size-dependent survival benefit



Only size-dependent survival benefit, no size-dependent viability benefit



Neither size dependent viability nor size-dependent survival benefit

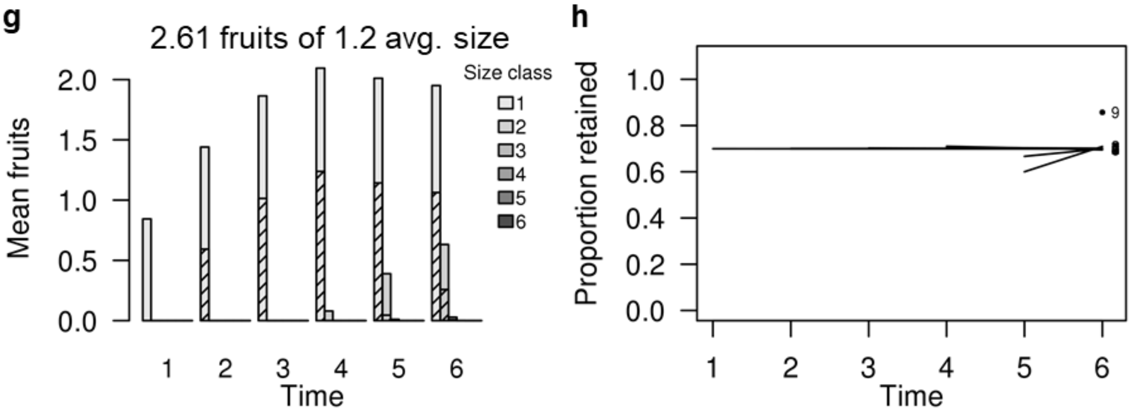
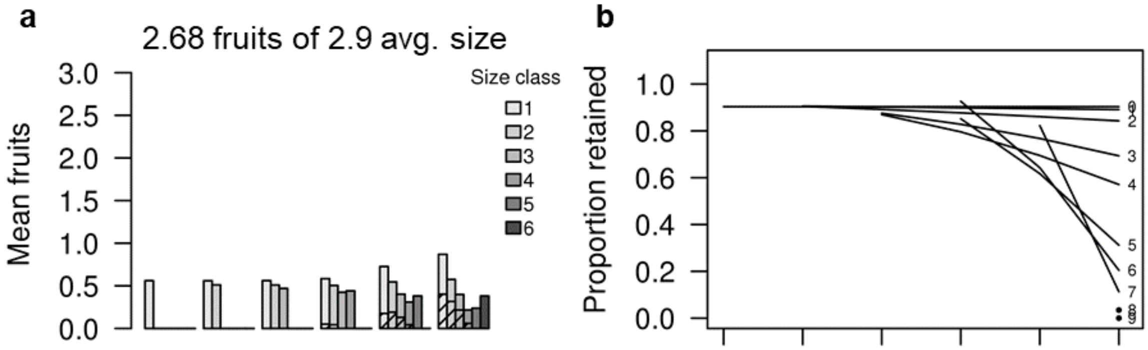
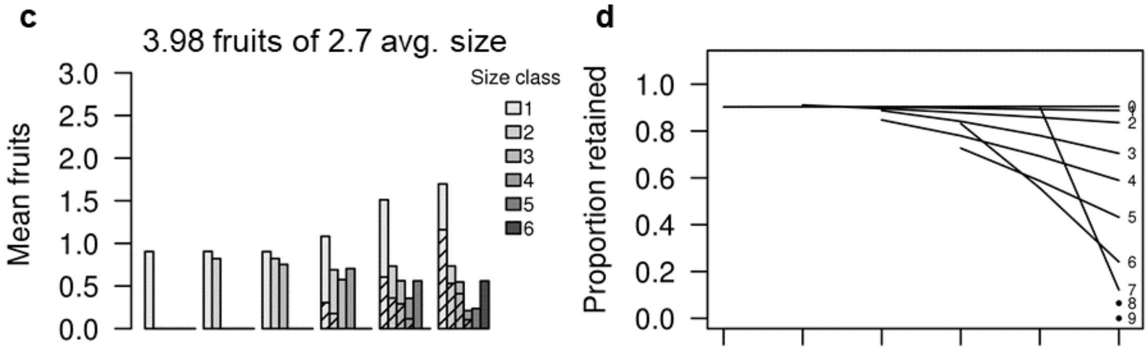


Figure S2.2. The effect of fruit size-dependency in seed viability (λ) and survival (μ) on the plant's optimal life history strategy from simulated populations of 10^6 plants. The survival of the smallest size class is lower than in the baseline model ($\sigma_{\tau=1}$ = 0.709). Fruits in each size class consist of "old" fruits that did not grow (slanted lines) and "new" fruits that were smaller the previous time step (no lines) or for size class 1 it indicates retained flowers. Sub-headings above panels show the average fruit number-size strategy at the end of the flowering period (table S2.3). The numbers following each line in et right hand side panels indicate the number of basal fruits. Each line is the mean prediction for the proportion of pollinated flowers retained at each time step. See table 1 for the baseline model parameter values. The other parameter values were $\sigma_{\tau=1}, \ldots, \sigma_{\tau=6}$ = 0.709, 0.722, 0.749, 0.804, 0.893, 0.999 (a, b, e, f) and σ_{τ} = 0.709 (c, d, g, h), and λ = 0 (e, f, g, h).

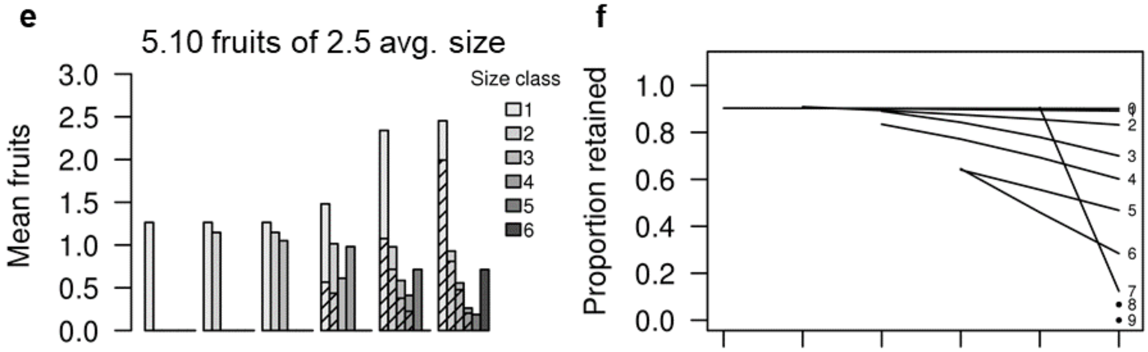
Very low pollination probability, p = 0.3



Low pollination probability, p = 0.5



High pollination probability, p = 0.7



Very high pollination probability, p = 0.9

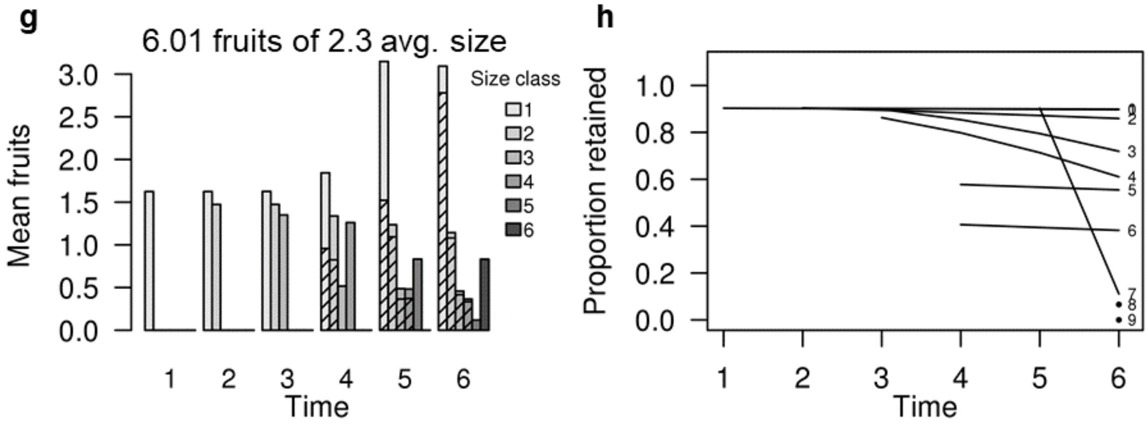


Figure S2.3. The effect of pollination probability, *p*, on the plant's optimal life history strategy from simulated populations of 10^6 plants. Fruits in each size class consist of "old" fruits that did not grow (slanted lines) and "new" fruits that were smaller the previous time step (no lines) or for size class 1 it indicates retained flowers. Sub-headings above panels show the average fruit number-size strategy at the end of the flowering period (table S2.1). The numbers following each line in the right hand panels indicate the number of basal fruits. Each line is the mean prediction for the proportion of pollinated flowers retained at each time step. See table 1 for the baseline model parameter values. The other parameter values were p = 0.3 (a, b), 0.5 (c, d), 0.7 (e, f), and 0.9 (g, h).

Table S2.1. Predictions of the Forward simulation model examining the effect of the number of flowers opening each time step and pollination probability. The numbers represent population means and 95% CIs in parentheses estimates as the 0.025 and 0.975 quantiles from the simulated population. The baseline model is highlighted.

Model type	***a***	***p***	**Fruiting plants (%)**	**Total fruits**	**Mean fruit size class**	**Total fitness**
Number of flowers opening each time step	1	0.6	97.37	2.68 (1, 5)	3.0 (1.0, 5.0)	0.51 (0.03, 1.09)
	3	0.6	100.00	6.14 (3, 10)	2.3 (1.7, 3.5)	0.95 (0.16, 1.23)
Pollination probability	2	0.3	95.39	2.68 (1, 5)	2.9 (1.0, 6.0)	0.48 (0.03, 1.11)
	2	0.5	99.66	3.98 (1, 7)	2.7 (1.7, 4.0)	0.69 (0.06, 1.14)
	2	0.6	99.92	4.57 (2, 7)	2.6 (1.8, 3.7)	0.78 (0.08, 1.17)
	2	0.7	99.99	5.10 (3, 8)	2.5 (1.8, 3.7)	0.86 (0.12, 1.18)
	2	0.9	100.00	6.01 (3, 9)	2.3 (1.8, 3.3)	1.00 (0.19, 1.21)

Table S2.2. Predictions of the Forward simulation model examining the effect of size-dependent viability (SDV) and size-dependent survival (SDS) benefit ($\sigma_{\tau=1}$ = 0.903). The numbers represent population means and 95% CIs in parentheses estimated as the 0.025 and 0.975 quantiles from the simulated population. The baseline model is highlighted.

Model type	λ	**SDS**	$\sigma_{\tau=1}$	**Fruiting plants (%)**	**Total fruits**	**Mean fruit size class**	**Total fitness**
SDV + SDS	100	Yes	0.903	99.92	4.57 (2, 7)	2.6 (1.8, 3.7)	0.78 (0.08, 1.17)
Only SDV	100	No	0.903	99.86	4.41 (2, 7)	2.4 (1.3, 3.7)	0.65 (0.06, 1.17)
Only SDS	0	Yes	0.903	99.92	5.32 (2, 9)	2.6 (1.9, 3.5)	5.32 (2.00, 9.00)
Neither SDV nor SDS	0	No	0.903	99.87	5.07 (2, 8)	1.6 (1.0, 2.4)	5.07 (2.00, 8.00)

Table S2.3. Predictions of the Forward simulation model examining the effect of size-dependent viability (SDV) and size-dependent survival benefit (SDS) ($\sigma_{\tau=1}$ = 0.709). The numbers represent population means and 95% CIs in parentheses estimated as 0.025 and 0.975 quantiles from the population.

Model type	λ	**SDS**	$\sigma_{\tau=1}$	**Fruiting plants (%)**	**Total fruits**	**Mean fruit size class**	**Total fitness**
SDV + SDS	100	Yes	0.709	96.89	2.77 (1, 5)	2.6 (1.0, 5.0)	0.41 (0.03, 1.10)
Only SDV	100	No	0.709	94.35	2.50 (1, 5)	2.3 (1.0, 5.0)	0.23 (0.03, 1.08)
Only SDS	0	Yes	0.709	96.74	2.95 (1, 6)	2.5 (1.0, 5.0)	2.95 (1.00, 6.00)
Neither SDV nor SDS	0	No	0.709	94.61	2.61 (1, 5)	1.2 (1.0, 2.0)	2.61 (1.00, 5.00)

Table S2.4. Predictions of the Forward simulation model examining the effect of fruit growth efficiency, *β*. The numbers represent population means and 95% CIs in parentheses estimated as 0.025 and 0.975 quantiles from the population. The baseline model is highlighted.

Growth efficiency	***β***	**Fruiting plants (%)**	**Total fruits**	**Mean fruit size class**	**Total fitness**
Very high	2.1	99.93	5.34 (2, 9)	2.6 (1.9, 3.7)	0.81 (0.08, 1.23)
High	2.3	99.93	5.32 (2, 9)	2.5 (1.8, 3.5)	0.80 (0.08, 1.22)
Low	2.5	99.92	4.57 (2, 7)	2.6 (1.8, 3.7)	0.78 (0.08, 1.17)
Very low	2.7	99.93	5.30 (2, 9)	2.4 (1.7, 3.3)	0.26 (0.08, 0.38)

Chapter 3

PHYTOPHAGOUS INSECT OVIPOSITION SHIFTS IN RESPONSE TO PROBABILITY OF FLOWER ABORTION OWING TO THE PRESENCE OF BASAL FRUITS

This chapter is published: *Jadeja S., Tenhumberg B. (2017) Phytophagous insect oviposition shifts in response to probability of flower abortion owing to the presence of basal fruits.* ***Ecology and Evolution*** *7(21):8770–8779. https://doi.org/10.1002/ece3.3426. Published under the Creative Commons license (CC BY 4.0.*

ABSTRACT

Phytophagous insects use a wide-range of indicators or associated cues to avoid laying eggs in sites where offspring survival is low. For insects that lay eggs in flowers, these unsuitable sites may be created by the host plant's resource allocation to flowers. In the sequentially flowering host plant, *Yucca glauca*, late-opening distal flowers are more likely to be aborted in the presence of already-initiated basal fruits because they are strong resource sinks. If flowers are aborted, all eggs of the phytophagous insect, *Tegeticula yuccasella*, within the flower die. We used the phytophagous insect *T. yuccasella* that lays eggs in and pollinates host plant *Y. glauca* flowers to test the hypothesis that phytophagous insect females are less likely to invest eggs in host plant flowers if basal fruits are present because they are more likely to be aborted. We also investigated potential predictors of arrival of *T. yuccasella* at inflorescences at the onset of flowering. These factors may influence a phytophagous insect's decisions to select oviposition sites. We carried out a behavioral experiment using wild-caught *T. yuccasella* females on manipulated inflorescences with distal flowers with basal fruits and without fruits. As potential predictors of *T. yuccasella* arriving at inflorescences we used floral display size and day of onset of flowering. In support of our hypothesis, our experimental results showed that *T. yuccasella* was significantly less likely to oviposit in distal flowers on inflorescences with basal fruits. We also found that *T. yuccasella* arrival was higher at inflorescences with larger floral display size and earlier in the flowering season. These findings uncover a novel indicator of unsuitable oviposition sites – presence of basal fruits, that phytophagous insects use to make oviposition decisions. Further, our study contributes to the growing body of evidence that shows that females prefer sites that increase the probability of survival of their offspring.

INTRODUCTION

A wide-range of phytophagous insects avoid laying eggs in host plants or plant parts that are unsuitable oviposition sites because they lead to a lower probability of offspring survival (Renwick & Chew 1994; Gripenberg et al. 2010; Mayhew 1997). To identify unsuitable oviposition sites, phytophagous insects use a variety of indicators, or tactile or chemical cues associated with those indicators. For example, for *Euura lasiolepis*, a shoot-galling sawfly, offspring survival is lower in shorter shoots of the willow, *Salix lasiolepis*, that are more likely to fall off which kills the fly's offspring (Craig et al.

1989). Flies used shoot length as an indicator of suitability of oviposition sites, and avoided shorter shoots (Craig et al. 1989). Some other indicators of unsuitable oviposition sites for phytophagous insects include, presence of specific plant secondary chemical compounds (Wennström et al. 2010), presence of host-marking pheromones laid during oviposition by conspecifics (Huth & Pellmyr 1999), fungal infection on oviposition sites that increases the likelihood of abortion of oviposition sites (Biere & Honders 2006), and age of plant parts where older plant parts may deteriorate before offspring can finish development (Heard 1995).

For phytophagous insects that lay eggs in flowers, offspring survival is likely to be strongly dependent on how plants allocate resources to flowers. Plants abort flowers due to resource limitation, and in many cases, show a predictable pattern of flower abortion (Stephenson 1981). For instance, in the sequentially flowering plant *Yucca glauca* (soapweed yucca) late-opening distal flowers have a higher probability of abortion (Jadeja and Tenhumberg, unpublished data) possibly because early developing fruits are strong resource sinks. Further, the probability of flower abortion in *Y. glauca* increases with increasing number of basal fruits (Jadeja and Tenhumberg, unpublished data). For the phytophagous insects *Tegeticula* spp. (yucca moths) that lay eggs in and pollinate *Yucca* spp. flowers, all eggs within aborted flowers die. Abortion of flowers and young fruits causes mortality of 95.5% of the *Tegeticula* sp. eggs (Shapiro & Addicott 2004). *Tegeticula* spp. are likely under selection to evolve and maintain oviposition strategies to reduce the loss of their eggs due to flower abortion (Wilson & Addicott 1998). In the first part of this paper, we explored the hypothesis that *T. yuccasella* uses the presence of basal fruits as an indicator of unsuitable oviposition sites. We made three predictions to test our hypothesis.

First, we predicted that, in the presence of basal fruits *T. yuccasella* will be less likely to oviposit in distal flowers. Second, we predicted that, if *T. yuccasella* choose to oviposit in distal flowers with basal fruits present, the number of ovipositions will be fewer than in flowers without basal fruits. To test these predictions, we carried out a field behavioral experiment using wild-caught *T. yuccasella* females (Figure 1). Third, we predicted that the number of ovipositions will decrease with an increasing number of basal fruits. To test this prediction, we carried out an observational study using the number of *T. yuccasella* larvae emerging from naturally-pollinated *Y. glauca* fruits as a proxy for the number of *T. yuccasella* ovipositions in flowers. In congeneric *T. altiplanella*, the number of ovipositions in flowers is positively correlated with the number of larvae emerging from fruits (Shapiro and Addicott, 2003).

Before phytophagous insect females decide to oviposit in a flower, they need to decide which inflorescences to explore as potential oviposition sites. Those decisions may be influenced by plant traits, environment and how synchronized insect and plant phenology is. Hence, in the second part of this paper, we explored factors predicting the arrival of *T. yuccasella* at inflorescences. Nectar-feeding pollinators visit plants with larger floral displays more frequently than plants with smaller displays (Eckhart 1991). In *Corydalis ambigua*, larger floral displays received both more frequent and longer visits by pollinators because larger floral displays likely signal higher rewards for pollinators (Ohara & Higashi 1994). For *T. yuccasella* larger floral displays likely indicate larger number of oviposition sites. Phytophagous insects may selectively visit inflorescences at certain locations within a population due to underlying microclimatic variables (Herrera 1995; Thompson 2001), such as shading or differences in temperature. In addition, arrival at inflorescences also depends on the synchrony between the phenologies of host plants and phytophagous insects. Since, *T. yuccasella* are difficult to observe away from inflorescences we used the relationship between time of onset of flowering and arrival of *T. yuccasella* at inflorescences to gain insights into the synchrony between host plants and phytophagous insects. We carried out an observational study to explore the effect of three variables: floral display size, shading, and timing of onset of flowering on the probability of arrival of and number of *T. yuccasella* at *Y. glauca* inflorescences at onset of flowering.

MATERIALS AND METHODS

Study system

We used *Tegeticula yuccasella* (Family: Prodoxidae) and *Yucca glauca* (Family: Agavaceae), as our study system. Both species inhabit arid habitats across North and South America, and obligately depend on each other for their sexual reproduction. *Yucca* spp. produce racemose usually unbranched inflorescences consisting of 17 to 140 buds (Kingsolver 1986, Svensson et al. 2011, Jadeja personal observation). The *Yucca* spp. flowering period is usually 15-30 days long (Powell 1992) during which each flowering inflorescence opens subsets of flowers sequentially from the bottom up. Flowers are receptive for approximately 2 days upon opening. After pollination *Tegeticula* spp. females lay their eggs in the flower’s ovary, and, the hatching larvae feed on the host plant seeds (Riley 1892). *Yucca* spp. populations retain on average less than 15% of their flowers as fruits (Kingsolver 1984; Pellmyr et al. 1997; Addicott 1998). Ninety five percent of the flowers that the plant aborts, are aborted within a week after they open (Pellmyr & Huth 1994). Causes of flower abortion include ovule damage by yucca moths during the process of oviposition (Marr & Pellmyr 2003) and herbivory by florivorous beetles and their larvae (*Carpophilus sp.*) (Huth and Pellmyr 1997, Jadeja personal observation).

Tegeticula yuccasella eclose and emerge from the soil when their host plant is in flower. Adult females live for three to five days while males live for two to three days (Rau 1945), with lab-reared adults from our study site generally surviving about a week in the laboratory (Jadeja, personal observation). Upon emergence, moths seek host plant flowers, with the help of the flower's fragrance (Rau 1945; Svensson et al. 2011). Moths are mostly active at night and rest inside the flowers during the day (Rau 1945). *Tegeticula* spp. mate in *Yucca* spp. flowers. After mating, females engage in pollen collection, oviposition and pollination behaviors. Since female moths have a relatively short lifespan we assume that they engage in oviposition and pollination behaviors soon after mating.

Female yucca moths seek suitable yucca flowers for oviposition. They prefer one to two day old flowers (Riley 1892). Females insert their ovipositor into the ovary and lay one egg during each insertion (Riley 1892; Pellmyr & Huth 1994; Rau 1945; Huth & Pellmyr 1999) and deposit host-marking pheromones (Kingsolver 1984; Huth & Pellmyr 1999). An average of 37.5-45% oviposition attempts fail (Pellmyr & Huth 1994; Huth & Pellmyr 1999; Segraves 2003) possibly because of disturbance by other moths and insects, bad weather, or females may not properly insert their ovipositor into the ovary

(Riley 1892). After *Tegeticula* spp. females complete oviposition they use their specialized mouthparts to push pollen down the opening in the stigma in multiple short up-down motions. A female may oviposit multiple times in a flower, but each oviposition may not be followed by a pollination event (Addicott & Tyre 1995; Tyre & Addicott 1993). However, each pollination event is always preceded by at least one oviposition event (Addicott & Tyre 1995). Females generally visit neighboring flowers, and spend a longer time pollinating and ovipositing than moving between flowers on an inflorescence, suggesting that they minimize the distance between oviposition sites (Pellmyr et al. 1997; Kingsolver 1984).

Within 7-10 days after oviposition, *Tegeticula* spp. larvae hatch and feed on the developing seeds within the maturing *Yucca* spp. ovary (Huth & Pellmyr 1999). Surviving *Tegeticula* spp. larvae emerge from fruits 30-40 days after oviposition (Huth & Pellmyr 1999; Humphries & Addicott 2004). The emerging larvae burrow into the soil, form a cocoon, and remain dormant for at least one fall and winter (Riley 1892). However, a large proportion of the larvae diapause for more than one year and for as long as four years (Riley 1892).

Oviposition in response to presence of basal fruits

Obtaining inflorescence treatments

We manipulated *Y. glauca* inflorescences for use in behavioral trials to test whether *T. yuccasella* are less likely to oviposit in late-opening distal flowers in the presence of basal fruits because they have a high likelihood of being aborted. We manipulated inflorescences following Jadeja and Tenhumberg (submitted) to obtain two inflorescence treatments – (1) inflorescences with late-opening distal flowers and no basal fruits, and (2) inflorescence with late-opening distal flowers and one to three basal fruits (see Appendix S1 for detailed methods). We protected 136 *Y. glauca* inflorescences that were yet to begin flowering from early May to mid-June 2016 at a mixed-grass prairie at the Cedar Point Biological Station (CPBS), Keith County, Nebraska, USA. We established inflorescences with one to three basal fruits by hand-pollinating three to six bottom flowers of the inflorescence. Overall, we could use 23 out of the 136 initially protected inflorescences in behavioral trials.

Obtaining yucca moths

We used field-collected *T. yuccasella* females for the behavioral trials. Wild-caught moths may vary in their oviposition due to differences in age and experience, but this is unlikely to bias the results of the experiment because moths were randomly assigned to both inflorescence treatments. One of the advantages of using wild-caught moths is avoiding possible artifacts introduced by rearing moths in the lab that may not be acclimatized to field conditions. We identified females by the dark brown scale-less abdominal tip that is visible on the underside of the posterior end of their abdomen where the ovipositor is located. We collected moths by baiting them using cut *Y. glauca* inflorescences that we kept outdoors at the field station in buckets with water and plant food solution (Miracle-Gro®) to keep the inflorescences fresh for longer. We replaced old inflorescences with newer cut inflorescences throughout the study period, as needed. Each evening we checked flowers on the cut inflorescences to collect *T. yuccasella* females.

We collected *T. yuccasella* females in 44 ml vials with holes in their caps for exchange of air. Upon collection, we visually checked the underside of the moths' heads for the presence of a pollen ball. If a *T. yuccasella* female did not have a pollen ball, we allowed her to collect pollen in a smoothie cup with one to two fresh *Y. glauca* flowers. We checked the moths regularly until midnight to see if they collected a pollen ball. We did not use moths without a pollen ball for the behavioral trials as they may not have mated, or may show different oviposition strategies than moths with pollen balls. We preferred to use moths with pollen balls in behavioral trials on the same night they were collected. However, when that was difficult due to the availability of inflorescences of each treatment and time taken by previous trials (on average longer than 80 min per trial), we kept moths in the lab at the field station and used them in trials on subsequent nights. We housed the moths individually in 44 ml vials with a moist cotton roll to prevent dehydration. To maintain the moths' circadian rhythm, we set the lighting in the lab to 12 h day light and 10 h night dark cycles, plus one hour each of gradual lighting and darkening during the mornings and evenings, respectively.

Behavioral trials

We carried out behavioral trials during the mid to late *Y. glauca* flowering period between June 6 to 20, 2016. We conducted trials at night between 8:30 pm and 2:30 am. We aimed to run focal moths in trials of both treatments to account for individual-level differences in oviposition. We alternated the order in which moths received both treatments to avoid confounding results with trial order. When possible, we conducted the second trial on the same night as the first trial with at least 20 min of rest period between the two trials. When a second trial was not possible on the same night due to unavailability of an inflorescence of the right treatment, lengthy previous trials, or stormy weather (affected sampling on two nights), we housed the moths in the lab as described earlier (see subsection "Obtaining yucca moths"), and used them during a subsequent sampling night, if possible. The time of the night when we run the trial (early versus late at night) may affect a moth's motivation and oviposition behavior. We avoided that from biasing our results by alternating the inflorescence treatment that was used at the beginning of a sampling night.

We carried focal *T. yuccasella* females with pollen balls in 44 ml vials to inflorescences in the field. After dark, we only used headlamps with dimmed red lights around collected moths as these are the least disturbing to the moths (Tyre and Addicott 1993, Jadeja, personal observation). Prior to each trial, we lowered the mesh cage around the inflorescence with the desired treatment (with or without basal fruits), selected three topmost receptive and herbivory-free experimental flowers, and removed the remaining flowers and buds. Then, we enclosed the inflorescence in a portable 101 cm tall and 24 cm diameter cylindrical trial cage made from a 0.18 mm thick clear acetate sheet with fine mesh sleeves attached on both ends, and an opening with a mesh sleeve attached towards the bottom of the cage to introduce the focal moth. We tied the trial cage to the tomato cage surrounding the inflorescence, ensuring the sides of the trial cage did not touch the inflorescence stalk or the experimental flowers, and allowed ample space for the moth to move.

We introduced the focal moth in a vial from the opening towards the bottom of the trial cage and opened the vial which marked the start of a behavioral trial. We recorded the focal moth's activity using the infrared light-based night-vision video recording feature of Sony® Handycam video recorders in the HDR-SR series. In four trials, we made part of the observations visually or using a voice recorder due to technical difficulties in operating the video recorder. Later we scored the recordings for each trial and quantified the moths' ovipositions. We considered the action of a focal moth inserting its ovipositor in the ovary of the flower and removing it after > 30 seconds as one oviposition event because inserting the ovipositor for a shorter time would not have resulted in the deposition of an egg (Addicott & Tyre 1995).

We discarded a trial if a focal moth did not exit the vial for 15 min from the start of the trial, and ended a trial if the moth did not begin ovipositing within 15 min from exiting the vial, did not oviposit 15 min after the last oviposition event or if a moth flew off the inflorescence stalk after its last oviposition event. We reused inflorescences where a focal moth did not oviposit in any of the flowers during a trial. Inflorescences where moths oviposited during a trial were not used in further trials to avoid host-marking pheromones to influence the focal moth's oviposition behavior. Overall, we obtained first trials from 18 moths and second trials from 11 moths that exited their vials (see Supplementary Table S1 for distribution of sample sizes).

Larval emergence in response to the presence of basal fruits

Yucca glauca open flowers sequentially from the bottom up. So, the number of basal fruits is an index of the number of fruits already formed when the collected fruit was a flower. These fruits represent flowers that moths oviposited in and that the plants retained. We predicted that *T. yuccasella* decreases the number of ovipositions with increasing number of basal fruits on naturally-pollinated inflorescences using larval emergence from a fruit as a proxy for the number of ovipositions in a flower. To check the suitability of our proxy we constructed an Individual-Based Model (Appendix S2). The model considered that *T. yuccasella* lays fewer eggs with increasing number of prior ovipositions (Huth & Pellmyr 1999), *Yucca* spp. selectively abort flowers with a high number of *Tegeticula* spp. eggs (Pellmyr & Huth 1994; Humphries & Addicott 2000; Shapiro & Addicott 2004). Flower abortion is unlikely affected by other sympatric *Tegeticula* sp. For instance, *T. corruptrix* occur later in the season and lay their eggs exclusively in fruits usually more than two weeks after pollination (Pellmyr et al. 1996, Jadeja, personal observation), which is after the period when plants abort flowers and early fruits (Pellmyr & Huth 1994, Jadeja, personal observation). Our simulation results show that only when moths decrease the number of ovipositions with increasing number of basal fruits can we expect a negative relationship between number of emerging larvae and number of basal fruits (Figure S2.3).

To test whether *T. yuccasella* in the field vary the number of oviposition in response to the presence of basal fruits, we collected all the full-grown fruits from the top third flowers of haphazardly selected naturally-pollinated *Y. glauca* inflorescences in late June and July in the years 2014, 2015, and 2016. Those flowers opened mid to late in the flowering season and had similar display sizes (Jadeja, personal observation). The fruits came from a 55 x 25 m patch of *Y. glauca* on the North-East slope of the Kingsley dam at Lake McConaughy, Keith County, Nebraska. This patch is 5 km from CPBS where we carried out the behavioral field experiment. We identified the top fruits using the relative position of the fruits and aborted flowers. When a flower aborts, its stalk (pedicel) is left behind and can be used to determine the flower’s position on the inflorescence prior to abortion. We labelled each collected fruit, kept them in individual containers at room temperature over the fall, and recorded the number of emerging *Tegeticula* spp. larvae from each fruit. Next, we quantified the number of fruits basal to each top fruit.

Larval emergence from *Y. glauca* fruits is low and highly variable (Jadeja, personal observation), and fruiting from the top third flowers is not very common in natural populations, particularly when inflorescences have already matured basal fruits (Jadeja and Tenhumberg, unpublished data). Therefore, in 2016 we increased our sample size by collecting top fruits from 18 inflorescences from outside the patch, but from within the same area. These fruits came from both, inflorescences with and without basal fruits.

At our study site, a non-pollinating congener of *T. yuccasella* – *T. corruptrix* lays eggs in fruits and has larvae that are morphologically indistinguishable from the pollinating *T. yuccasella* larvae. In contrast, the adults of these moth species can be easily morphologically distinguished. To determine the relative proportions of *T. yuccasella* and *T. corruptrix* larvae at our study site, we used reared larvae collected from *Y. glauca* fruits in summer 2014 and 2015 as part of a different study. We allowed larvae to burrow in soil-filled cans. We covered the cans with cling wrap with holes to allow exchange of air but prevent excessive loss of soil moisture. We maintained the cans at room temperature (21-27 ºC) during the fall, spring and summer, and colder temperatures (5 ºC or 18 ºC) during the winter, except during transportation when it was not feasible to regulate the temperature. We added a small quantity of water to the cans approximately once every two months to moisten the soil. Adults from some of the larvae collected in

2014 emerged in 2015 and 2016, and adults from some of the larvae collected in 2015, emerged in 2016. After moths eclosed in 2016, we terminated larval rearing.

Predictors of T. yuccasella arrival at onset of flowering

Each morning of the flowering season, we checked inflorescences protected for the field experiment and noted when the first flower on an inflorescence opened (onset of flowering). In addition, we recorded (1) how many *T. yuccasella* arrived at the inflorescence, (2) how many flowers opened as an index of size of the floral display, (3) the basal diameter of the rosette from which the inflorescence was emerging as an index of plant size, (4) the straight-line distance to the nearest red cedar tree (*Juniperus virginiana*) that may provide an index of the presence of shade over the inflorescence, and (5) the Universal Transverse Mercator (UTM, Zone 14T, datum WGS 84) Easting and Northing coordinates to account for spatial autocorrelation, if any.

Tegeticula yuccasella rested on mesh sleeves of protected inflorescences during the day as the sleeves prevented them from accessing the flowers. We considered *T. yuccasella* on mesh sleeves as having arrived at the inflorescences. This was done before we manipulated inflorescences for the field experiment. We obtained *T. yuccasella* arrival data from 111 out of the 136 initially protected inflorescences after discarding 25 inflorescences that either dried or were damaged before onset of flowering. These 111 inflorescences were located over a distance of 352 m along the West-East direction (UTM Easting, Zone 14T, datum WGS 84) and 844 m along the North-South direction (UTM Northing, Zone 14T, datum WGS 84). The elevation ranged from 971 m to 1023 m above sea level.

Statistical analysis

Oviposition in response to presence of basal fruits

We used a generalized linear mixed-effects model (GLMM) with binomial error distribution to determine whether the probability of *T. yuccasella* oviposition in behavioral trials differed between treatments and trial order. The response variable was the proportion of flowers with at least one oviposition. Next, we used a linear mixed-effects model (LMM) to determine whether the number of *T. yuccasella* oviposition in behavioral trials with at least one oviposition differed between treatments and trial order. The response variable was the log-transformed number of ovipositions in a trial with at least one oviposition. The predictor variables were presence of basal fruits (inflorescence treatment) and trial order (first or second trial), and the random effects were moth identity and trial night. We used backward model selection to identify the minimum adequate model for our experimental data using a significance cut-off of 0.05 (see Supplementary Tables S2-S3 for the results from the full models).

Larval emergence in response to the presence of basal fruits

We analyzed the number of larvae emerging from fruits from top third flowers using GLMMs with a Poisson error distribution with inflorescence identity as a random effect. The fixed effects were number of basal fruits and year. Year was treated as a categorical variable.

Predictors of T. yuccasella arrival at onset of flowering

We used a Spearman's rank correlation analysis to quantify correlations between all factors we measured. In our statistical models, we only included predictor variables that were not highly correlated (maximum correlation coefficient was less than 0.5 for predictor variables in each model). Further, we checked for spatial autocorrelation in the probability and number of moths arriving at inflorescences and found no significant spatial autocorrelation. There was no significant spatial autocorrelation in the number (Moran's I = 0.018, p = 0.4) and probability (Moran's I = 0.18, p = 0.2) of moths arriving at inflorescences at onset of flowering (see Figure S3.1ab for semivariograms). Hence, we did not consider the coordinates of the inflorescences in our analysis.

We analyzed the probability of moths arriving at onset of flowering using a generalized linear model (GLM) with binomial distribution of errors. The response variable for the full model was presence/absence of moths at onset of flowering and predictor variables were number of open flowers, day of onset of flowering, basal diameter, and distance to nearest tree (see Supplementary Tables S7-S8 for model selection details). We analyzed the number of moths arriving at inflorescences conditional on moths being present using a generalized additive model (GAM) with Poisson distribution of errors to capture the complex nonlinear response of the number of moths arriving and day of onset of flowering. The response variable for the full model was the number of moths at the inflorescence, and the predictor variables were number of flowers open, smooth splined day of onset of flowering, basal diameter, and distance to nearest tree (see Supplementary Tables S10-S11 for model selection details). In both models, we considered the date the first inflorescence started flowering as the first day of onset of flowering.

For the observational data, we used an information theoretic approach (Burnham & Anderson 2002) to identify the final model for the probability of arrival and number of moths arriving at onset of flowering. To account for the small sample sizes we used the corrected Akaike Information Criterion (AICc). We show the effect of each predictor variable on the response variable in the final model by holding other predictor variables at their median values.

We carried out all statistical analyses in R version 3.3.2 (2016-10-31) (R Core Team 2016), using packages lme4 (Bates et al. 2015), mgcv (Wood 2016), and nlme (Pinheiro et al. 2016).

RESULTS

Oviposition in response to presence of basal fruits

Tegeticula yuccasella oviposited at least once in 55% of the trials (n = 29 trials). Of these, 63% of the trials were on inflorescences without basal fruits. The total number of *T. yuccasella* ovipositions in trials with at least one oviposition ranged from 3 to 109 ovipositions across the three experimental flowers, with an average of 19 ± 7 (mean ± SE) ovipositions (n = 16 trials). The number of ovipositions in individual flowers with at least one oviposition during the experiment ranged from 2 to 52 ovipositions with an average of 12 ± 2 (mean ± SE) ovipositions (n = 25 flowers across 16 trials).

Our analysis showed that the presence of basal fruits significantly reduced the proportion of flowers with at least one oviposition. Moths oviposited on average in 1-2 out of 3 flowers when no fruits were present and in 0-1 out of 3 flowers when basal fruits were present (p = 0.048, Figure 2a, Supplementary Table S4). Additionally, the presence of basal fruits did not significantly reduce the total number of ovipositions in trials with at least one oviposition (p = 0.61, Figure 2b, Supplementary Table S5).

Larval emergence in response to the presence of basal fruits

Overall, not many larvae emerged from fruits. In only 22 % of the top fruits (n = 243 fruits) one or more larvae developed successfully. The average number of larvae emerging from fruits of top third flowers was 0.3 ± 0.04 (mean ± SE, n = 243 fruits). In all three years, the number of basal fruits did not affect the number of larvae emerging from top fruits (p > 0.7, Figure 3a-c, Supplementary Table S6). Adult moths emerging from reared larvae showed that the proportion of non-pollinating moths (*T. corruptrix*) was 11% (n = 28 moths) and 4% (n = 24 moths) in 2015 and 2016, respectively.

Predictors of T. yuccasella arrival at onset of flowering

Inflorescences opened 7.5 ± 0.5 (mean ± SE) flowers with a maximum of 25 flowers at onset of flowering (n = 111 inflorescences). The first inflorescence started flowering on 26th May 2016 and the last inflorescence started flowering on 16th June 2016. Each inflorescence flowered for about 1 to 2 weeks.

The probability of *T. yuccasella* arriving at onset of flowering increased significantly with increasing number of flowers open, and decreased over the flowering season (Figure 4a-b, Supplementary Table S9). There was a 0.48 probability of moths arriving at inflorescences with one open flower, which almost doubled to 0.97 when 25 flowers were open ($p = 0.003$, Figure 4a). Further, there was a greater than 0.90 probability of moths arriving at inflorescences with onset of flowering within the first 10 days of the flowering season. However, the probability of arrival reduced to less than 0.2 at the end of the flowering season ($p < 0.0002$, Figure 4b).

On inflorescences visited by *T. yuccasella*, the average number of moths arriving was 3.5 ± 0.5 (mean ± SE) moths with a maximum of 26 moths. The number of *T. yuccasella* arriving significantly increased with increasing number of open flowers, and significantly changed non-linearly over the flowering season (Figure 5a-b, Supplementary Table S12). The number of moths arriving was 4 on inflorescences with one open flower, and more than tripled to 11 on inflorescences with 25 open flowers ($p = 0.001$, Figure 5a). Further, the number of moths arriving peaked close to the middle of the flowering season on the 13th day with 5 moths arriving on average. The number of moths more than halved to less than 2 moths arriving at inflorescences with the most delayed onset of flowering ($p < 0.0001$, Figure 5b).

DISCUSSION

Oviposition in response to presence of basal fruits

Yucca glauca flowers are more likely to be aborted in the presence of basal fruits (Jadeja and Tenhumberg, unpublished data). Further, all *Tegeticula* spp. eggs in flowers that are later aborted die (Huth & Pellmyr 1999; Shapiro & Addicott 2004). Hence, we hypothesized that *T. yuccasella* will be less likely to invest eggs in distal flowers on inflorescences with basal fruits. As expected, the probability of *T. yuccasella* oviposition was lower in flowers on inflorescences with basal fruits. These results support our prediction that *T. yuccasella* will avoid laying eggs in flowers with a higher probability of abortion. Possible proximate cues for *T. yuccasella* to reject distal flowers with basal fruits as oviposition sites include tactile and/or chemical cues from fruits and/or flowers. There is overwhelming empirical evidence to show that many lepidopterans use multiple plant-based cues to identify suitable oviposition sites and reject unsuitable ones, both within and between host plant species (reviewed in Renwick & Chew 1994; Wennström *et al.* 2010; Ryuda *et al.* 2013; Mukae *et al.* 2016). Identifying specific cues that females use to respond to presence of basal fruits is an avenue for further research.

A strategy to avoid oviposition in distal flowers may benefit *T. yuccasella* and similar phytophagous insect females in different ways. First, it may save females from losing a large proportion of their eggs in years with a large number of inflorescences with basal fruits. This benefit would be large during certain years and at certain sites in host plants like *Yucca* spp. where the frequency of distal flowers with and without basal fruits may vary across space and time because fruiting is highly resource limited (Pellmyr & Huth 1994; Huth & Pellmyr 1997; Humphries & Addicott 2004) and variable (Addicott 1998; Kingsolver 1986). Second, short-lived females like *Tegeticula* spp. that are time-limited in their ability to deposit eggs may benefit from avoiding the opportunity costs of spending time ovipositing in flowers that are unlikely to form fruits. Likewise, females of an egg-limited species in the same scenario would also benefit from selecting sites that are more likely to give each egg a higher chance of survival.

The number of ovipositions in flowers accepted as oviposition sites is another measure of the female's egg investment in flowers. We predicted that in our experiment, if *T. yuccasella* choose to lay eggs in flower with basal fruits, they will lay fewer eggs than in flowers without basal fruits. However, contrary to expectations, *T. yuccasella* did not lay significantly fewer eggs in flowers on inflorescences with basal fruits. It is possible that *T. yuccasella* do not decrease the number of eggs they lay in response to presence of basal fruits. This suggests that their strategy is limited to determining whether a flower is a suitable oviposition site, and does not involve determining number of eggs to oviposit. Alternatively, it is likely that we could not detect the expected pattern due to a high variation in the number of ovipositions among trials. The number of eggs laid may vary due to differences in the number of ovipositions by wild-caught moths. For example, wild-caught moths may have varied in their age-related oviposition strategy. Older moths nearing the end of their life may oviposit more eggs in each flower they visit, which may increase variation in the ovipositions we observed. An example of the effect of life expectancy on oviposition behavior comes from parasitic wasps (Roitberg et al. 1993; Roitberg et al. 1992). Parasitic wasps have a low rate of ovipositing in already parasitized hosts. However, when parasitic wasps perceive they are near the end of their life they increase their rate of ovipositing in already parasitized hosts.

Our study shows a novel way phytophagous insects can increase their fitness – a tendency to avoid ovipositing in distal flowers in the presence of basal fruits because they have a higher probability of abortion (Jadeja and Tenhumberg, unpublished data).

Tegeticula yuccasella is also an obligate pollinator of *Y. glauca.* The consequences of such oviposition behavior on the complex eco-evolutionary dynamics between mutualist partners is beyond the scope of this paper. However, our results suggest that *T. yuccasella* has evolved an oviposition strategy that increases the number of surviving larvae which is in line with theory and empirical studies that show that phytophagous insect females prefer to oviposit in sites that are better for larval performance and survival (Mayhew 1997; Gripenberg et al. 2010). Our investigation is also in line with egg-laying site choice of female anurans that prefer to oviposit in ponds with a faunal composition that provides the best chances of survival for their offspring (Resetarits 1996).

Ideally, we would have designed an experiment allowing females to choose between flowers with and without basal fruits in a trial to identify a female's oviposition preference. However, this was not possible with inflorescences attached to plants in the field because the inflorescences were very often located many meters away from each other. In order to present a female moth with both inflorescence treatments simultaneously in a choice experiment, we would have had to cut inflorescences and place them besides each other in a trial cage. Cutting inflorescences could have affected the chemical cues used by the female moth to assess a flower's probability of abortion. To avoid the risk of losing chemical cues of the flower's probability of abortion we used inflorescences attached to the plants in the field that prevented us from designing a choice experiment.

Larval emergence in response to the presence of basal fruits

The number of larvae emerging from fruits is an index of the number of *Tegeticula* sp. ovipositions (Shapiro & Addicott 2003). Since the probability of flower abortion decreases with increasing number of basal fruits (Jadeja and Tenhumberg, unpublished data) we originally expected fewer larvae to emerge from distal fruits with increasing number of basal fruits. However, in our field experiment, we did not detect a significant decrease in the number of oviposition in the presence of basal fruits. In line with our experimental results, our field observational study shows that the number of larvae emerging from fruits of naturally-pollinated top third flowers did not decrease with increasing number of basal fruits.

There are three possible explanations for the absence of a relationship between the number of emerging larvae and number of basal fruits. First, the probability of an egg to survive in a flower may influence a *T. yuccasella* female's decision to accept a flower as an oviposition site, but once a flower has been accepted, the female may not decrease the number of ovipositions in response to increasing number of basal fruits. Hence, when flowers with basal fruits are retained, we do not see a decrease in the number of larvae emerging from their fruits. Second, *T. yuccasella* larvae may experience higher density-dependent larval mortality in fruits without basal fruits where we expected a larger number of larvae. This may result in the same number of larvae independent of the number of ovipositions. A study has documented density-dependent larval mortality in congeneric *T. altiplanella* (Shapiro & Addicott 2003).

Third, the true pattern of larval emergence may be masked by our inability to morphologically distinguish larvae of pollinating *T. yuccasella* and congeneric non-pollinating *T. corruptrix*. It is possible that later-occurring, non-pollinating *T. corruptrix* lay more eggs in fruits with basal fruits, or their larvae have a higher probability of survival in fruits with basal fruits due to weak competition with *T. yuccasella* larvae. This would result in a negative relationship between the number of *T. yuccasella* and *T. corruptrix* larvae emerging from fruits. As a result, there may be no overall differences in the total number of larvae emerging as number of *T. yuccasella* larvae increase. For instance, in two out of three years, the number of pollinating and non-pollinating *Tegeticula* spp. larvae emerging from *Y. filamentosa* fruits was negatively correlated (Marr et al. 2001). However, the presence of the *T. corruptrix* larvae is unlikely to explain the results from our study because *T. corruptrix* larvae occurred in low frequency at our study site. Out of the lab-reared adult moths that eclosed in 2015 and 2016 only 11% (3 out of 28 moths) and 4% (1 out of 24 moths) were non-pollinating *T. corruptrix*. Therefore, we believe it is unlikely that our inability to morphologically distinguish larvae from pollinating and non-pollinating *Tegeticula* spp. has contributed to no relationship between the number of larvae emerging and number of basal fruits.

Predictors of T. yuccasella arrival at onset of flowering

Both the probability and number of moths arriving at inflorescences increased with larger floral display sizes at onset of flowering. Pollinator preference for plants with larger floral displays has been well-established in nectar-feeding pollinators (Eckhart 1991; Ohara & Higashi 1994; Buide 2005; Thompson 2001). We show this pattern holds true for the non-nectar seeking *T. yuccasella* too. Larger floral displays with more open flowers may increase the probability of a moth finding an inflorescence through visual and/or chemical cues. The presence of a larger number of flowers may also increase the probability of finding receptive flowers that have not yet been oviposited in. In addition, larger floral displays may increase the likelihood of finding mates because it attracts a larger number of moths.

Finally, both the probability and number of *T. yuccasella* arriving was very low on inflorescences with late onset of flowering. This result may be explained by a mismatch between the availability and abundance of the *T. yuccasella* and flowering host plants. In our study, *T. yuccasella* abundance may have been low later in the flowering season. If so, we expect *T. yuccasella* arrival to be independent of day of onset of flowering in years with a greater synchrony between the availability of the *T. yuccasella* and host plants. Alternatively, this result may be explained by the presence of other inflorescences that started flowering earlier in the flowering season that co-occur with inflorescences with late onset of flowering. Therefore, late in the flowering season, more competition among a larger number of flowering inflorescences may reduce the chances of finding moths at a particular inflorescence. Further, after onset of flowering inflorescences usually have larger floral displays than at onset of flowering (Jadeja, personal observation). We have already shown in this study that *T. yuccasella* are more likely to arrive at inflorescences with larger floral displays. Therefore, the low probability and number of *T. yuccasella* on inflorescences with late onset may also be due to *T. yuccasella* preferring larger floral displays of already flowering inflorescences.

In conclusion, the result from our observational study show that floral display size and timing of onset of flowering, are likely important in influencing *T. yuccasella* decisions to arrive at inflorescences. These factors may also influence the female's decisions to invest eggs in inflorescences, and the distribution of eggs and fruiting success across inflorescences in a flowering season. It is likely that these results are applicable to other phytophagous insect species.

ACKNOWLEDGEMENTS

We thank two anonymous reviewers for comments that helped improve our manuscript considerably. We thank the Cedar Point Biological Station staff for logistical support at the field site and N Nielson for permission to access the Kingsley dam surface. We also thank field assistants Tiffany Riffle and Masiel Maza for help with field work. This study was supported by funds from the J. Ve. Srb Memorial Fund to SJ and TR, Sigma Xi Grants-in-Aid of Research Award to SJ, Warren and Edith Day Dissertation Travel Award to SJ, the Janovy Fund Award to MM, and funds from the Center for Great Plains Studies to SJ.

REFERENCES

Addicott, J.F., 1998. Regulation of mutualism between yuccas and yucca moths: population level processes. *Oikos*, 81(1), pp.119–129.

Addicott, J. & Tyre, A. 1995. Cheating in an obligate mutualism: How often do yucca moths benefit yuccas? *Oikos*, 72(3), pp.382–394.

Bates, D. Mächler, M., Bolker, B. M., & Walker, S. C., 2015. Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, 67(1), pp.1–51.

Biere, A. & Honders, S.C. 2006. Coping with third parties in a nursery pollination mutualism: *Hadena bicruris* avoids oviposition on pathogen-infected, less rewarding *Silene latifolia*. *New Phytologist*, 169(4), pp.719–727.

Buide, M.L. 2005. Pollination ecology of *Silene acutifolia* (Caryophyllaceae): floral traits variation and pollinator attraction. *Annals of Botany*, 97(2), pp.289–297.

Burnham, K.P. & Anderson, D.R. 2002. *Model selection and multimodel inference: a practical information-theoretic approach*, New York: Springer.

Craig, T.P., Itami, J.K. & Price, P. 1989. A strong relationship between oviposition preference and larval performance in a shoot- galling sawfly. *Ecology*, 70(6), pp.1691–1699.

Eckhart, V.M. 1991. The effects of floral display on pollinator visitation vary among populations of *Phacelia linearis* (Hydrophyllaceae). *Evolutionary Ecology*, 5(4), pp.370–384.

Gripenberg, S. Mayhew, P. J., Parnell, M., & Roslin, T. 2010. A meta-analysis of preference-performance relationships in phytophagous insects. *Ecology Letters*, 13(3), pp.383–393.

Heard, T.A. 1995. Oviposition preferences and larval performance of a flower-feeding weevil, *Coelocephalapion aculeatum*, in relation to host development. *Entomologia Experimentalis et Applicata*, 76(2), pp.195–201.

Herrera, C.M. 1995. Microclimate and individual variation in pollinators : flowering plants are more than their flowers. *Ecology*, 76(5), pp.1516–1524.

Humphries, S. & Addicott, J. 2000. Regulation of the mutualism between yuccas and yucca moths: intrinsic and extrinsic factors affecting flower retention. *Oikos*, 89(2), pp.329–339.

Humphries, S.A. & Addicott, J.F. 2004. Regulation of the mutualism between yuccas and yucca moths: intrinsic and extrinsic patterns of fruit set. *Canadian Journal of Botany*, 82(5), pp.573–581.

Huth, C.J. & Pellmyr, O. 1997. Non-random fruit retention in *Yucca filamentosa*: consequences for an obligate mutualism. *Oikos*, 78(3), pp.576–584.

Huth, C.J. & Pellmyr, O. 1999. Yucca moth oviposition and pollination behavior is affected by past flower visitors: evidence for a host-marking pheromone. *Oecologia*, 119(4), pp.593–599.

Kingsolver, R. 1984. *Population biology of a mutualistic association: Yucca glauca and Tegeticula yuccasella*. PhD Thesis. University of Kansas, Lawrence.

Kingsolver, R. 1986. Vegetative reproduction as a stabilizing feature of the population dynamics of *Yucca glauca*. *Oecologia*, 69(3), pp.380–387.

Marr, D., Brock, M. & Pellmyr, O. 2001. Coexistence of mutualists and antagonists: exploring the impact of cheaters on the yucca–yucca moth mutualism. *Oecologia*, 128(3), pp.454–463.

Marr, D.L. & Pellmyr, O. 2003. Effect of pollinator-inflicted ovule damage on floral abscission in the yucca-yucca moth mutualism: the role of mechanical and chemical factors. *Oecologia*, 136(2), pp.236–243.

Mayhew, P.J. 1997. Adaptive patterns of host-plant selection by phytophagous insects. *Oikos*, 79(3), pp.417–428.

Mukae, S. Ohashi, T., Matsumoto, Y., Ohta, S., & Ômura, H. 2016. d-pinitol in Fabaceae: an oviposition stimulant for the common grass yellow butterfly, *Eurema mandarina*. *Journal of Chemical Ecology*, 42(11), pp.1122–1129.

Ohara, M. & Higashi, S. 1994. Effects of inflorescence size on visits from pollinators and seed set of *Corydalis ambigua* (Papaveraceae). *Oecologia*, 98(1), pp.25–30.

Pellmyr, O. & Huth, C.J. 1994. Evolutionary stability of mutualism between yuccas and yucca moths. *Nature*, 372, pp.257–260.

Pellmyr, O., Leebens-Mack, J. & Huth, C. 1996. Non-mutualistic yucca moths and their evolutionary consequences. *Nature*, 380, pp.155–156.

Pellmyr, O. Massey, L. K., Hamrick, J. L., & Feist, M. A. 1997. Genetic consequences of specialization: yucca moth behavior and self-pollination in yuccas. *Oecologia*, 109(2), pp.273–278.

Pinheiro, J. Bates, D., DebRoy, S., Sarkar, D., EISPACK, Heisterkamp, S., ... R-core. 2016. nlme: Linear and nonlinear mixed effects models. 3.1-126.

Powell, J.A. 1992. Interrelationships of yuccas and yucca moths. *Trends in Ecology & Evolution*, 7(1), pp.10–15.

R Core Team 2016. *R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.*

Rau, P. 1945. The yucca plant, *Yucca filamentosa*, and the yucca moth, *Tegeticula* (*Pronuba*) *yuccasella* Riley: An ecologico-behavior study. *Annals of the Missouri Botanical Garden*, 32(4), pp.373–394.

Renwick, J.A.A. & Chew, F.S. 1994. Oviposition behavior in Lepidoptera. *Annual Review of Entomology*, 39(1), pp.377–400.

Resetarits, W.J. 1996. Oviposition site choice and life history evolution. *Integrative and Comparative Biology*, 36(2), pp.205–215.

Riley, C. V. 1892. The yucca moth and yucca pollination. *Missouri Botanical Garden Annual Report*, 3, pp.99–158.

Roitberg, B. Mangel, M., Lalonde, R., van Alphen, J. J. M., & Vet, L. 1992. Seasonal dynamic shifts in patch exploitation by parasitic wasps. *Behavioral Ecology*, 3(2), pp.156–165.

Roitberg, B. Sircom, J., Roitberg, C., van Alphen, J. J. M., & Mangel, M. 1993. Life expectancy and reproduction. *Nature*, 364, pp.108–108.

Ryuda, M. Calas-List, D., Yamada, A., Marion-Poll, F., Yoshikawa, H., Tanimura, T., & Ozaki, K. 2013. Gustatory sensing mechanism coding for multiple oviposition stimulants in the swallowtail butterfly, *Papilio xuthus*. *The Journal of Neuroscience*, 33(3), pp.914–924.

Segraves, K. 2003. Understanding stability in mutualisms: Can extrinsic factors balance the yucca-yucca moth interaction? *Ecology*, 84(11), pp.2943–2951.

Shapiro, J.M. & Addicott, J.F. 2003. Regulation of moth–yucca mutualisms: mortality of eggs in oviposition-induced “damage zones.” *Ecology Letters*, 6, pp.440–447.

Shapiro, J. & Addicott, J.F. 2004. Re-evaluating the role of selective abscission in moth/yucca mutualisms. *Oikos*, 105(3), pp.449–460.

Stephenson, A.G. 1981. Flower and fruit abortion: proximate causes and ultimate functions. *Annual Review of Ecology and Systematics*, 12(1981), pp.253–279.

Svensson, G.P., Pellmyr, O. & Raguso, R.A. 2011. Pollinator attraction to volatiles from virgin and pollinated host flowers in a yucca/moth obligate mutualism. *Oikos*, 120, pp.1577–1583.

Thompson, J.D. 2001. How do visitation patterns vary among pollinators in relation to floral display and floral design in a generalist pollination system? *Oecologia*, 126(3), pp.386–394.

Tyre, A. & Addicott, J. 1993. Facultative non-mutualistic behaviour by an “obligate” mutualist:“Cheating” by yucca moths. *Oecologia*, 94(2), pp.173–175.

Wennström, A. Hjulström, L. N., Hjältén, J., & Julkunen-Tiitto, R. 2010. Mother really knows best: host choice of adult phytophagous insect females reflects a within-host variation in suitability as larval food. *Chemoecology*, 20(1), pp.35–42.

Wilson, R.D. & Addicott, J.F. 1998. Regulation of mutualism between yuccas and yucca moths: is oviposition behavior responsive to selective abscission of flowers? *Oikos*, 81(1), p.109.

Wood, S. 2016. Package “mgcv”: Mixed GAM Computation Vehicle with GCV/AIC/REML Smoothness Estimation.

FIGURES

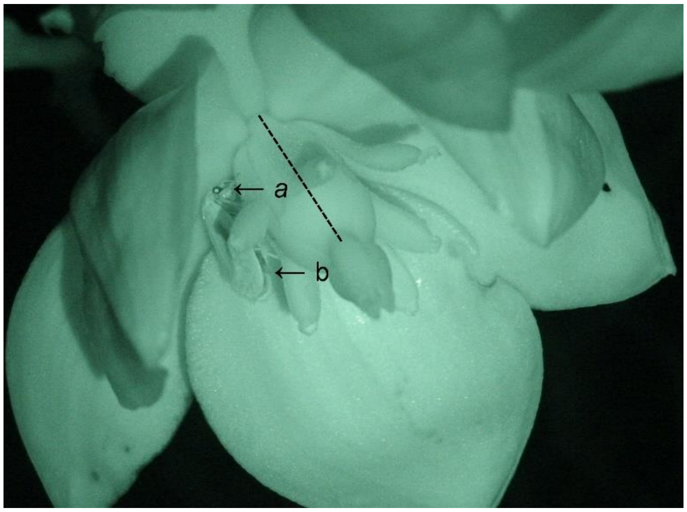


Figure 1. Wild-caught *Tegeticula yuccasella* female (yucca moth) with a pollen ball under her head (arrow a), resting in a *Yucca glauca* flower during a behavioral trial. The moth is on the left-hand side of the flower's ovary (dashed line running along its length). The posterior end of the abdomen of the moth (arrow b) bears an ovipositor that the moth inserts in the flower's ovary to lay an egg. This image was captured using the infrared light-based night-vision feature of a Sony® Handycam video recorder in the HDR-SR series.

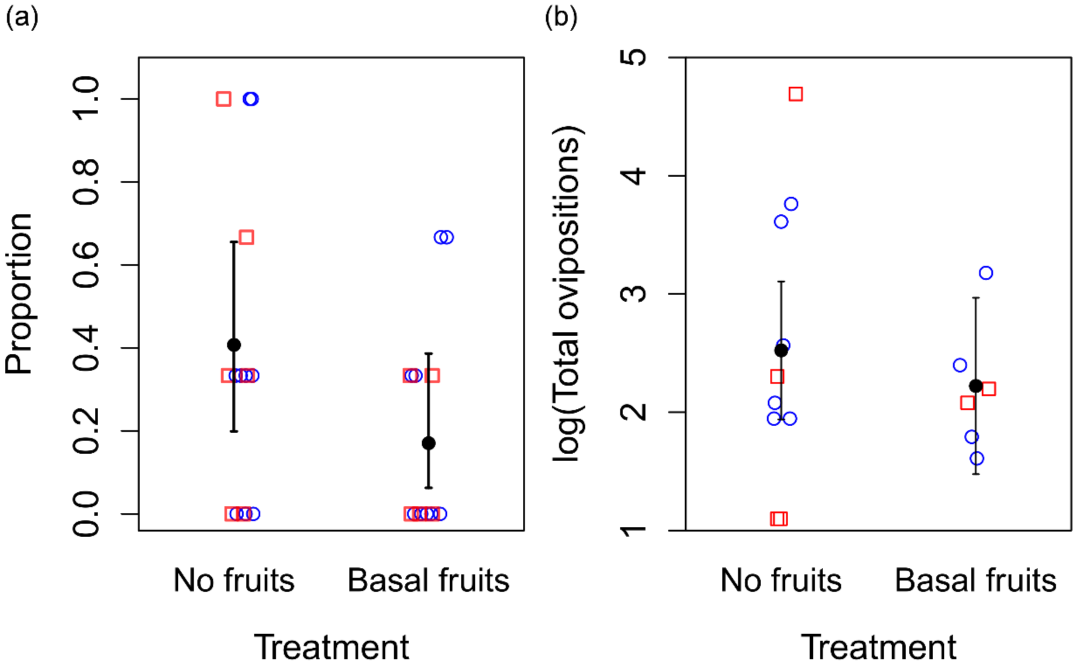


Figure 2. (a) The proportion of flowers with at least one oviposition is significantly lower on inflorescences with the presence of one to three basal fruits than on inflorescences without basal fruits. (b) There is no significant difference in the total number of ovipositions in trials with at least one oviposition between inflorescences with basal fruits and inflorescences without basal fruits. Points are jittered along their x-axis to visualize overlapping points. Open circles are first trials and open squares are second trials. Filled points and error bars are model predicted means and 95% CIs, respectively, from the simplified models with only the presence of basal fruits as a predictor variable (n = 29 trials).

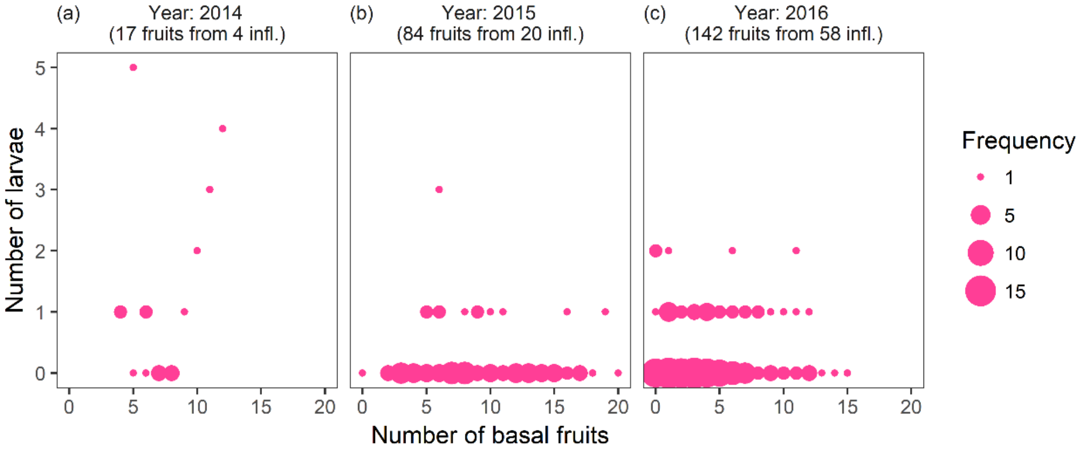


Figure 3. The number of larvae emerging from fruits from top third flowers is not predicted by the number of basal fruits across three years (a,b,c). Points are fruits, and the size of the points is proportional to the frequency of observations. Sample sizes are shown in parentheses above figure panels where infl. stands for inflorescences.

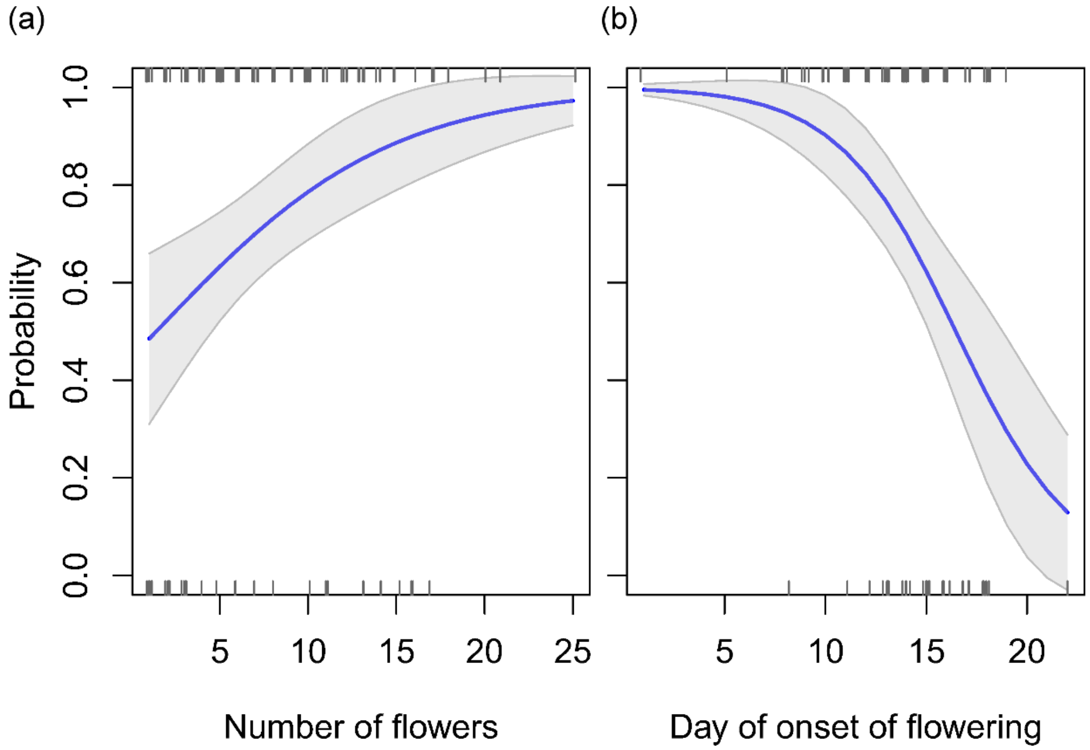


Figure 4. The probability of moths arriving at inflorescences at onset of flowering was (a) positively correlated with the number of flowers open at onset of flowering, and (b) negatively correlated with the day of onset of flowering. In 2016 the first day of onset of flowering (day 1) was May 26th. Lines and shaded areas show model predicted means (solid lines) and 95% CIs, when the other variables are at their median value. Rugs show observed presence and absence of moths (n = 111 inflorescences).

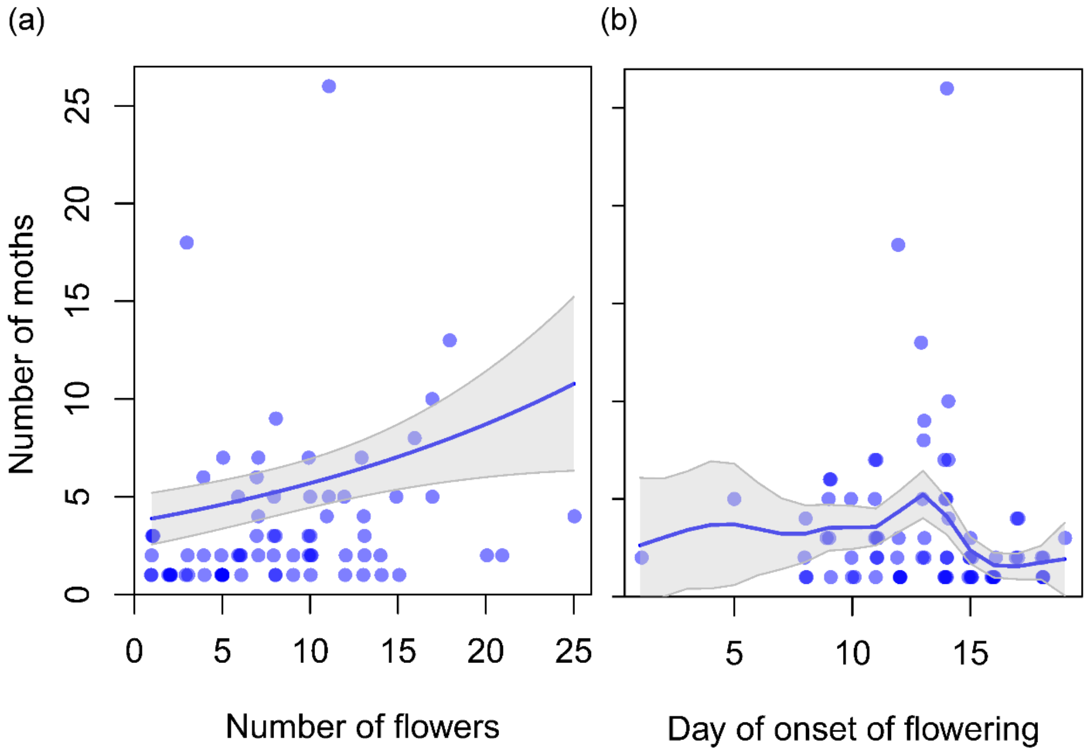


Figure 5. On inflorescences where moths arrived at onset of flowering, the number of moths arriving (a) increased with increasing number of flowers open at onset of flowering, and (b) changed in a complex non-linear pattern with day of onset of flowering. In 2016 the first day of onset of flowering (day 1) was May 26th. Points are inflorescences. Darker points are overlapping points. Lines and shaded areas show model predicted means (solid lines) and 95% CIs when the other variable is held at its median value (n = 76 inflorescences).

SUPPLEMENTARY MATERIAL

Appendix S1

Details of methods used to obtain inflorescence treatments

We selected 136 *Y. glauca* inflorescences that were yet to begin flowering from early May to mid-June 2016 at a mixed-grass prairie at the Cedar Point Biological Station (CPBS), Keith County, Nebraska, USA. We protected the inflorescences from deer herbivory using tomato cages with sides wrapped with 2.54 cm hex netting. When the growing inflorescences were strong enough, we covered them with long cylindrical mesh cages made from fine tulle fabric sleeves with wire rings of approximately 20.5 cm diameter to prevent yucca moths from visiting flowers and florivorous beetles (*Carpophilus sp.*) from damaging flowers. Before placing the mesh cages, we removed all visible florivorous beetles from the inflorescence. At the onset of flowering, we alternately assigned each inflorescence to one of the two treatments.

We established inflorescences with one to three basal fruits by hand-pollinating three to six bottom flowers of the inflorescence. We used pollen from fresh donor flowers from protected inflorescences at least 25 m away from recipient plants. We collected donor flowers in a cooler with a small quantity of ice to keep the flowers cool, and utilized their pollen within three hours of collection. We transferred pollen from one anther lobe of a donor flower to the stigmatic opening of a recipient flower using a toothpick. Thereafter, we used a size 00 brush to push the pollen inside the stylar canal. All hand-pollinated flowers received pollen from the same pollen donor. We thoroughly cleaned both the toothpick and the brush between pollen donors to prevent transfer of pollen of mixed genotypes.

Thirty-five percent hand pollinations resulted in fruits. If hand-pollinated inflorescences did not retain any basal fruits, we considered it a treatment without basal fruits, and we reassigned the treatments on subsequently flowering inflorescences to ensure a sufficient number of inflorescences of each treatment were available at any time during the flowering season.

Inflorescences were checked daily to determine whether they were ready to be used in a behavioral trial. An inflorescence was considered ready for use in a behavioral trial when at least three undamaged receptive (1-2 days old) flowers were available from the top third buds of the inflorescence.

We discarded inflorescences with damage from deer and with florivorous beetles left inside mesh cages. Further, we also discarded inflorescences that dried before flowering or finished flowering sooner than we could use them in a behavioral trial. In 2016, flowering was about 10 days shorter than the previous year likely due to a warmer summer (Jadeja, personal observation). Overall, we could use 23 out of the 136 initially protected inflorescences in behavioral trials.

Appendix S2

Description of Individual-Based Model

We developed an Individual-Based Model (IBM) to support our prediction that with increasing number of basal fruits fewer yucca moth larvae will emerge from distal fruits because of fewer ovipositions in those flowers with increasing number of basal fruits. We simulated flowering, moth arrival and oviposition, and fruiting in sequentially flowering inflorescences. Our model assumes that oviposition is a hierarchical process. After arriving at a flower, a female first decides if the flower is suitable for oviposition at all, and if it is then the female decides how many eggs to oviposit. This process is consistent with the bimodal distribution of number of ovipositions from our experiment described in the main text.

Binomial probability of ovipositing at least one egg, P_{ovi}. Yucca moths are less likely oviposit in flowers with prior ovipositions (Huth & Pellmyr 1999) probably to reduce competition for resources and because flowers containing many eggs have a higher chance of being aborted (Pellmyr & Huth 1994; Humphries & Addicott 2000; Shapiro & Addicott 2004). Hence, we assumed that when a moth arrives at a flower, her probability of ovipositing at least one egg, P_{ovi}, will decrease with increasing number of prior ovipositions, E. To implement this behavior we used the following sigmoidal function that we adapted from Louda et al. (2011) (Figure S2.1a):

$$P_{ovi}(E) = e^{-\left(\frac{E}{12}\right)^3} \qquad (equation\ 1)$$

In some simulations, we assumed that moth oviposition probability will decrease linearly with increasing number of basal fruits because previous work (Jadeja and Tenhumberg, submitted) indicated that flower abortion is higher in the presence of basal fruits, *B* (Figure S2.1b).

$$P_{ovi}(E,B) = e^{-\left(\frac{E}{12}\right)^3} \times \left(1 - \frac{B}{29}\right) \qquad (equation\ 2)$$

Number of ovipositions, λ: The model assumes that the number of ovipositions is Poisson distributed with mean $\lambda = 6$. We explored two scenarios. One scenario assumes that the number of ovipositions is independent of the presences of basal fruits. The other scenario assumes that the number of ovipositions decreases with increasing number of basal fruits, *B*.

$$\lambda(B) = 6 \times \left(1 - \frac{B}{29}\right) \qquad (equation\ 3)$$

In *Yucca glauca* less than 15% of the flowers are retained (Kingsolver 1984; Pellmyr et al. 1997; Addicott 1998). Our model assumes that the probability of retaining a flower decreases with the number of ovipositions, *E*, and with increasing number of basal fruits, *B* (equation 4, Figure S2.2).

$$P_{ret}(E,B) = e^{-\left(\frac{E}{20}\right)^5} \times \left(1 - \frac{B}{29}\right) \qquad (equation\ 4)$$

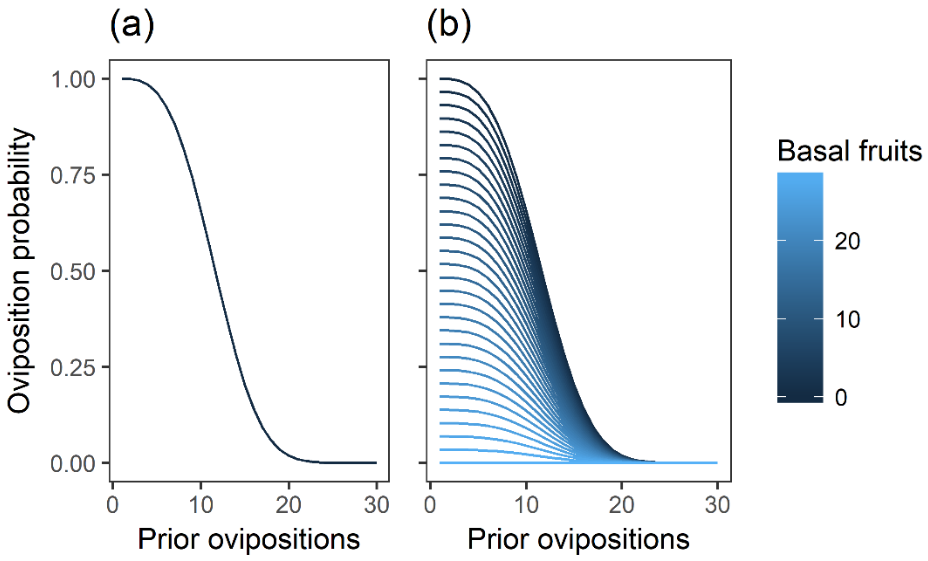


Figure S2.1. Probability of ovipositing at least one egg in flowers with increasing number of prior ovipositions from previous moth visits. In (a) oviposition probability only depends on number of prior ovipositions (equation 1), and in (b) oviposition probability decreases linearly with the number of basal fruits (equation 2).

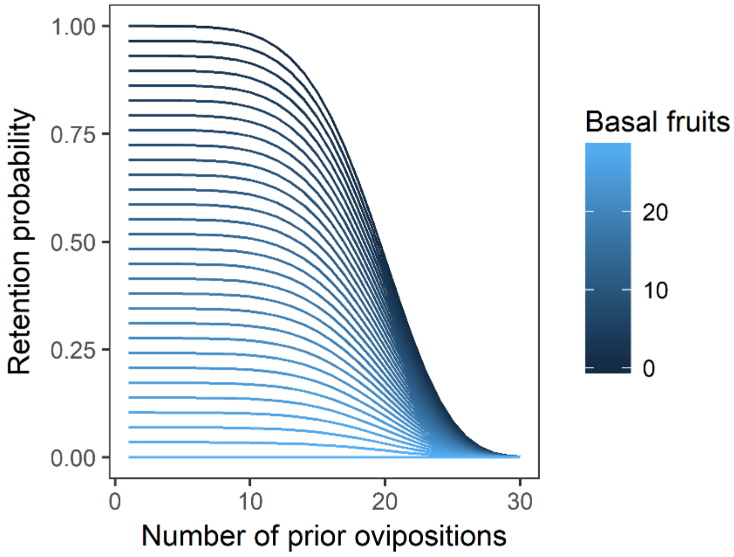


Figure S2.2. Probability of retaining flowers.

We simulated the following four scenarios: (1) P_{ovi} and λ are independent of B, (2) P_{ovi} decreases with B, but λ is independent of B, (3) P_{ovi} is independent of B, but λ decreases with B, and (4) P_{ovi} and λ decrease with B. For each scenario we simulated the fate of 10,000 inflorescences with 30 sequentially opening flowers each. Each simulation started with the bottom-most flower opening on all inflorescences. In our model, one flower per inflorescence opened each time step, and each flower was receptive for only one time step. Hence, flower position and time in the flowering season were correlated in our model, which is in line with the general flowering pattern of *Yucca* spp. Each time step the model cycled through every newly opened flower of all inflorescences and recorded the number of ovipositions per retained flower. For each flower, the model went through the following sequence of events:

The model determined the number of moths arriving, M, by drawing a random number from a Poison distribution with a mean of 5. When more than one moth arrived (M>1), we assumed moths arrived one after another during the time step and not simultaneously. For each moth, we first determined whether the moth laid at least one egg by drawing a random number from a binomial distribution (yes/no) given by $\mathrm{P_{ovi}}$ (equation 1, or equation 2). In case of yes, we determined the number of ovipositions by drawing a random number from a zero-truncated Poisson distribution with the mean of 6 (constant) or given by equation 3. Once the model cycled through all arriving moths it determined whether a flower is retained. If a flower did not receive any ovipositions it was aborted because of lack of pollination (yucca moths are obligate pollinators of *Yucca* spp.). If a flower received at least one oviposition, the model determined whether the flower was retained by drawing a random number from a binomial distribution (yes/no) given by P_{ret} (equation 4).

We analyzed the simulation results by quantifying the number of eggs in fruits of top third flowers and the associated number of basal fruits. Assuming that the survival of eggs in retained flowers is not influenced by the number of basal fruits our simulation results show that only when moths decrease the number of ovipositions with increasing number of basal fruits, $\lambda(B)$ (equation 3) can we expect a negative relationship between number of emerging larvae and number of basal fruits (Figure S2.3). This supports our notion that the number of emerging larvae is a proxy for the number of ovipositions to test whether *T. yuccasella* will decrease the number of ovipositions in response to number of basal fruits.

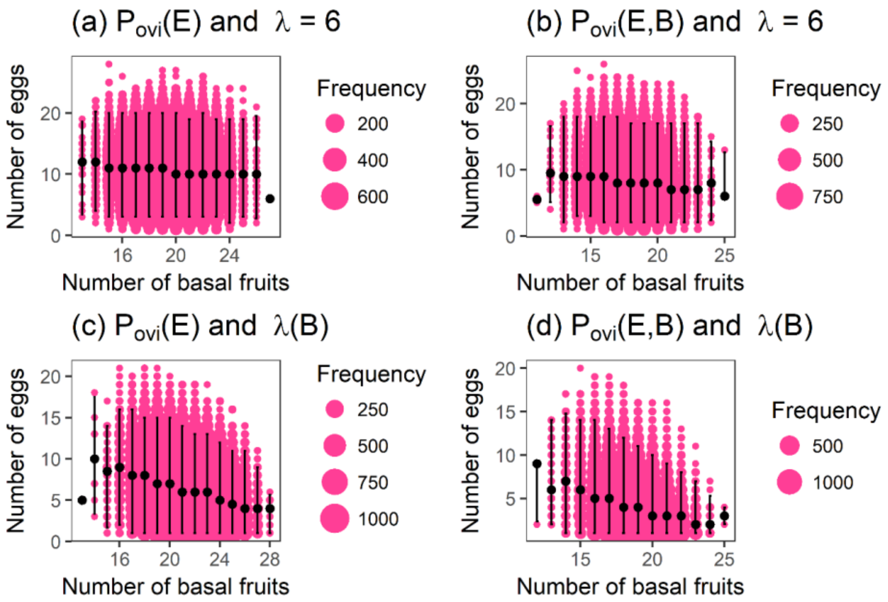


Figure S2.3. Predicted relationships between number of basal fruits and number of egg from simulations of four scenarios: (1) P_{ovi} and λ are independent of B, (2) P_{ovi} decreases with B, but λ is independent of B, (3) P_{ovi} is independent of B, but λ decreases with B, and (4) P_{ovi} and λ decrease with B. Points are fruits from simulated flowers, and point size is proportional to the frequency of points. Black points and error bars are median, and upper and lower 95% quantiles of number of larvae for each number of basal fruits, respectively.

References

Humphries, S. & Addicott, J., 2000. Regulation of the mutualism between yuccas and yucca moths: intrinsic and extrinsic factors affecting flower retention. *Oikos*, 89(2), pp.329–339.

Huth, C.J. & Pellmyr, O., 1999. Yucca moth oviposition and pollination behavior is affected by past flower visitors: evidence for a host-marking pheromone. *Oecologia*, 119(4), pp.593–599.

Louda, S.M. et al., 2011. Priority resource access mediates competitive intensity between an invasive weevil and native floral herbivores. *Biological Invasions*, 13(10), pp.2233–2248.

Pellmyr, O. & Huth, C.J., 1994. Evolutionary stability of mutualism between yuccas and yucca moths. *Nature*, 372, pp.257–260.

Shapiro, J. & Addicott, J.F., 2004. Re-evaluating the role of selective abscission in moth/yucca mutualisms. *Oikos*, 105(3), pp.449–460.

Appendix S3

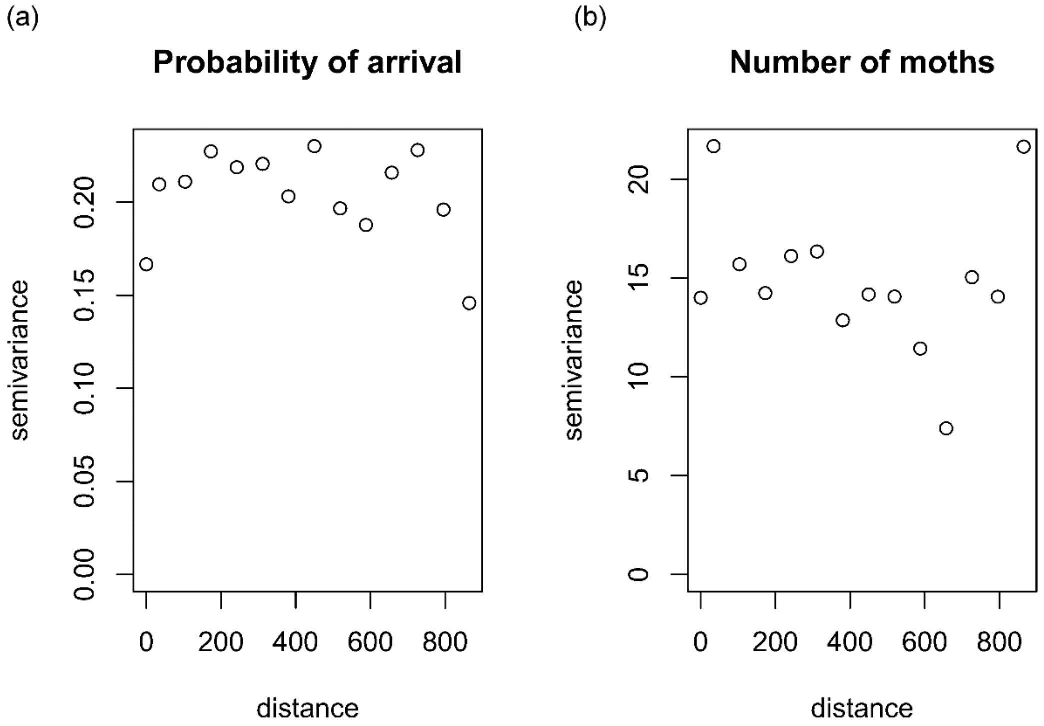


Figure S3.1. Semivariograms for the (a) probability and (b) number of *T. yuccasella* arriving at inflorescences at onset of flowering. A high nugget effect (semivariance at 0 distance on x-axis) compared to the sill (upper limit of the semivariogram) suggests a very little spatial autocorrelation in the probability and number of moths arriving at inflorescences at onset of flowering.

Table S1. Distribution of number of trials carried out during the experiment. We discarded all the trials where moths did not exit their vials.

Trial order	Inflorescence treatment				Total
	no fruits		basal fruits		
	Analyzed	Discarded	Analyzed	Discarded	
first	9	0	9	3	21
second	6	0	5	2	13

Table S2. Full model for the proportion of flowers with at least one oviposition during a trial with presence of basal fruits and trial order as predictors. The model is a generalized linear mixed-effects model with binomial error distribution, and with moth identity and night of trial as random effects. SE indicates standard errors. Variance of random effects - identity of moths and trial night is 0.50 ± 0.71 SD and 0.39 ± 0.62 SD, respectively. (n = 29 trials, from 18 moths over 11 trial nights).

Parameter	**Estimated mean**	**SE**	**z value**	**Pr(>|z|)**
(Intercept) no basal fruits, first trials	-0.31	0.56	-0.55	0.58
basal fruits	-1.25	0.63	-1.98	0.048
second trials	-0.37	0.66	-0.56	0.58

Table S3. Full model for log-transformed total number of ovipositions in a trial with at least one oviposition, with presence of basal fruits and trial order as predictors. The model is a linear mixed-effects model with moth identity and night of trial as random effects. SE indicates standard errors. Standard deviation for random effects - trial night is 0.15, and identity of moths is 0.0001 with 0.9 residuals. (n = 16 trials from 11 moths over 10 trial nights).

Parameter	**Estimated mean**	**SE**	**df**	**t value**	**Pr(>|t|)**
(Intercept) no basal fruits, first trials	2.62	0.39	9	6.76	0.001
basal fruits	-0.32	0.53	1	-0.61	0.65
second trials	-0.26	0.53	1	-0.49	0.71

Table S4. Final model for the proportion of flowers with at least one oviposition during a trial with presence of basal fruits as a predictor. The model is a generalized linear mixed-effects model with binomial error distribution, and with moth identity and night of trial as random effects. SE indicates standard errors. Variance of random effects - identity of moths and trial night is 0.27 ± 0.52 SD and 0.48 ± 0.7 SD, respectively. (n = 29 trials, from 18 moths over 11 trial nights).

Parameter	**Estimated mean**	**SE**	**z value**	**Pr(>|z|)**
(Intercept) no basal fruits	-0.38	0.52	-0.72	0.47
basal fruits	-1.21	0.61	-1.98	0.048

Table S5. Model for log-transformed total number of ovipositions in a trial with at least one oviposition, with presence of basal fruits as a predictor. The model is a linear mixed-effects model with moth identity and night of trial as random effects. The predictor variable, trial order, was removed during backward selection. Standard deviation for random effects - trial night is 0.21, and identity of moths is 0.00005 with 0.91 residuals. (n = 16 trials from 11 moths over 10 trial nights). The model shows a non-significant effect of the presence of basal fruits on the number of ovipositions in trials where eggs were laid. Further model simplification during backward selection shows that this model is not significantly different from the null model (LRT = 0.39, p = 0.53).

Parameter	**Estimated mean**	**SE**	**df**	**t value**	**Pr(>|t|)**
(Intercept) no basal fruits	2.52	0.32	9	7.93	<0.0001
basal fruits	-0.30	0.51	2	-0.59	0.61

Table S6. Model for number of larvae emerging from fruits from top third flowers on naturally-pollinated inflorescences. The model is a generalized linear mixed-effects model with Poisson error distribution, and with inflorescence identity as a random effect. Predictor variables are number of basal fruits and year. Year was treated as a categorical variable. SE indicates standard errors. Variance of random effect - inflorescence identity is 0.33. (n = 243 fruits from 82 inflorescences).

Parameter	**Estimated mean**	**SE**	**t value**	**Pr(>|t|)**
(Intercept) year 2014	-0.2	0.49	-0.4	0.69
number of basal fruits	0.01	0.04	0.33	0.74
year 2015	-1.87	0.52	-3.60	0.0003
year 2016	-1.43	0.47	-3.08	0.002

Table S7. Full model for the probability of arrival of moths at onset of flowering with day of onset of flowering (onset day), number of flowers open at onset of flowers (flowers), basal diameter of the rosette (basal dia.), and the distance to the nearest tree (tree dist.) as predictors. The model is a generalized linear model with Binomial error distribution. (n = 111 inflorescences).

Parameter	**Estimated mean**	**SE**	**z value**	**Pr(>|z|)**
(Intercept)	3.28	1.85	1.77	0.08
tree dist.	0.005	0.03	0.15	0.88
basal dia.	0.02	0.02	0.97	0.33
onset day	-0.33	0.09	-3.61	0.0003
flowers	0.14	0.05	2.78	0.005

Table S8. Candidate set of models for the probability of arrival of moths on the day of onset of flowering of an inflorescence (model syntax) with number of parameters (k), corrected Akaike Information criteria (AICc), differences in AICc (ΔAICc), Akaike weights (AICcWt), cumulative Akaike weights (Cum.Wt), and Log-likelihood (LL). GLM with binomial family of errors was used. Best approximating model is highlighted in grey.

Model syntax	**k**	**AICc**	**ΔAICc**	**AICcWt**	**Cum.Wt**	**LL**
onset day + flowers	3	118.38	0	0.57	0.57	-56.08
basal dia. + onset day + flowers	4	119.59	1.21	0.31	0.88	-55.61
tree dist. + basal dia. + onset day + flowers	5	121.76	3.38	0.10	0.98	-55.6
basal dia. + onset day	3	126.6	8.22	0.01	0.99	-60.19
onset day	2	127.32	8.94	0.01	1	-61.61
basal dia. + flowers	3	134.26	15.88	0	1	-64.02
flowers	2	135.03	16.64	0	1	-65.46
basal dia.	2	136.52	18.14	0	1	-66.21
Null	1	138.81	20.43	0	1	-68.39
tree dist.	2	140.86	22.48	0	1	-68.38

Table S9. Final model for the probability of moths arriving at onset of flowering with day of onset of flowering (onset day) and number of flowers open at onset of flowers (flowers) as predictors. The model is a generalized linear model with binomial error distribution. (n = 111 inflorescences).

Parameter	**Estimated mean**	**SE**	**z value**	**Pr(>|z|)**
(Intercept)	4.61	1.29	3.59	0.0003
onset day	-0.34	0.09	-3.78	0.0002
flowers	0.15	0.05	3.01	0.003

Table S10. Full model for the number of moths arriving at onset of flowering with smoothed day of onset of flowering (onset day), number of flowers open at onset of flowers (flowers), basal diameter of the rosette (basal dia.), and the distance to the nearest tree (tree dist.). The model is a generalized additive model with Poisson error distribution. (n = 76 inflorescences where moths arrived at onset of flowering).

Parameter	**Estimated mean**	**SE**	**z value**	**Pr(>|z|)**
(Intercept)	-0.91	0.33	-0.28	0.006
tree dist.	-0.008	0.009	-0.84	0.40
basal dia.	-0.0006	0.004	-0.14	0.89
flowers	0.04	0.01	3.10	0.002
Smoothing spline			**X^2**	**p-value**
s(onset day)			31.08	0.0001

Table S11. Candidate set of models for the number of moths arriving at inflorescences on day of onset of flowering (model syntax) with corrected Akaike Information Criteria (AICc), differences in AICc (ΔAICc), Akaike weights (AICcWt), cumulative Akaike weights (Cum.Wt), and Log-likelihood (LL). Generalized additive model with Poisson family of error was used with smoothing spline on day of onset of flowering. Best approximating model is highlighted in grey.

Model syntax	**AICc**	**ΔAICc**	**AICcWt**	**Cum.Wt**	**LL**
s(onset day) + flowers	396.51	0	0.69	0.69	-188.54
basal dia. + s(onset day) + flowers	399.02	2.51	0.20	0.89	-188.53
tree dist. + basal dia. + s(onset day) + flowers	401.06	4.55	0.07	0.96	-191.85
s(onset day)	402.94	6.43	0.03	0.99	-208.22
basal dia. + s(onset day)	405.19	8.68	0.01	1	-216.07
flowers	420.60	24.09	0	1	-215.37
basal dia. + flowers	422.23	25.72	0	1	-188.54
Null	434.19	37.68	0	1	-188.21
basal dia.	434.90	38.39	0	1	-191.73
tree dist.	435.07	38.56	0	1	-208.22

Table S12. Final model for the number of moths arriving at onset of flowering with smoothed day of onset of flowering (onset day) and number of flowers open at onset of flowers (flowers) as predictors. The model is a generalized additive model with Poisson error distribution. (n = 76 inflorescences where moths arrived at onset of flowering).

Parameter	**Estimated mean**	**SE**	**z value**	**Pr(>|z|)**
(Intercept)	0.8	0.14	5.74	<0.0001
flowers	0.04	0.01	3.27	0.001
Smoothing spline			**X^2**	**p-value**
s(onset day)			32.02	<0.0001