Direct Consumption of Cotton Pollen Improves Survival and Development of Cheiracanthium inclusum (Araneae: Miturgidae) Spiderlings

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ABSTRACT Cheiracanthium inclusum (Hentz) (Araneae: Miturgidae), a spider that is common in cotton, Gossypium hirsutum L., foliage, was tested for the impact of consumption of cotton pollen on its survival and development under conditions of prey limitation. Immature C. inclusum readily consumed cotton pollen grains that were pierced, crushed, and macerated with the mouthparts while being extraorally digested. When reared on a diet of cotton pollen alone, spiderlings survived 178% longer than those given only water (14.45 versus 5.2 d). When provided with a prey-limited diet (Five eggs of Helicoverpa zea, a favored prey) access to cotton pollen improved the likelihood of spiderlings molting to the second instar from 0 to 22%. This increased from 60 to 84% for spiderlings provided with 10 eggs plus access to pollen. Addition of cotton pollen also extended the survival of spiders fed five or 10 eggs. Supplemental consumption of pollen seems adaptive for this spider and may lead to increased survival and retention of spiders in cotton fields.

KEY WORDS pollenivory, spider development, nonprey resource, conservation, biological control

Nocturnally active, foliage-occupying cursorial spiders can be important predators of pests in various crops (Ruberson and Greenstone 1998; Amalin et al. 2001; Pfannenstiel and Yeargan 2002; Pfannenstiel 2005, 2005b). These spiders have been demonstrated to use naturally occurring food resources, other than prey, such as extrafloral nectars (Patt and Pfannenstiel 2008, 2009; Taylor and Pfannenstiel 2008, 2009, Chen et al. 2010; Pfannenstiel and Patt 2012) and supplemental artificial diets, such as wheat, soy powders, and yeasts, when reared in the laboratory (Patt et al. 2012). Another abundant nonprey resource that might be used by these spiders is pollen. However, there are very few published records of spider pollenivory. The original report described incidental consumption of pollen by web-building spiders as they consume their webs that have become coated with pollen grains (Smith and Mommsen 1984). However, direct pollenivory by web-building spiders has been observed previously (Ludy 2004, Peterson et al. 2010). Vogelel and Greissl (1989) also documented extended survival by a flower-inhabiting crab spider when pollen or simulated nectar was available. Only one reported test of pollenivory by spiders did not demonstrate pollen feeding (Carrel et al. 2000).

Because cursorial spiders feed on a variety of motionless prey and nonprey foods (Pfannenstiel 2008a,b; Taylor and Pfannenstiel 2008, Pfannenstiel and Patt 2012), it was hypothesized that they also might consume pollen as a supplemental food and that pollenivory might augment their survival and development. This guild of spiders is common in cotton, Gossypium hirsutum L. (Pfannenstiel 2005b), which has an extended period of flowering and pollen availability. During its flowering period, it is not uncommon to observe pollen grains that have fallen from the flowers to leaves (unpublished data). Therefore, the value of cotton pollen to augment spider survival and development was tested using Cheiracanthium inclusum (Hentz) (Araneae: Miturgidae), a species that is common in cotton.

Materials and Methods

Spider colonies were initiated from C. inclusum collected from cotton and soybean Glycine max (L.) Merr., fields at the research farm of the USDA laboratory in Weslaco, TX. Field-collected individuals were placed in a 90 by 15-mm plastic petri dish with a moistened dental wick and held at 26 ± 1°C and a photoperiod a 14:10 (L:D) h. Spiders were fed Helicoverpa zea (Boddie) eggs ad libitum. C. inclusum develops well on a diet of frozen H. zea eggs (Pfannenstiel 2008a). H. zea eggs for prey were obtained from a laboratory colony reared by modified methods of Ignoffo (1965). On reaching adulthood, male and female spiders were paired and mating observed. Females were then fed H. zea eggs ad libitum until oviposition. Egg masses collected this way were monitored daily for spiderling emergence. Freshly emerged

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spiderlings were placed individually in 90 by 15-mm petri dishes with a moistened dental wick as a water source. All spiders used for assays were first instars that had just emerged from the silken sac enclosing an egg mass.

Pollen was collected from greenhouse-grown cotton ('Deltapine 54-15 BR') plants that had been direct seeded four to a 5-liter pot. After the cotton plants began to flower, they were checked daily. Newly opened or 1-d-old blossoms were clipped from the plant and the pollen was tapped into a 90 by 15-mm petri dish. The pollen was then inspected under a microscope to make sure there were no arthropods present. If any arthropods were observed they were removed from the dish to ensure that the cotton pollen was devoid of prey. Because the amount of pollen collected in any one day varied considerably, typically three to four consecutive days of pollen collection were stored in a single petri dish. The pollen was stored in a refrigerator at 4°C until used. For each feeding: pollen from a single set of collection dates was presented to spiders in all treatments. In all studies feeding; pollen from a single set of collection dates was compared with individuals receiving only pollen grains being consumed could be determined, noted and are described here. When the number of events. General aspects of feeding behavior were demonstrated ready acceptance and feeding on pollen mass.

During the setup and evaluation of the following studies, spiders were observed for pollen feeding events. General aspects of feeding behavior were noted and are described here. When the number of pollen grains being consumed could be determined, they were quantified.

The survival of spiderlings with access to cotton pollen was compared with individuals receiving only water. Newly emerged spiderlings of C. inclusum were placed individually into 90 by 15-mm plastic petri dishes with a moistened dental wick and a small amount of cotton pollen or into a petri dish with the moistened dental wick but no pollen as a control (n = 25 spiderlings for each). One difficulty that was occasionally encountered in this study was that the pollen grains became statically charged within the petri dish. This feeding occurred during the daytime, despite these spiders being strictly nocturnal in the field (Pfannenstiel 2008a). To ensure spiderlings became prey limited in this study, prey levels between 15 and 40% of the previously observed consumption levels were selected or in this case five and 10 H. zea eggs per individual (17 and 34% of ad libitum consumption, respectively). The value of pollen consumption for development was then evaluated when C. inclusum spiderlings were presented with cotton pollen and fed five or 10 H. zea eggs and contrasted to eggs and no-pollen controls. Initial sample sizes were 27 for each treatment; however, several escaped during the first night resulting in the final sample sizes of: five eggs with no pollen, n = 25; five eggs with pollen, n = 27; 10 eggs with no pollen, n = 25; and 10 eggs with pollen, n = 26. Eggs for the study were deposited by H. zea moths onto green florist paper and then stored frozen at −20°C for <1 month. Newly emerged spiderlings were placed individually in a 90 by 15-mm plastic petri dish with a moistened dental wick, and one of H. zea egg treatments (five or 10 eggs) with or without cotton pollen. Additional water was added to the dental wick for both treatments twice weekly, and fresh pollen was added to the appropriate dishes every 3–4 d for 2 wk (four times). No additional H. zea eggs were added to the petri dishes during the study. Spiders were observed daily for molting or mortality. Survival curves for each egg treatment were compared for each level of pollen feeding by using PROC LifeTest (SAS Institute 2000), and the log-rank test (chi-square) was used to determine significance. To determine the impact of treatments on the proportion of individuals molting to the second instar, we used the Fisher’s exact test. The time to develop to the second instar was compared between the pollen and no-pollen treatments within each level of H. zea eggs by using t-tests when the sample size was sufficient.

Results

Spiderlings were observed feeding directly on cotton pollen. Spiders were most frequently observed to feed on pollen immediately after initial placement in a petri dish. This feeding occurred during the daytime, despite these spiders being strictly nocturnal in the field (Pfannenstiel 2008b; unpublished data). Spiderlings fed on pollen by picking up several grains (range, 2–5; n = 7 for C. inclusum) and manipulating them between their fangs, chelicerae, maxillae, and labium. Pollen grains could be picked up as multiple individual grains or as clumps. The spiderlings pierced, crushed, and macerated the pollen grains while digesting them externally, frequently rotating the moist bolus of pollen grains and digestive juices. Spiders manipulated the mass of pollen grains and digestive juices for many minutes (not quantified but can be ≥10 min) until the spider switched from manipulating the moist food bolus to imbibing the juices over 10 or 20 s. After the mix of digestive juices and pollen was imbibed, a mostly dry mass of the pollen exines was discarded. Several moments after discarding the bulk of the indigestible portions, a second smaller mass was ex-
truded from between the maxillae and the labium and also was discarded.

Consumption of cotton pollen alone significantly improved survival of spiderlings over those provided only water ($\chi^2 = 44.71$, df = 1, $P < 0.0001$) (Fig. 1). The mean percentage of extension of survival due to feeding on cotton pollen versus access to water alone for *C. inclusum* was 178% (mean survival of 14.45 versus 5.2 d for cotton pollen and water, respectively). Spiderlings fed cotton pollen when prey-limited were significantly more likely to molt to the second instar than those feeding on prey alone (Fig. 2). None of the spiderlings fed only five eggs (0/25) became second instars versus 22% (6/27) of those fed five eggs and cotton pollen ($P = 0.023$; Fisher’s exact test). Spiderlings fed 10 eggs with and without pollen also showed an increase in development to the second instar, with 60% molting without pollen (15/25) and 84.6% (22/26) with pollen ($P = 0.064$; Fisher’s exact test) (Fig. 2).

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The mean developmental time from emergence to molting to the second instar was not significantly different between treatments ($t = 1.44$, df = 34, $P = 0.1597$), although there was a trend for *C. inclusum* spiderlings feeding on 10 eggs with pollen to develop faster (mean = 8.27 ± 0.25 d; n = 22) than for those feeding on 10 eggs alone (mean = 8.86 ± 0.33 d; n = 15). For all spiders feeding on cotton pollen along with *H. zea* eggs, survival was extended versus feeding on eggs alone (Fig. 3). Increasing the number of eggs from five to 10 caused a significant increase in survival time ($\chi^2 = 15.655$, df = 1, $P < 0.0001$); however, the addition of pollen muted the differences between egg treatments, and there was no significant difference in the survival curves for spiders given either five or 10 eggs when they were provided with pollen ($\chi^2 = 0.0173$, df = 1, $P = 0.8955$).

**Discussion**

Recent studies have vastly expanded the range of nonprey foods that spiders readily, and arguably regularly, consume. Floral and extrafloral nectars (Voigelei and Greissl 1989, Taylor and Foster 1996, Jackson et al. 2001, Taylor and Bradley 2009, Taylor and Pfannenstiel 2009), insect honeydew (Pfannenstiel and Patt 2012), yeast and plant-derived proteins (Patt et al. 2012), and pollen (Smith and Mommsen 1984, Voigelei and Greissl 1989, Meissle and Romeis 2009) have all been shown to benefit spider survival or weight gain. Spiders have historically been considered obligate predators (Foelix 1982); however, it seems that they are much more omnivorous than previously realized. The expanded resource use range of these spiders probably increases their ability to establish and build populations in crops or habitats that contain these nonprey resources, as has been shown in other omnivorous predators (Eubanks and Styrsky 2005, Lundgren 2009, Welch et al. 2012). Increased establishment and population growth due to nonprey resources, particularly in annual crops, should lead to...
greater impact on pests than might otherwise be possible. Ruben and Handel (1999) observed increases in plant fitness due to the presence of spiders whose densities were increased because of plant-provided, nonprey resources.

Although there has been some discussion on how the spiders consume the pollen (Smith and Mommsen 1984, Peterson et al. 2010), observations made here indicate that the pollen grains are pierced, crushed, and broken open by the fangs in concert with the other mouthparts, and the contents are then extraradially digested and imbied by the spiders.

The improved survival of *C. inclusum* spiderlings feeding on cotton pollen was greater on a percentage basis than that reported by other studies. Survival was extended 147% for *C. inclusum* compared with just water. Smith and Mommsen (1984) observed that survival was doubled for second-instar *Araneus diadematus* Clerck and increased 56% for third instars feeding on birch (*Betula* spp.) pollen. Vogelei and Greisll (1989) observed a 63% increase in survival for *Thomisus onustus* Walkenaer feeding on *Erigeron an- nuus* (L.) Pers. pollen and 105% when feeding on *Bellis perennis* L. pollen (both Asteraceae). *C. inclusum* is a highly active forager that moves nearly continuously during the night; *A. diadematus* is an orb weaver, and *T. onustus* is primarily a sedentary sit-and-wait predator associated with flowers. The extended survival time for *C. inclusum* provided with cotton pollen, whereas significant, was much shorter in duration than observed on cotton extralfloral nectar (52.6 d, 870% longer) or mealybug honeydew (37.9 d, 626% longer survival) (Pfannenstiel and Patt 2012). However, when pollen is available in cotton, the extralfloral nectar also would be readily available; these resources are probably complementary, or synergistic, in value.

The digestive physiology of spiders is poorly understood. If spiders evolved to feed only on prey, they should have difficulty digesting and assimilating nectar and pollen because these nonprey foods have different nutritive constituents than prey. Predators that are adapted to pollenivory have been shown to possess the requisite enzymes to digest pollen (Lundgren 2009). Spiders may possess at least some of these enzymes (Mommsen 1978); however, whether pollenivory is an evolved trait is unknown, as is the degree to which spiders generally feed on pollen.

Previous reports of spider pollenivory have demonstrated increased survival (Smith and Mommsen 1984, Vogelei and Greisll 1989) or weight gain (Meissle and Romeis 2009). This is the first documentation that pollenivory also might support spider development. Taylor and Pfannenstiel (2009) demonstrated that the availability of extralfloral nectar allowed *C. inclusum* to develop, mature, and reproduce on fewer prey than when nectar was not available. Similarly, the availability of yeast or artificial diets containing proteins and sugars also improved the ability of spiders to develop and mature (Patt et al. 2012). The results observed here with first-instar *C. inclusum* suggest that the benefits of pollenivory could be similar.

These spiders are more frequently observed feeding on lepidopteran eggs in crops containing extralfloral nectaries (cotton) than in adjacent crops that do not have extralfloral nectar (soybean and corn) (Pfannenstiel 2005b). In retrospect, pollen also may be contributing to the observation of higher densities of these spiders in cotton versus soybean (with little shed pollen and no extralfloral nectaries) or corn (no extralfloral nectaries but copious pollen during a brief anthesis period). In cropping systems or habitats where pollens and nectars are regularly available, population densities of these spiders may consistently be higher, resulting in increased mortality of pests. Alternatively, our knowledge of spider use of nonprey resources may help us devise strategies to augment spider populations with other predators through enhancing retention and survival of immigrating spiders.

Acknowledgments

Sincere thanks are extended to Frank De La Fuente who assisted in conducting these studies. Joe M. Patt and T. E. Cottrell and two anonymous reviewers improved on earlier drafts of this manuscript.

References Cited

Amanelinejad, D., M. R. Reiskind, J. E. Peña, and R. McSorley. 2001. Predator behavior of three species of sac spider attacking citrus leafminer. J. Arachnol. 29: 72–81.

Carrel, J.E.H., K. Burgess, and D. M. Shoemaker. 2000. A test of pollen feeding by a linyphiid spider. J. Arachnol. 28: 243–244.

Chen, X., Y. Chen, L. Wu, Y. Peng, J. Chen, and F. Liu. 2010. A survey of nectar feeding by spiders in three different habitats. Bull. Insectol. 63: 203–208.

Cubanks, M. D., and D. J. Sterksy. 2005. Effects of plant feeding on the performance of omnivorous predators. In F. L. Wackers, P.C.J. van Rijn, and J. Bruin (eds.). Plant-provided food for carnivorous insects. Cambridge University Press, Cambridge, United Kingdom.

foelix, R. 1982. Biology of Spiders. Harvard Univ. Press, Cambridge, MA.

Jackson, R., S. D. Pollard, X. J. Nelson, G. B. Edwards, and A. T. Barrion. 2001. Jumping spiders (Araneae: Salti- cidae) that feed on nectar. J. Zool. (Lond.) 255: 25–29.

Ignoffo, C., M. 1965. The nuclear-polyhedrosis virus of *Heliothis zea* (Boddie) and *Heliothis virescens* (Fabricius). II. Biology and propagation of diet-reared *Heliothis*. J. Invertebr. Pathol. 7: 217–226.

Ludy, C. 2004. Intentional pollen feeding in the spider *Araneus diadematus* Clerck, 1757. Newsl. Br. Arachnol. Soc. 101: 4–5.

Lundgren, J. G. 2009. Relationships of natural enemies and non-prey foods. Progress in biological control, vol. 7. Springer, New York.

Meissle, M., and J. Romeis. 2009. The web-building spider *Theridion impressum* (Araneae: Theridiidae) is not adversely affected by Bt maize resistant to corn rootworms. Plant Biotechnol. 7: 645–656.

Mommsen, T. P. 1978. Comparison of digestive a-amylases from two species of spiders (*Tegenaria atrica* and *Copi- ennus salei*). J. Comp. Physiol. 127: 355–361.

Patt, J. M., and R. S. Pfannenstiel. 2008. Odor-based recognition of nectar in cursorial spiders. Entomol. Exp. Appl. 127: 64–71.
Patt, J. M., and R. S. Pfannenstiel. 2009. Characterization of restricted area searching behavior following consumption of prey and non-prey food in a cursorial spider, *Hibana futilis*. Entomol. Exp. Appl. 132: 13–20.

Patt, J. M., R. S. Pfannenstiel, W. G. Meikle, and J. J. Adamczyk. 2012. Supplemental diets containing yeast, sucrose, and soy powder enhance the survivorship, growth, and development of prey-limited cursorial spiders. Biol. Control (in press). doi: 10.1016/j.biocontrol.2011.02.004.

Peterson, J. A., S. A. Romero, and J. D. Harwood. 2010. Pollen interception by linyphiid spiders in a corn agroecosystem: implications for dietary diversification and risk-assessment. Arthropod Plant Int. 4: 207–217.

Pfannenstiel, R. S. 2005. Nocturnal predation and their impact on lepidopteran eggs in annual crops: what we don’t see does help us!, pp. 463–471. In Proceedings of the 2nd International Symposium on Biological Control of Arthropods, 12–16 September 2005, Davos, Switzerland. FEHTET-2005-08. USDA Forest Service, Morgantown, WV.

Pfannenstiel, R. S. 2008a. Development of the cursorial spider, *Cheiracanthium inclusum* (Araneae: Miturgidae), on eggs of *Helicoverpa zea* (Lepidoptera: Noctuidae). J. Entomol. Sci. 43: 418–422.

Pfannenstiel, R. S. 2008b. Spider predators of lepidopteran eggs in south Texas field crops. Biol. Control 46: 202–205.

Pfannenstiel, R. S., and J. M. Patt. 2012. Feeding and survival of two nocturnal cursorial spiders on extraloral nectar and honeydew sugars. Biol. Control (in press).

Pfannenstiel, R. S., and K. V. Yeargan. 2002. Identification and diel activity patterns of predators attacking *Helicoverpa zea* (Lepidoptera: Noctuidae) eggs in soybean and sweet corn. Environ. Entomol. 31: 232–241.

Ruberson, J. R., and M. H. Greenstone. 1998. Predators of budworm/bollworm eggs in cotton: an immunological study, pp. 1095–1098. In Proceedings of the Beltwide Cotton Conferences, Nashville, TN. National Cotton Council, Memphis, TN.

Ruhren, S., and S. N. Handel. 1999. Jumping spiders (Salticidae) enhance the seed production of a plant with extraloral nectaries. Oecologia 119: 227–230.

SAS Institute. 2000. SAS/STAT® user’s guide, version 8. SAS Institute, Cary, NC.

Smith, R. B., and T. P. Mommsen. 1984. Pollen feeding in an orb-weaving spider. Science 226: 1330–1332.

Taylor, R. M., and R. A. Bradley. 2009. Plant nectar increases survival, molting, and foraging in two foliage wandering spiders. J. Arachnol. 37: 232–237.

Taylor, R. M., and W. A. Foster. 1996. Spider nectarivory. Am. Entomol. 82–86.

Taylor, R. M., and R. S. Pfannenstiel. 2008. Nectar feeding by wandering spiders on cotton plants. Environ. Entomol. 37: 996–1002.

Taylor, R. M., and R. S. Pfannenstiel. 2009. How dietary plant nectar affects the survival, growth, and fecundity of a cursorial spider *Cheiracanthium inclusum* (Araneae: Miturgidae). Environ. Entomol. 38: 1379–1386.

Vogelei, A., and R. Greissl. 1989. Survival strategies of the crab spider *Thomisus onustus* Walckenaer 1806 (Chelicerata, Arachnida, Thomisidae). Oecologia 80: 513–515.

Welch, K., R. S. Pfannenstiel, and J. M. Harwood. 2012. The role of generalist predators in terrestrial food webs: lessons for agricultural pest management. In G. M Gurr, S. D Wratten, and W. E. Snyder (eds.), Biodiversity and insect pests: key issues for sustainable management. Wiley Blackwell, New York.

Received 29 September 2011; accepted 29 December 2011.