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Indirect effects on mutualisms: parasitism of bumble bees and pollination service to plants

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Abstract. Researchers increasingly recognize the important role of mutualisms in structuring communities and view positive interactions in a community context rather than as simple pairwise interactions. Indirect effects, such as those that predators have on lower trophic levels, are a key process in community ecology. However, such top-down indirect effects have rarely been extended to mutualisms. Antagonists of one mutualist have the potential to negatively affect the second mutualist through negative effects on their partner, and the magnitude of such effects should vary with mutualism strength. Bumble bees are ecologically and economically important pollinators that are an ideal system to determine if such indirect effects play an important role in mutualisms. Bumble bees are attacked by an array of parasites and predators, and they interact with a range of plants that vary in their dependence on bumble bees for reproduction. We tested whether variation in parasitism rates by *Nosema bombi*, *Crithidia bombi*, and conopid flies correlated with reproduction of greenhouse-raised plants placed in the field. At multiple sites over two years, we studied four plant species that varied in reliance on bumble bees as pollinators. We found a consistent negative relationship between *Nosema* parasitism and measures of pollination for *Trifolium pratense* and *Solanum carolinense*, plant species with high bumble bee visitation, whereas *Rudbeckia hirta* and *Daucus carota,* plant species with generalized pollination, experienced no impacts of *Nosema*. However, both *Crithidia* and conopids showed inconsistent relationships with pollination service. Although these patterns are correlational, they provide evidence that parasites of bumble bees may have negative indirect effects on plants, and that mutualism strength can moderate the magnitude of such effects.

Key words: Bombus *spp.; bumble bee; conopid;* Crithidia bombi; *indirect effects; mutualism;* Nosema bombi; *parasitism; pollination service; western Massachusetts, USA*.

Introduction

One of the greatest challenges faced by ecologists is creating general, predictive theories out of the bewildering complexity in even the simplest communities. A key concept in our understanding of communities is that of the trophic cascade, where predators have indirect positive effects on producers through negative effects on herbivores (Paine 1980). Trophic cascades have been investigated in a variety of ecosystems (Pace et al. 1999), and have informed predictions about ecosystem productivity and abundance of different trophic levels (e.g., Carpenter et al. 1985). The more generalized form of this framework, that of indirect effects, has proven useful in describing many general patterns in community ecology. However, from its earliest conception, it has been recognized that "trophic levels" do not encompass all the complexity of interactions that give communities their characteristics (Polis 1991). For example, the inclusion of omnivory has dramatic effects on community dynamics (e.g., Polis and Strong 1996). Predictions from research on trophic interactions can be extended to further understanding of indirect effects of antagonists on ubiquitous non-trophic interactions such as mutualisms.

Researchers are increasingly recognizing the important role of mutualisms in structuring communities (Stachowicz 2001), and are viewing positive interactions in the context of community effects, rather than as simple pairwise interactions (Stanton 2003, Suttle 2003, Knight et al. 2006). Despite this, the potential for trophic interactions to indirectly affect mutualisms has rarely been examined. Mutualists do not interact solely with their mutualist partner, but also with their antagonists, such as predators and parasites. Antagonists of one mutualist have the potential to negatively affect the second mutualist through negative effects on their partner. There are numerous examples of predators that have impacts on mutualistic interactions (Letourneau and Dyer 1998, Dukas 2005) but our understanding of when and why these impacts occur, and how they affect other components of the community, may be

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improved by extending our knowledge of indirect effects. Furthermore, mutualisms range from strongly specialized and obligate to very general and facultative, and the potential for negative multitrophic effects through mutualist interactions will probably be influenced by the degree to which each partner relies on the other.

Bumble bees (Apidae, *Bombus* spp.) are an excellent system for investigating the impacts of mutualism strength on top-down effects of predators. They are abundant and ecologically important pollinators in North America and elsewhere (Kearns and Thomson 2001), and are managed for crop pollination (Velthuis and van Doorn 2006). Bumble bees are attacked by a wide range of antagonists (Schmid-Hempel 1998, Dukas 2005) and interact with numerous plant species that vary in their dependence on bumble bees for pollination (Waser et al. 1996). This variation in the strength of the mutualism allows us to examine the impact of bumble bee parasites on a range of plant species. Furthermore, there is ongoing concern that transmission of pathogens from managed colonies could impact populations of wild bumble bees, and that pathogens may have contributed to range-wide declines of some species (Colla et al. 2006, Cameron et al. 2011, Meeus et al. 2011, Szbado et al. 2012). Thus, studying bumble bees allows us to examine the effects of parasites in a native wild pollinator guild that has applied significance in agriculture and conservation.

Predators of pollinators can potentially have strong negative effects on plant reproduction (Suttle 2003, Knight et al. 2006), but no work has examined whether parasites and parasitoids may have similar impacts, despite their prevalence (Gillespie 2010, Kissinger et al. 2011, Goulson et al. 2012). Bumble bees are attacked by a number of parasites and parasitoids that may have multitrophic effects by changing both bumble bee abundance and foraging behavior. Several species of parasitoid conopid flies (Diptera, Conopidae) attack foraging bumble bees on the wing, and lay their eggs inside the bee's abdomen. The infectious protozoan *Crithidia bombi* (Zoomastigophora, Trypanosommatidea; *Crithidia* hereafter) is a common gut parasite that can be contracted by bees at flowers via fecal transmission (Durrer and Schmid-Hempel 1994). The microsporidium *Nosema bombi* (Microsporidia; Nosematidae; *Nosema* hereafter) is another common parasite of bumble bees that is most likely transmitted from adults to larvae within a colony (Meeus et al. 2011). Evidence suggests that parasitoid flies, *Crithidia*, and *Nosema* can affect colony reproduction and thus bumble bee population dynamics (Schmid-Hempel and Schmid-Hempel 1988, Meeus et al. 2011). Many parasites that attack bumble bees can also affect foraging behavior. For example, *Crithidia* reduces bees' ability to use floral information to distinguish between rewarding and non-rewarding flowers, and slows their ability to learn novel flower types (Gegear et al. 2005, 2006). Thus, either by affecting bumble bee populations or changing bumble bee foraging behavior, parasites and parasitoids could alter pollinator service to plants. Negative population effects of parasites are likely to reduce pollination service to plants. However, behavioral impacts could be positive or negative. Increased time per flower due to parasitism could increase pollination service, whereas decreased floral constancy could increase heterospecific pollen deposition plant and reduce seed set (Galen and Gregory 1989).

Parasites of bumble bees are common in western Massachusetts, USA, with considerable variation across sites and years (Gillespie 2010). We tested whether variation in the parasitism rates by *Nosema*, *Crithidia*, and conopids correlated with reproduction of greenhouse-raised plants that were placed into field sites. By using greenhouse plants placed at a site on a single date, we took a "snapshot" of pollination service and were able to simultaneously determine parasitism levels. We predicted that there would be a negative correlation between parasite infection levels in bumble bees and pollinator service across sites. Furthermore, these correlations should be stronger for plant species that rely primarily on bumble bees for pollination, and weaker for more generalist plant species.

Methods

Field sites

Bees were collected in old fields throughout western Massachusetts during the summers of 2007 and 2008 (Appendix A). Sites were between 1.4 and 34 ha in area, were generally mowed once a year, and had flora typical of North American old fields: *Asclepias* sp., (Asclepiaceae), *Solidago* spp. (Asteraceae), *Trifolium* spp. and *Vicia* sp.(Fabaceae). During 2007, bees were collected in 13 meadows; each site was sampled twice in July and August between 09:00 hours and 17:00 hours, on sunny days with minimal cloud cover (Appendix A). During 2008, bees were collected in 26 meadows (Appendix A), including the same ones used in 2007 but encompassing a greater geographic area. Thirty bees were collected throughout the habitat from a variety of flowering plants, resulting in a haphazard sample of the bee species present. Every effort was made to collect every bee seen, to avoid biased sampling of species that are easier to catch (Otterstatter 2004, Gillespie 2010). Initially, we collected primarily bumble bee workers to consistently sample one guild of bees, but on some sampling dates there were not enough for adequate sampling. At eight out of 13 meadows in 2007, and eight out of 26 meadows in 2008, when sufficient workers could not be collected, males were also collected (Appendix A). In both years, we measured flowering plant diversity and density at each site on the same dates as the bee samples, as in Gillespie (2010), and Appendix A. Flowering plant data were used to calculate a rough estimate of floral resource density as a potential determinant of parasite abundance, as well as conspe-

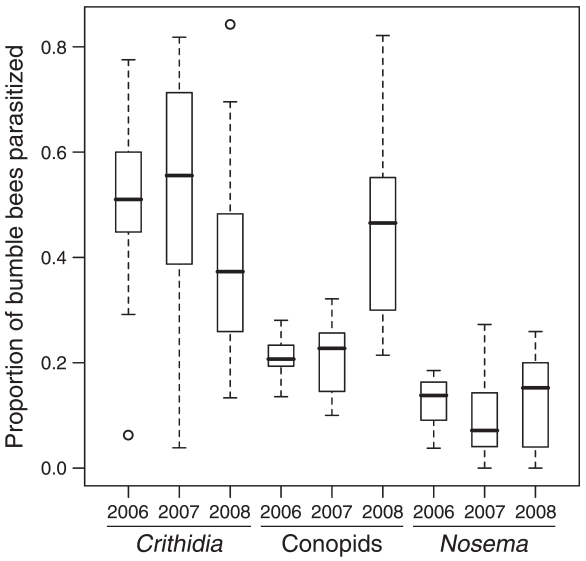

FIG. 1. Boxplot of parasitism rates for 2006–2008. Black bars represent the median. Upper and lower bounds of the boxes are the 25th and 75th percentile, respectively. The whiskers represent the lowest and highest data still within 1.5 interquartile ranges of the upper or lower quartile. Outliers (open circles) are any points outside this range. Sample sizes are as follows: 2006, 9 sites sampled once; 2007, 13 sites sampled twice; 2008, 26 sites sampled once. 2006 data were from Gillespie (2010) and are included here for comparison.

cific density for each of our focal plant species. In 2007 only, we estimated bumble bee abundance by walking two 20-m transects twice on each sampling day, counting the number of bumble bees seen within 1 m on each side. Bumble bee abundance was calculated as the average number of bumble bees per transect walk.

Estimating parasitism

Bumble bees collected from each field site were brought to the lab and provided with sugar water ad libitum until either they died from parasites or several weeks had passed, at which time they were sacrificed for dissection. Prior to dissection, bees were identified to species using the DiscoverLife key (*available online*).[4] Common species sampled included *Bombus impatiens*, *B. vagans*, *B. griseocollis, B. ternarius*, and *B. bimaculatus* (95% of total), whereas *B. fervidus*, *B. perplexus*, and *B. citrinus* constituted less common species. Because several species were present in fewer than 15 of 52 total sampling dates over both years, bumble bee species composition was strongly confounded with site. It was thus inappropriate for us to attempt to disentangle the role of parasitism for each *Bombus* species on pollination services because species composition was strongly confounded with site and sampling date. Thus, for the purposes of this experiment, we consider all *Bombus* as one taxonomic unit.

[4] http://www.discoverlife.org/mp/20q?search=Apoidea

Bees were dissected within 24 hours of death and inspected for the presence of conopid parasitoids. The gut was then removed, ground in a drop of water on a slide, and inspected at 100× magnification for spores of *Crithidia* and *Nosema*. If conopid larvae had consumed the entire gut, screening for microorganisms was not possible, leading to different sample sizes for different parasites. Bees that contained conopid larvae were returned to the growth chamber so that the larvae could pupate. Parasitoid larvae were stored for 2 months at 8°C, returned to room temperature, and allowed to emerge. They were then killed, pinned, and keyed to genus (Smith and Peterson 1987). All reared conopids were identified as either *Physocephala furcillata* (Williston) or *P. tibialis* (Say) (J. Gibson, *personal communication*).

In 2008, bees were dissected as in 2007, except that tools were sterilized with a Bunsen burner between dissections to avoid cross contamination of parasite DNA. All bees were inspected for conopid larvae. Gut samples from all bees were preserved in 100% ethanol in a −40°C freezer. Infection with *Crithidia* and *Nosema* was detected using PCR as a more time-efficient process than dissection. A subsample of bees were both dissected and analyzed with PCR to confirm accuracy. All six bees with positive *Nosema* detection by dissection also had positive detection by PCR, as did 75% of the 35 bees with *Crithidia*. The discrepancy between dissection and PCR detections for *Crithidia* could reflect errors in either process. However, *Crithidia* and *Nosema* parasitism rates in 2008 were similar to those in 2006 and 2007 (Fig. 1; Gillespie 2010), suggesting that the PCR protocol gave results consistent with visual assessment of parasitism (Fig. 1).

Estimating pollination service

We removed the potential effects of site-level variation in plant quality by using greenhouse plants to measure pollination service. In 2007, we grew individuals of *Trifolium pratense* L. (Fabaceae) and *Solanum carolinense* L. (Solanaceae) from seed in the greenhouse (see Plate 1). In 2008 we grew *Trifolium pratense*, *Rudbeckia hirta* L. (Asteraceae), and *Daucus carota* L. (Apiaceae) (all referred to by genus names hereafter). *Solanum* was not used in 2008, as plants either failed to germinate or flower that year. These species vary from annual to short-lived perennial, but all can be induced to flower in their first year of growth. Details of seed sources and greenhouse methods are in Appendix B. All are primarily outcrossing through various mechanisms (Harkess and Lyons 1994, Proctor et al. 1996, Elle 1999, Dhar et al. 2006). These plants represent a range of flower types that probably differ in their dependence on bumble bees for pollination, with *Trifolium* and *Solanum* relatively more dependent (Plath 1925, Elle 1999), and *Rudbeckia* and *Daucus* less so (Wilkinson et al. 1991, Townsend and Levey 2005, Rao and Stephen 2009). *Solanum* flowers are buzz-pollinated, a pollina-

tion mechanism done primarily by bumble bees (Elle 1999), whereas *Trifolium* is pollinated effectively by a small range of bee species, of which bumble bees are efficient pollinators in seed production (Rao and Stephen 2009). *Rudbeckia* and *Daucus* have floral morphologies that are associated with more generalist pollination systems (Waser et al. 1996, Goulson et al. 2009), and a wide range of insect species visit them in the field, when compared to *Solanum* and *Trifolium* (S. Gillespie, *personal observation*). In our results, bumble bees comprised 56% and 87% of visits to *Trifolium* and *Solanum*, respectively, and only 0.6% and 0.2% of visits to *Rudbeckia* and *Daucus*, supporting our assertion that the former are largely dependent on bumble bees for pollination and the latter are not.

Plants were placed at field sites during bee-sampling dates to estimate pollination service (see Plate 1). Because these species require pollinators and were in an insect-free greenhouse except for one day in the field, the only seed set that they achieve should be due to pollinator visits during that day. When possible, five plants of each available species were selected for each sampling date and site. One mature, open flower on each plant was marked, and other flowers were either placed in mesh bags or removed to maintain consistent display size. For species with composite flowering structures (all but *Solanum*), we considered a single inflorescence to be the unit of display, and refer to such structures as "flowers" throughout. On some sampling dates, not enough plants were flowering. Where possible, we then used two flowers from the same plant to keep the patch size at five open flowers for all sites, but on certain days fewer than five flowers were used, or some plant species were excluded. Thus, sample sizes are variable between plant species and sites. This was accounted for by averaging measures of pollination service within individual plants before calculating site-level measures on each sampling date; these site-level measures were the unit of replication used for analyses. Plants were placed at each field site before 09:00 hours on the same day that we collected bumble bees to quantify parasitism at that site. Plants were placed in a single patch, in proximity to wild plants of the same species, to ensure that visitation reflected naturally occurring levels at that site. To quantify pollinator visitation, we observed each experimental patch of each species for four 15-minute periods, two in the morning and two in the afternoon. We recorded each insect visitor's order (e.g., Diptera, Lepidoptera, Hymenoptera), and classified bees as solitary bees, honey bees, or bumble bees. Plants were returned to the greenhouse at the end of the day. In addition, in 2008 only, a receptive stigma was removed from each observed flower and squashed with fuchsin dye suspended in glycerol (Baker et al. 1967). We counted the number of conspecific and heterospecific pollen grains on the stigma under a compound microscope. Increased conspecific pollen deposition could reflect more pollinator visits, increased conspecific pollen carried per bee, or greater transfer pollen efficiency, and higher pollen deposition is often correlated with greater seed number or quality (Quesada et al. 1993). Heterospecific pollen deposition can have negative impacts on seed set in some species through stigmatic clogging (Wilcock and Neiland 2002).

In both years, seeds from marked flowers were collected, counted, and weighed for *Solanum*, *Trifolium*, and *Rudbeckia*. *Daucus* produced no seed. Seed set was calculated at the level of the floral display unit for *Solanum*, *Trifolium*, and *Rudbeckia*. For both *Triolium* and *Rudbeckia*, which have inflorescences, the maximum number of seeds that could be pollinated during their one day in the field depends on the number of receptive stigmas that day. To account for this, for *Trifolium* we counted the number of florets open per flower on the sampling date as an estimate of potential seed set. Proportion of seed set was then calculated as the total seeds set divided by the number of open florets per flower. Both total and proportion of seed set were analyzed for *Trifolium*. For *Rudbeckia*, because the inflorescence is a head with hundreds of tiny stigmas exerted at the same time, we visually estimated the percentage of potential florets that were open. This proved to be a poor predictor of seed set and was excluded from analysis ($n = 130$, $P > 0.1$). Thus, only total seed set was analyzed for *Rudbeckia*. *Solanum* flowers are not inflorescences; thus, total seed set per flower was used to measure reproduction. For all species that reproduced, seed mass was included as a measure of pollination service. Seed mass can be an important correlate of seed quality and viability (Stanton 1984), and is thought to be affected by the genetic quality of the offspring (Temme 1986).

Data analysis

For each year, we treated sampling date as our unit of replication. Because sites were sampled twice in 2007, we analyzed data with and without site included. Site was never significant, and thus was excluded from all subsequent analyses. Thus, all measures of parasitism and pollination service are calculated at the level of sampling date. For 2007, for each site $\times$ sample date combination we calculated the proportion of bees infected with each parasite, the average number of bumble bees per transect walk, and for *Trifolium* and *Solanum*, we calculated the per flower visitation by all visitors and for bumble bees alone, as well as the average seed set and seed mass. To account for variable patch sizes for experimental plants, we calculated overall visitation rate as the total number of insect visitors seen per observed flower on a plant species on a given day. We did not calculate visitation per unit time because every plant species was observed for the same amount of time per sampling date. Where two flowers were observed on the same plant, measures of pollination service were averaged within plant, and then within sampling date. For 2008, we also calculated the average

TABLE 1. Major relationships in regression analysis of parasitism effects on bumble bees: significant positive (+) and negative (−) and nonsignificant (ns) relationships; "x" indicates variables that were part of a significant interaction term but were not significant as main effects.

	Explanatory variables							
Year, plant, and trait	*Crithidia*	*Nosema*	Conopids	Date	Conspecific density	Visitation	Conspecific pollen	Heterospecific pollen
A) 2007								
Trifolium								
Visitation	−	ns	−	ns	ns			
Total seed set	ns	ns	ns	−	ns	+		
Proportion seed set	ns	−	ns	−	ns	ns		
Seed mass	ns	ns	x	−	ns	+		
Solanum								
Visitation	ns	ns	ns	ns	ns			
Total seed set	ns	x	ns	x	ns	ns		
Seed mass	−	−	ns	−	ns	ns		
B) 2008								
Trifolium								
Visitation	ns	−	ns	ns	ns			
Conspecific pollen	ns	−	ns	ns	ns	ns		
Heterospecific pollen	ns	ns	ns	ns	ns	ns		
Total seed set	ns	ns	ns	−	−	ns	+	ns
Proportion seed set	ns	−	ns	ns	ns	ns	+	ns
Seed mass	ns	ns	ns	ns	ns	ns	ns	ns
Rudbeckia								
Visitation	ns	ns	ns	−	ns			
Conspecific pollen	ns	ns	ns	ns	ns	ns		
Heterospecific pollen	ns	ns	ns	−	ns	ns		
Total seed set	+	ns	ns	ns	ns	ns	ns	+
Seed mass	ns	x	x	x	ns	ns	ns	ns
Daucus								
Visitation	ns	ns	ns	−	ns	ns		
Conspecific pollen	ns	ns	ns	ns	ns	ns		
Heterospecific pollen	ns	ns	ns	ns	ns	ns		

Notes: Alpha levels were set using a Bonferroni correction adjusted by the number of regression analyses conducted for each plant species. For *Solanum* and *Daucus,* which had three regression analyses, $\alpha = 0.0167$; for *Trifolium* in 2007, $\alpha = 0.012$; for *Rudbeckia*, $\alpha = 0.01$, and for *Trifolium* in 2008, $\alpha = 0.0085$. Blank cells are variables that were not included in that analysis. Appendix C contains full statistical tables.

heterospecific and conspecific pollen deposition per stigma for each focal plant. All other visitation and reproductive measures were calculated in 2008 as in 2007, excluding bumble bee abundance, which was not measured in 2008.

All analyses were conducted in R (R Development Core Team 2009). We verified normality of variables using visual methods (Zuur 2009). To better meet assumptions, for 2007 data we log-transformed bumble bee abundance, flowering plant density, and overall visitation to *Trifolium* and *Solanum*. For 2008, we log-transformed flower density, overall visitation to *Trifolium* and *Rudbeckia*, heterospecific and conspecific pollen for *Trifolium* and *Rudbeckia*, and *Trifolium* seed set. We then standardized (z-transformed) response and explanatory variables and checked explanatory variables for collinearity. All variance inflation factors were lower than 3 except for overall visitation and bumble bee visitation. Because bumble bee visitation and overall visitation were highly positively correlated, and collinear for *Trifolium* and *Solanum* (*Trifolium* in 2007, $r = 0.96$; *Solanum*, $r = 0.96$; *Trifolium* in 2008, $r = 0.63$), and bumble bees were only a small percentage of floral visitors for *Rudbeckia* and *Daucus* (0.6% and 0.3%, respectively), we used overall visitation as our measure of pollinator visitation in all analyses, recognizing that bumble bees made up very few of the visitors to *Rudbeckia* and *Daucus* and were the primary visitors to *Trifolium* and *Solanum*. To test whether any of our environmental variables were related to measures of parasitism, for each parasite and year we used linear regression to test whether date, flowering plant density, plant diversity, bumble bee abundance, or proportion of the sample that was male affected parasitism rates.

Because of the hierarchical nature of our pollination service data (parasitism could affect visitation, which could in turn change pollen deposition, and thus seed set), we conducted multiple linear regression analyses for each year for each focal plant species. Explanatory variables that were included in all analyses included conspecific plant density, *Crithidia*, *Nosema*, and conopid infection, sampling date, and all date × parasite interactions. In addition, we included the effect of bumble bee abundance for 2007 analyses, but not 2008,

TABLE 1. Extended.

Explanatory variables			
Bombus abundance	*Crithidia* × date	*Nosema* × date	Conopid × date
+	ns	ns	ns
ns	−	ns	ns
+	ns	ns	ns
ns	ns	ns	−
+	ns	ns	ns
ns	ns	+	ns
	ns	ns	ns
	ns	ns	ns
	ns	ns	ns
	ns	ns	ns
	ns	ns	ns
	ns	ns	ns
	ns	ns	ns
	ns	ns	ns
	ns	ns	ns
	ns	ns	ns
	ns	ns	ns
	ns	−	−
	ns	ns	ns
	ns	ns	ns
	ns	ns	ns

as it was not measured that year. The first set of analyses addressed factors that could explain pollinator visitation to each focal plant. The second set of analyses was only conducted for 2008 data, addressed factors that could explain conspecific and heterospecific pollen deposition, and included visitation as an additional explanatory variable. The final set of analyses asked what factors might affect seed set or seed mass. Explanatory variables were the same as for the second set of analyses, but for 2008, also included heterospecific and conspecific pollen deposition. To illustrate any significant parasite × date interactions, we arbitrarily divided sampling dates in half, into "early" and "late" categories, and plotted separate regression lines for each category. For each analysis we used nested model selection to find the best model; nonsignificant interactions and main effects were sequentially removed based on likelihood-ratio tests (Zuur 2009). For both the full model prior to model selection and for the final model, we verified adherence to assumptions of parametric statistics by examining residual patterns for normality and independence between explanatory and response variables. Because of the large number of tests being conducted (21 total), we adjusted our cutoff for significance using Bonferroni corrections (Sokal and Rohlf 1995). We adjusted the P value within each species and year based on the number of regression analyses done, rather than experiment-wide, to attempt to reduce detection of spuriously significant P values but avoid overly conservative tests that can discount consistent, but not highly significant, P values, as are often found in field studies (Moran 2003). Adjustments for all species are detailed with the statistical results in Appendix C. Only results significant at the adjusted P value are discussed.

RESULTS

Parasitism rates and factors affecting parasitism

Overall parasitism rates for 2007 have been described elsewhere (Gillespie 2010), and are summarized in Fig. 1. In 2008, *Crithidia* infected, on average, 42% of bees at a site (range 13–84%), *Nosema* infected 14% of bees (range 0–29%), and conopids infected 44% of bees (range 2–82%; Fig. 1). In 2007, for all parasites, parasitism rates were not related to any measured factor ($n = 26$; all $P > 0.1$). In 2008, conopid and *Crithidia* parasitism was not explained by any measured factor ($n = 26$; all $P > 0.1$) whereas *Nosema* parasitism increased with sampling date ($n = 26$; transformed slope = 0.00328; $t = 3.399$; $P = 0.0025$).

Plants and parasites

Because of the complexity of our results, we summarize the direction and significance of patterns in Table 1 and will discuss here only patterns related to parasitism for each plant. P values and regression coefficients for each model are described in Appendix C.

Trifolium.—Over both years, multiple measures of pollination service to *Trifolium* plants were lower at sites with high *Nosema* parasitism, but pollination service was inconsistently related to parasitism by *Crithidia* and conopids (Table 1; Appendix C: Tables C1 and C2). In 2007, *Trifolium* proportion seed set declined with increasing *Nosema* parasitism (Fig. 2A), and visitation decreased with *Crithidia* and conopid parasitism. There was a significant conopid × date interaction such that seed mass increased with increasing conopid parasitism earlier in the season, but was unrelated later in the season. In 2008 *Trifolium* visitation, conspecific pollen deposition and proportion seed set both declined with increasing *Nosema* parasitism (Fig. 2B). Total seed set declined with increasing *Crithidia* infection. Heterospecific pollen deposition and seed mass were not affected by any factor in 2008.

Solanum.—For the bumble bee specialist *Solanum*, plants at sites with higher parasitism by *Nosema* had lower reproduction, although visitation was unaffected (Table 1; Appendix C: Table C3). *Solanum* seed set and seed mass declined with increasing *Nosema* parasitism (Fig. 2C), and there was a significant date × *Nosema* interaction affecting seed set such that *Solanum* seed numbers declined with *Nosema* parasitism early in the

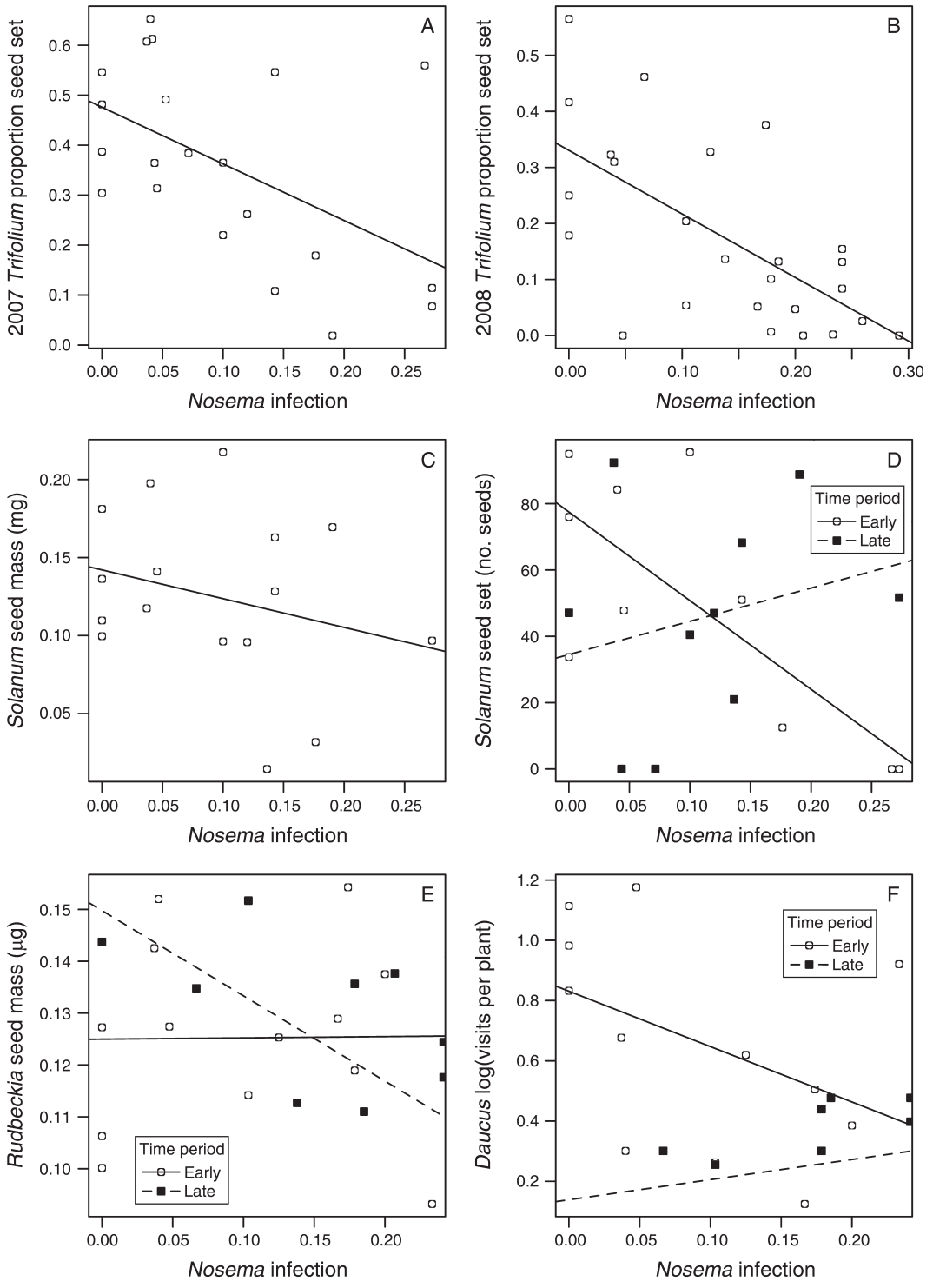

FIG. 2. Relationships between measures of pollination service and *Nosema* parasitism are stronger for plants with high *Bombus* visitation (*Trifolium, Solanum*) than for those with less (*Rudbeckia*, *Daucus*). *Trifolium* proportion seed set is negatively related to *Nosema* parasitism in both (A) 2007 and (B) 2008. (C) *Solanum* seed mass is always negatively related to *Nosema*, whereas (D) *Solanum* seed set is negatively related to *Nosema* early in the season, but not later. (E) *Rudbeckia* seed mass is unrelated to *Nosema* early in the season, but negatively related later, while (F) *Daucus* visitation (log-transformed) declines with *Nosema* parasitism early in the season, but not late. To visualize the nature of interactions between parasitism and date, the data were arbitrarily divided into "early" sampling dates (solid line, open symbols) and "late" sampling dates (dashed lines, closed symbols; C, E, and F). Unstandardized data are presented for ease of interpretation.

PLATE 1. *Bombus impatiens* foraging on greenhouse-grown *Solanum carolinense* in the field. Photo credit: S. D. Gillespie.

season, not later (Fig. 2D). Seed mass also declined with *Crithidia* parasitism.

Rudbeckia.—Pollination service to pollinator-generalist *Rudbeckia* plants was not consistently affected by parasitism (Table 1; Appendix C: Table C4). Parasitism was not related to visitation or to heterospecific or conspecific pollen deposition. Seed set increased with increasing *Crithidia* infection and, although there was no overall relationship between parasitism and seed mass, there were significant *Nosema* × date and conopid × date interactions affecting seed mass. Seed mass of *Rudbeckia* declined with increasing *Nosema* parasitism, but only early in the season, and weakly overall (Fig. 2E). *Rudbeckia* seed mass declined over time at sites with low conopid parasitism, but remained steady over time at higher conopid parasitism.

Daucus.—Parasitism did not consistently affect pollination service to pollinator-generalist *Daucus* plants (Table 1; Appendix C: Table C5).

DISCUSSION

We found a remarkably consistent negative relationship between *Nosema* parasitism and multiple measures of pollination service for two bumble bee-dependent plant species, *Solanum* (used only in 2007) and *Trifolium* (both years; Appendix C: Tables C1 and C2). Conversely, there was no consistent relationship between *Nosema* parasitism and pollination service to *Daucus* and *Rudbeckia*, although there was a significant *Nosema* × date interaction for *Rudbeckia* seed mass (Appendix C: Table C4). *Daucus* did not set any seed, possibly due to insufficient pollen deposition in a single day in the field. Thus we cannot say whether *Nosema* parasitism would be related to *Daucus* seed set; however, it is clear that it had no negative relationship with visitation or pollen deposition. Overall, this pattern supports our hypothesis that bumble bee-dependent plant species will experience negative indirect effects of parasites, and provides the first evidence that parasites of bumble bees may have top-down effects on pollination ecosystem services.

Our results follow expectations based on multitrophic effects in traditional predator–herbivore–plant systems. In general, top-down effects should be weaker where food webs are more complex than simple three-level food chains. For example, both omnivory and mutualisms between lower trophic levels are predicted to reduce positive indirect effects of predators on primary producers (Polis and Strong 1996, Knight et al. 2006). This occurs because these secondary trophic links are a source of negative effects on producers that counteract the positive impacts the predator has in reducing herbivory. Indeed, it has been argued that low-diversity food webs are required for true trophic cascades to occur at all (Strong 1992), and theoretical research shows that strong links between trophic levels lead to stronger top-down effects (Herendeen 2004). Similarly, in indirect effects involving pollination mutualisms, plants such as *Rudbeckia* or *Daucus* with generalized pollination systems may be able to compensate for reductions in one group of mutualists by receiving increased visitation from others. By contrast, plants such as *Solanum,* which rely primarily on bumble bees, are more vulnerable. Overall, our results suggest that, much as in trophic cascades, pollination network complexity will reduce the magnitude of top-down effects of antagonists on mutualisms.

In contrast to *Nosema*, measures of pollination for bumble bee-dependent and independent plants showed a mix of positive and negative relationships with *Crithidia* and conopid parasitism (Table 1; Appendix C). *Crithidia* is not considered to be a virulent pathogen, and has documented negative effects on host colony fitness only when its host is stressed (Brown et al. 2000, Logan et al. 2005). Similarly, although conopids kill their host within about 10 days of parasitism, during this time the bumble bee continues to forage and contribute to the colony (Schmid-Hempel 1998, Gillespie 2010). Given that a worker's average foraging life span is only ~14 days (Kearns and Thomson 2001), this may not represent a great reduction in the worker's contribution to either colony fitness or pollination activity. Unlike pathogens that spread within a colony, the effects of a conopid parasitoid are isolated to a single bee, and may have minimal impacts on pollinator abundance and thus pollination service to flowers. By contrast, *Nosema* is highly virulent, and transmissible within a colony (Rutrecht and Brown 2009), so we expect to see stronger effects of this pathogen on pollination service.

Because our data are observational, it is important to consider whether confounding variables related to parasitism may impact pollination service. For example, high host density is frequently related to high levels of parasitism (Costamagna et al. 2004), and a positive correlation between seed set and parasitism could derive from higher parasitism levels at sites with higher bumble bee host density and, thus, higher pollinator visitation. Although there was no correlation between our measure of bumble bee abundance and any parasite, productive sites with more non-*Bombus* pollinators could also have higher bumble bee parasitism if, for example, highly productive sites have more resources for adult conopids, or host more vigorous bumble bee colonies that can maintain high *Crithidia* parasite loads. This may explain the positive relationships between *Crithidia* parasitism and seed set in *Rudbeckia*. Bumble bees were not common visitors of these plants, but highly productive sites may have higher *Crithidia* parasitism and higher overall pollinator abundance, thus leading to a positive correlation between parasitism and seed set in this species. However, such a pattern would not explain the negative relationships between *Trifolium* and *Solanum* pollination service and *Nosema* parasitism. Overall, the inconsistent patterns found for *Crithidia* and conopids may be due to confounding variables with differing impacts on pollination of different plant species. However, none of these alternate hypotheses would explain the consistent negative relationship between *Nosema* parasitism and reproduction in species pollinated by bumble bees, lending support to the hypothesis that trophic impacts of parasites are the underlying mechanism.

The concept of the trophic cascade was first formulated to describe predator effects on lower trophic levels via reductions in the population of their prey (density-mediated indirect interactions, DMII; Abrams 1995). Due to its potential role in bumble bee species decline in North America, *Nosema* seems likely to have strong negative impacts on bumble bee populations (Goulson et al. 2008, Cameron et al. 2011). Such negative effects on bumble bee abundance could then cascade down to bumble bee-pollinated plants. However, our results show no relationship between *Nosema* infection and bumble bee abundance as measured by transects on our sampling dates. Alternately, *Nosema* may have differential impacts on the abundance of specific bumble bee species without changing overall bumble bee abundance. Species-level variation in susceptibility to both *Crithidia bombi* and *Nosema bombi* has been documented in Europe (Brown et al. 2000). Such bumble bee species-specific susceptibility could change the composition of the bumble bee community under high levels of parasitism. This could result in a correlation between parasitism rates and pollination service if bumble bee species vary in their effectiveness as pollinators for different plants. Our limited sampling days at each site do not provide sufficient data to draw any conclusions about these effects. However, given ongoing concern about the role of parasites in bumble bee species decline (Colla et al. 2006, Goulson et al. 2008), the differential susceptibilities of bumble bee species to parasites is a key subject for further investigation.

Prey behavior, such as predator avoidance, frequently plays a role in multitrophic impacts of predators (trait-mediated indirect effects, TMII; Werner and Peacor 2003, Schmitz et al. 2004). Like predators, parasites and pathogens could have indirect effects on lower trophic levels via behaviorally mediated indirect effects on their host. Evidence suggests that bee behavior may play a role in the indirect effects that we documented. In our analysis of *Nosema* and *Trifolium* pollination in 2008, we found that *Nosema* was negatively related to visitation, but was also negatively related to pollen deposition and seed set after controlling for visitation (Appendix C: Table C2). This suggests that behavioral impacts may reduce both the number of bees visiting a plant and the quality of pollination service those bees provide. Changes in pollinator behavior may explain the less intuitive result that high *Nosema* infection was negatively associated with seed mass in *Solanum*. For example, reduced seed size can be a consequence of inbreeding depression (e.g., Vaughton and Ramsey 1997), which could be affected by *Nosema* infection if infected bees tend to have shorter flight distances and thus transfer pollen between more closely related plants. However, little is known about behavioral impacts of *Nosema* infection, so the role of TMII in the patterns documented here remains to be determined. Because pollination benefits to plants are frequently driven by pollinator behavioral decisions (e.g., host fidelity), it seems likely that TMII will be important in pollination systems.

Our research examines mutualisms in the context of multitrophic effects, merging a key ecological interac-

tion with a major concept in community ecology to gain insight into the role of mutualisms in a community context. Our results suggest that food web complexity in the form of generalized pollination syndromes attenuates top-down effects of pathogens and parasites. Overall, we show that parasites and antagonists of bumble bees, an important pollinator group, may have top-down effects on the ecosystem services that these organisms provide.

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Supplemental Material

Appendix A

Field site descriptions, sampling dates, and ratio of female workers to males caught on each date (F:M) (*Ecological Archives* E094-037-A1).

Appendix B

Detailed description of methods for floral community sampling, greenhouse rearing of plants, and PCR (*Ecological Archives* E094-037-A2).

Appendix C

Detailed statistical tables for analyses of parasite effects on plant reproduction (*Ecological Archives* E094-037-A3).