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## Abstract

The microsporidian *Nosema pyrausta* Paillot is a common and widespread pathogen of European corn borer, *Ostrinia nubilalis* (Hübner), in North America and Europe. *Nosema* negatively affects European corn borer longevity and fecundity. In this study, we used flight mills to examine the effects of *Nosema* infection on flight activity of unmated males and females at 1 and 3 days after pupal eclosion, taking the level of infection, as measured by number of *Nosema* spores per mg of tissue, into consideration. *Nosema* infection had a significant negative effect on distance, duration and speed of the longest uninterrupted flight, as well as on total distance and duration of flight of 1-day-old males, but not of 3-day-old males or females of either age. However, when insects with a light infection (<15 spores/mg) were pooled with uninfected moths, significant negative effects of a moderate/heavy infection ( $\geq 15$  spores/mg) were observed for most flight parameters in 1-day-old, but not 3-day-old, moths of both sexes. The magnitude of reduction was often substantial: e.g., distance and duration were sevenfold, and 3.5-fold less, respectively, in 1-day-old females with a moderate/heavy infection. Flight distance and duration were significantly negatively correlated with level of *Nosema* infection in 1-day-old, but not 3-day-old, moths of both sexes. The percentage of moths of either sex or age with a moderate/heavy *Nosema* infection flying given distances was less than that of lightly infected (<15 spores/mg) moths. Among uninfected adults, forewing length was positively correlated with several measures of flight performance in males, but not females. However, some performance measures were correlated with wing length in females when infected with *Nosema*. *Nosema* infection was associated with reduced adult weight, but not forewing length, suggesting that negative effects of *Nosema* infection on flight performance are related to reduced energy reserves rather than smaller body size *per se*.

## Keywords

*Ostrinia nubilalis*, dispersal, flight mill, microsporidia, wing length

## Disciplines

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## Abstract

The microsporidian *Nosema pyrausta* Paillot is a common and widespread pathogen of European corn borer, *Ostrinia nubilalis* (Hübner), in North America and Europe. *Nosema* negatively affects European corn borer longevity and fecundity. In this study, we used flight mills to examine the effects of *Nosema* infection on flight activity of unmated males and females at 1 and 3 days after pupal eclosion, taking the level of infection, as measured by number of *Nosema* spores per mg of tissue, into consideration. *Nosema* infection had a significant negative effect on distance, duration and speed of the longest uninterrupted flight, as well as on total distance and duration of flight of 1-day-old males, but not of 3-day-old males or females of either age. However, when insects with a light infection (<15 spores/mg) were pooled with uninfected moths, significant negative effects of a moderate/heavy infection (≥15 spores/mg) were observed for most flight parameters in 1-day-old, but not 3-day-old, moths of both sexes. The magnitude of reduction was often substantial: e.g., distance and duration were sevenfold, and 3.5-fold less, respectively, in 1-day-old females with a moderate/heavy infection. Flight distance and duration were significantly negatively correlated with level of *Nosema* infection in 1-day-old, but not 3-day-old, moths of both sexes. The percentage of moths of either sex or age with a moderate/heavy *Nosema* infection flying given distances was less than that of lightly infected (<15 spores/mg) moths. Among uninfected adults, forewing length was positively correlated with several measures of flight performance in males, but not females. However, some performance measures were correlated with wing length in females when infected with *Nosema*. *Nosema* infection was associated with reduced adult weight, but not forewing length, suggesting that negative effects of *Nosema* infection on flight performance are related to reduced energy reserves rather than smaller body size *per se*.

## Introduction

The European corn borer, *Ostrinia nubilalis* (Lepidoptera: Crambidae), is a major pest of corn, *Zea mays* L., in North America, Europe and Asia. Before the introduction of transgenic Bt corn targeting lepidopteran pests, the European corn borer was responsible

for over \$1 billion in damage and control measures annually in the U.S. alone (Mason et al. 1996). Management of this insect historically has been through combinations of cultural, host-plant resistance, and chemical methods (Mason et al. 1996; Rice and Ostlie 1997; Pilcher and Rice 2001). In addition, the microsporidian intracellular parasite

*Nosema pyrausta* Paillot (Microsporidia: Nosematidae) has played an important role in regulating natural European corn borer populations in both Europe and North America (Lewis et al. 2009).

Even though Bt corn hybrids now offer the grower essentially complete protection against the European corn borer, there are some concerns that arise from the increasing reliance on this single management tactic in North America. One concern is that as adoption of Bt corn increases, so too does selection pressure on the insect to evolve resistance (Gould 1998; Ferré and Van Rie 2002; Bates et al. 2005; Siegfried et al. 2007; Tabashnik et al. 2008). Apprehension of widespread failure of Bt corn due to development of insect resistance is substantial enough that growers in the U.S. are required by law to plant a percentage of their corn hectares to non-Bt hybrids to serve as a refuge (Bourguet et al. 2005; EPA 2006; Sivasupramaniam et al. 2007). The refuge functions as a nursery for production of susceptible adults to mate with any resistant adults that may emerge from the Bt corn so that, given the high-dose of the Bt toxin in the plant and the resulting functional recessiveness of the resistance trait (Tabashnik et al. 2004; Alves et al. 2007; Crespo et al. 2009), all offspring will be susceptible. This strategy is credited in part with the continued efficacy of Bt corn against the European corn borer in North America (Tabashnik et al. 2003, 2008; Gassmann et al. 2008).

Another concern is potential unintended effects of Bt corn on non-target organisms, including natural enemies (Pilcher et al. 1997; Andow and Zwahlen 2006; Lundgren et al. 2009). Although there are some minor negative effects on some non-target insects (Lövei et al. 2009), extensive studies are in broad agreement that acute negative effects of Bt corn expressing Cry1Ab on non-targets are rare or absent (Pilcher et al. 1997; Daly and Buntin 2005; Naranjo et al. 2005). Most reported reductions in natural enemy populations are apparently indirect, and attributable to the reduction in the target host (pest) population (Naranjo et al. 2005; Pilcher et al. 2005). Although some research has examined the effects of entomopathogens on pests in transgenic crops (Reardon et al. 2004; Raymond et al. 2006), little is known about the indirect effects of host population decline. There is growing evidence that European corn borer populations are being suppressed by Bt corn at a regional scale in the U.S. Corn Belt (Steffey and Gray 2008), although definitive studies have not yet been published. Because it is an obligate parasite of European corn borer, the

potential for a concomitant and more severe reduction in *N. pyrausta* prevalence must be considered (Pierce et al. 2001; Sisterson and Tabashnik 2005; Lundgren et al. 2009). Maintenance of *N. pyrausta* in the environment is important to growers who choose not to plant Bt hybrids, and will be important in quickly re-establishing its role as a regulator of European corn borer populations if this insect develops resistance to Bt corn. Furthermore, *Nosema* infection increases susceptibility of European corn borer to the Bt toxin Cry1Ab (Reardon et al. 2004), which could help slow the rate of resistance evolution (Carrière and Tabashnik 2001; Lundgren et al. 2009). Consequently, changes in pathogen density caused by changes in pest density affect the influence of natural enemies on the evolution of resistance (Lundgren et al. 2009). Lewis et al. (2009) concluded from evidence in the literature that *N. pyrausta* can persist at a low level in the presence of even modest populations of European corn borer. Nevertheless, it is unknown how low European corn borer populations will be driven by maximum adoption of Bt corn, and whether those populations will be large enough to maintain *Nosema*.

European corn borer flight activity and consequent dispersal impact both of these concerns, i.e. development of resistance (Caprio and Tabashnik 1992; Roderick 1996; Andow and Ives 2002; Sisterson et al. 2004) and reduction in pathogen prevalence (Chen and Feng 2006). European corn borers can be infected by *N. pyrausta* both horizontally through ingestion of spores and vertically from mother to offspring, and both contribute to maintenance of the pathogen in a population (Lewis et al. 2006). Because infection is not spread from males to females during copulation (Lopez 2008) and mobility of larvae is spatially limited (Caffrey and Worthley 1927; Ross and Ostlie 1990), geographic dispersal of *N. pyrausta* and introduction to uninfected European corn borer populations depends on dispersal of infected female adults followed by oviposition of infected eggs.

Long-distance dispersal and gene flow over perhaps >100 km per generation seems to be normal for European corn borer (Bourguet et al. 2000; Krumm et al. 2008; Kim et al. 2009), but nothing is known about sublethal effects of *N. pyrausta* infection on flight behaviour. Pathogens and parasites often reduce flight performance of the insect host (Schiefer et al. 1977; Seyoum et al. 1994; Bradley and Altizer 2005; Overton et al. 2006; Schilder and Marden 2007), but not always (Akbulut and Linit 1999; Chen and Feng 2006), and may even increase

flight activity (Berry et al. 1988; Mayack and Naug 2009). Furthermore, parasitization may reduce adult size or alter wing morphology, and thus exert an indirect effect on flight performance (Villacide and Corley 2008). Female spruce budworms, *Choristoneura fumiferana* (Clemens) (Lepidoptera: Tortricidae), suffering a moderate-heavy infection by *Nosema fumiferanae* (Thompson), weighed less and had shorter wings than uninfected females (Eveleigh et al. 2007). Infection reduced emigration by females, but did not affect male tendency to emigrate (Eveleigh et al. 2007) or flight duration in a wind tunnel (Sanders and Wilson 1990). If European corn borer flight behaviour is negatively affected by *N. pyrausta* infection, infected Bt-resistant moths emerging from the same natal field may be more likely to mate with one another. Likewise, infected susceptible moths not dispersing from the refuge may be less likely to encounter resistant moths. Under either scenario, even a small percentage of moths failing to disperse because of *N. pyrausta* infection could potentially accelerate the rate of resistance evolution.

In a previous study of flight performance of uninfected European corn borers on flight mills, we found differences that depended on age and sex, with the most pronounced difference between 1-day-old females and males (Dorhout et al. 2008). Although flight capacity of moths was substantial at any age up to 5-day-old, unmated 1-day-old females were most likely to engage in long uninterrupted flights, apparently reflecting an obligate migratory behaviour. In this study, we used flight mills to compare flight performance of 1- and 3-day-old male and female European corn borers uninfected or infected with *N. pyrausta*. We also examined the effects of *N. pyrausta* infection on adult weight and forewing length, as well as the relationship of forewing length to flight performance.

## Materials and Methods

### Insect culture

The adult weight portion of the study was conducted in 1967 using a European corn borer laboratory colony initiated, reared, and infected with *N. pyrausta* as described by Lewis et al. (1971). Weight of adults from parallel infected and uninfected colonies was determined to the nearest 0.1 mg on a Roller-Smith balance (Newark, NJ) for moths aged 1, 2 and 3 day after eclosion from the pupa. The flight mill portion of the study was conducted in 2006 and 2007 using adults obtained from a laboratory colony maintained

at the USDA-Agricultural Research Service, Corn Insects and Crop Genetics Research Unit, Ames, IA, under conditions similar to the previous colony. The recent colony was established from wild females captured in central Iowa in 2005 using methodology described by Saleh et al. (1995). The first two generations were heat treated in a water bath at 43.3°C for 30 min to eliminate any microsporidial infection (Raun 1961). Subsequently, egg masses were collected separately from caged females paired with males. After death, females were checked for *Nosema* spores, and offspring of any that were infected were discarded. Males were not inspected because sexual transmission of *N. pyrausta* does not occur (Lopez 2008). In addition, five larvae from each family were examined for spores, but none were infected. Offspring from 50 uninfected females were reared to adulthood and used to produce the next generation via mass matings.

From this uninfected colony, an infected colony was initiated by exposing 3–4 day old larvae from 40–50 egg masses to *N. pyrausta* spores in each of four 25-cm diameter rearing dishes. Spores were obtained as described by Reardon et al. (2004). Three to four days after hatch, 15 ml of a  $1 \times 10^6$  spores/ml solution were applied to the surface of meridic wheat germ diet, but without the microsporidial growth inhibitor Fumidil B, as described by Lewis and Lynch (1969).

To prevent mating before flight assays, pupae were placed individually in small jelly cups and checked twice daily for emergence. Emerged adults were held in cages by age cohort and sex with access to drinking water as described by Dorhout et al. (2008). Moths were classified as 1-day-old if they emerged the previous night or early on the morning before flight.

### Flight behaviour

Flight behaviour of *Nosema*-infected and uninfected moths was characterized using round-about flight mills (Clarke et al. 1984; Coats et al. 1986; Dorhout et al. 2008). Each adult was attached to one of 16 flight mills with an inverted U-shaped disposable harness similar to those developed by Luo et al. (2002) and described in detail by Dorhout et al. (2008). The moths were sedated for about 30 s with ether during the process of gluing the harness to the dorsal anterior-most part of the abdomen. The flight mills were housed together on two banks of shelving along three walls in a single environmental chamber maintained at 24°C, 16 : 8 (L : D) and 80% RH.

Moths were attached to the mills 3–4 h before dark. Flight before lights were turned off was minimal, and only flight activity occurring during the 8 h of darkness was analyzed (Dorhout et al. 2008). Rotation of the flight arm (1-m circumference) was detected by an infrared eye and relayed to a computer, where software (Beerwinkle et al. 1995) tracked time and revolutions per minute for each mill. Designation of a discrete or uninterrupted flight was attributed to blocks of time with at least some flight activity in each consecutive 1-min interval.

## Experiments

Moths from the parallel *Nosema*-infected and uninfected colonies were tested in mixed groups of age and infection status based on daily availability, except that only one sex was flown per night to avoid potential confounding effects of pheromone. The lack of pheromone contamination was confirmed by Kruskal–Wallis tests comparing flight performance variables of males flown the night after females were tested vs. those of males flown more than one night after females were tested, and there were no significant differences (data not shown). After flight, abdomens posterior to the harness attachment point were removed and stored at  $-20^{\circ}\text{C}$  until processing for *Nosema* spore counts. All moths were inspected for spores regardless of colony because it was possible that some from the uninfected colony might be infected and *vice versa*. The abdomens were weighed and homogenized, and spores counted using a hemacytometer at  $400\times$  magnification. Spore counts are expressed as spores per mg tissue (Raun et al. 1960). The forewings were removed and mounted on card stock, and images obtained by optical scanning. Length of both forewings was obtained as the major axis length using the web-based UTHSCSA ImageTool (<http://ddsdx.uthscsa.edu/Imagetool.asp>). Because there could be slight differences in the point at which the wing was detached from the body, the longest measure obtained from either of the two wings was used in all analyses.

## Data analysis

Data from moths that were found dead on the flight mills the morning after testing were discarded, because there was no way to determine when they died, and because their behaviour prior to death was unlikely to be comparable to that of moths that were vigorous enough to survive through the night. Mea-

asures of flight behaviour included duration and distance of the longest uninterrupted flight, total cumulative duration and distance over the night, average speed during the longest-duration uninterrupted flight and total number of flights during the night. Shapiro–Wilk normality tests (Royston 1995) indicated the data for all of these parameters were not normally distributed, so the non-parametric Kruskal–Wallis test (Daniel 1990) was employed, using Statistix 7 software (Analytical Software 2000). Differences were considered significant at  $\alpha = 0.05$  in one-tailed tests, under the expectation that an effect of *Nosema* infection would be in the direction of reduced flight performance. An exception is the number of flights per night, which was conducted as a two-tailed test because infection could be reasonably expected to have either positive or negative effects on this parameter. Because distributions of the data for flight parameters are all severely skewed, medians are presented rather than means. Effects of infection intensity, as measured by spores per mg tissue, on flight parameters, weight, and forewing length were assessed by calculating Pearson correlation coefficients (Analytical Software 2000). Variation about the median for adult weight and forewing length are visualized with box and whisker plots, where the hinges (or upper and lower bounds of the box) encompass the upper and lower quartiles, whiskers terminate at the most extreme datum point within  $1.5\times$  of the inner spread (i.e., length of the box), and data points outside this range are presented individually as potential outliers, as described by Velleman and Hoaglin (1981) and as implemented by Statistix 7 software (Analytical Software 2000).

## Results

### *Nosema* infection and flight performance

Distance, duration, and speed of the longest uninterrupted flight, as well as total distance and duration of flight of 1-day-old male European corn borers were all negatively affected by *Nosema* infection (table 1). Total duration of flight of infected 1-day-old males was roughly half that of uninfected 1-day-old males, while total distance flown was fivefold less, related in part to a 35% reduction in flight speed. In contrast, none of the flight parameters for 3-day-old males, or 1- or 3-day-old females was significantly affected by *Nosema* infection.

However, when insects with a light infection (arbitrarily defined as moths infected with  $<15$

**Table 1** Median performance of unmated European corn borer adults uninfected vs. infected with *Nosema pyrausta*, or with no/low infection (<15 spores/mg tissue) vs. moderate/high infection (≥15 spores/mg tissue), at indicated ages on laboratory flight mills during 8 h of darkness

Sex	Age (day)	<i>Nosema</i> infection status	n	Distance (m) of longest uninterrupted flight	Total distance (m) of all flights	Duration (min) of longest uninterrupted flight	Total duration (min) of all flights	Speed (m/s) of longest uninterrupted flight	Total number of flights
M	1	Uninfected	23	842	5370	38.0	218.0	1.23	19
		Infected	35	254*	1048*	15.0*	100.0*	0.80*	19
		<15 spores	38	787	2691	41.5	204.0	0.94	19
		≥15 spores	20	188**	623**	14.0*	87.5**	0.81*	18
	3	Uninfected	11	562	4029	32.0	244.0	1.09	27
		Infected	18	204	841	15.0	120.5	0.58	27
		<15 spores	17	452	1273	31.0	218.0	0.85	27
		≥15 spores	12	249	841	15.0	120.5	0.58	25
F	1	Uninfected	25	2840	4538	73.0	173.0	2.04	7
		Infected	29	1152	6769	50.0	248.0	1.38	14
		<15 spores	39	2840	7486	76.0	248.0	1.78	7
		≥15 spores	15	425*	1114*	21.0*	162.0	1.21**	15*
	3	Uninfected	15	976	4554	45.0	246.0	1.22	35
		Infected	36	1298	3392	47.5	233.5	1.26	18
		<15 spores	37	1554	4554	58.0	239.0	1.38	20
		≥15 spores	14	762	2379	21.5	195.0	0.97	22

\*Significant difference ( $P \leq 0.05$ ) between moths of specified infection status within a given sex and age.

\*\* $P \leq 0.01$ ; \*\*\* $P \leq 0.001$ .

Mann–Whitney U-test, all one-tailed except total number of flights.

spores/mg tissue) were pooled with those with no infection, some significant negative affects of *Nosema* on flight performance were observed in 1-day-old, but not 3-day-old, moths of both sexes (table 1). The magnitude of effects on 1-day-old males were similar to those comparing simply infected and uninfected. In the case of 1-day-old females, those with an infection of ≥15 spores/mg flew maximum and total distances nearly sevenfold less than those with no or light infection (table 1). Duration of the longest uninterrupted flight was about 3.5-fold less in moths with ≥15 spores/mg, but the reduction in total time spent in flight during the night was not significant. Flight speed was reduced by 32% in 1-day-old

females infected with ≥15 spores/mg, but the total number of flights taken during the night was double that of uninfected and lightly infected moths. Trends among parameters for 3-day-old females were in the same direction but were not significant.

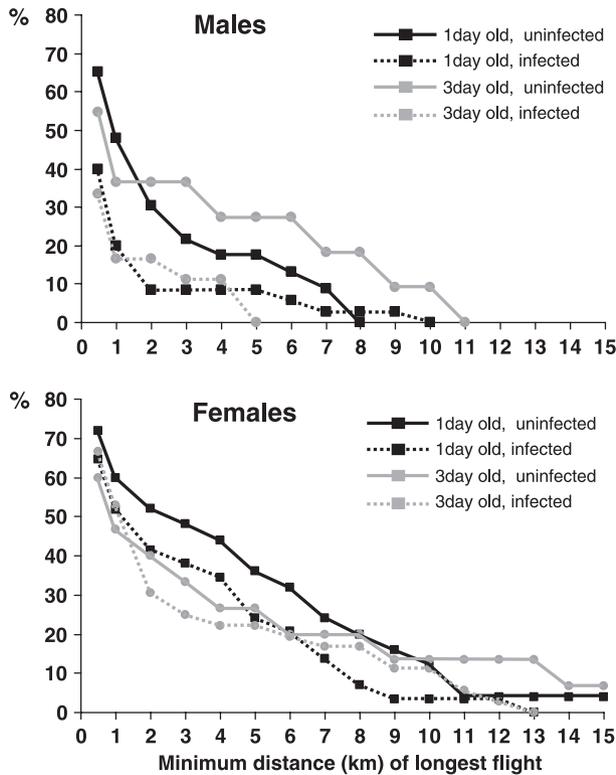
Measures of flight distance and duration were significantly negatively correlated with level of *Nosema* infection, measured by spores per mg tissue, for 1-day-old males and females (table 2). However, there were no significant correlations between flight parameters and level of infection for 3-day-old moths of either sex.

*Nosema* infected males were less likely to make a maximum continuous flight of given duration than

**Table 2** Pearson correlation coefficients of *Nosema pyrausta* spore count (spores/mg tissue) to indicated flight parameters measured during 8 h of darkness on laboratory flight mills for unmated *Nosema*-infected European corn borer males and females at indicated ages

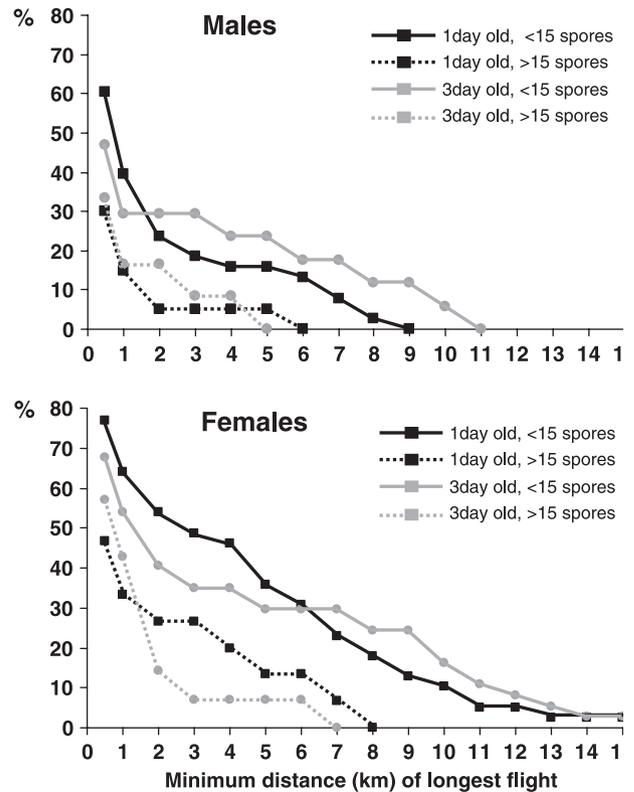
Sex	Age (day)	n	Distance of longest uninterrupted flight	Total distance of all flights	Duration of longest uninterrupted flight	Total duration of all flights	Speed of longest uninterrupted flight
M	1	35	-0.35*	-0.44**	-0.42*	-0.48**	-0.30
	3	18	0.20	-0.05	0.52*	0.01	0.04
F	1	29	-0.37*	-0.47*	-0.39*	-0.46*	-0.33
	3	36	-0.18	-0.06	-0.17	0.06	0.06

\* $P \leq 0.05$ , \*\* $P \leq 0.01$ .



**Fig. 1** Decumulation curves showing the percentage of unemated male and female European corn borers of different ages and *Nosema* infection status engaging in flight activity resulting in indicated minimum distance of the longest uninterrupted flight on flight mills during 8 h of darkness. Solid lines indicate uninfected moths, dashed lines indicate infected moths.

uninfected males, with the most pronounced differences observed in 3-day-old males and in the percentage of 1-day-old males flying at least 1 or 2 h (fig. 1). For example, almost 30% of 3-day-old uninfected males made a continuous flight of at least 5 km, whereas no infected 3-day-old males made a flight of that distance. Differences among infected or uninfected females of either age were small (fig. 1). Differences were more pronounced for females of both ages when moths with a light infection (<15 spores/mg) were pooled with uninfected moths and compared to those with infections of  $\geq 15$  spores/mg tissue (fig. 2), suggesting that females are more tolerant of light infections than males in terms of flight performance. No males of either age with  $\geq 15$  spores/mg made a continuous flight of 6 km, whereas among those with <15 spores/mg, 13% of 1-day-old males made a flight of at least 6 km and 18% of 3-day-old males made a flight of at least 7 km. Females tended to fly farther than males in general, but the differences related to *Nosema* infec-



**Fig. 2** Decumulation curves showing the percentage of unemated male and female European corn borers of different ages and *Nosema* infection status engaging in flight activity resulting in indicated minimum distance of the longest uninterrupted flight on flight mills during 8 h of darkness. Solid lines indicate uninfected or lightly infected (<15 spores/mg) moths, dashed lines indicate moderately to heavily infected ( $\geq 15$  spores/mg) moths.

tion in the former were just as pronounced as in males (fig. 2). In 1-day-old non- or lightly infected females, 77% made a flight of at least 0.5 km, compared to only 47% of those with  $\geq 15$  spores. No 1-day-old females with  $\geq 15$  spores made a flight of at least 8 km, compared to 18% of those with <15 spores. Similar percentages of 3-day-old females at the two levels of infection flew at least 1 km, but only 14% with  $\geq 15$  spores made a flight of at least 2 km while 41% of those with <15 spores made a flight of at least 2 km. No 3-day-old females with  $\geq 15$  spores made a flight as long as 7 km, compared to 30% of those with <15 spores which flew at least that far.

#### Forewing length and flight performance

The forewing length of uninfected 1-day-old males was significantly positively correlated with the distance and speed of the longest uninterrupted flight,

**Table 3** Pearson correlation coefficients of forewing length to indicated flight parameters measured during 8 h of darkness on laboratory flight mills for unmated European corn borer adults uninfected or infected with *Nosema pyrausta*, or with no/low infection (<15 spores/mg tissue) vs. moderate/high infection ( $\geq 15$  spores/mg tissue), at indicated ages

Sex	Age (day)	<i>Nosema</i> infection status	n	Distance of longest uninterrupted flight	Total distance of all flights	Duration of longest uninterrupted flight	Total duration of all flights	Speed of longest uninterrupted flight
M	1	Uninfected	18	0.49*	0.57*	0.45	0.49*	0.62**
		Infected	32	0.63***	0.56***	0.68***	0.48**	0.48**
		<15 spores	32	0.56***	0.60***	0.53**	0.53**	0.60***
		$\geq 15$ spores	18	0.62**	0.45	0.64**	0.37	0.39
	3	Uninfected	11	0.32	0.51	0.43	0.69*	0.16
		Infected	18	0.29	0.29	0.18	0.12	0.22
		<15 spores	17	0.40	0.55*	0.44	0.58*	0.32
		$\geq 15$ spores	12	0.02	0.10	-0.01	0.12	-0.03
F	1	Uninfected	19	0.33	0.39	0.39	0.41	-0.03
		Infected	18	0.36	0.44	0.32	0.50*	0.30
		<15 spores	25	0.28	0.27	0.34	0.31	-0.12
		$\geq 15$ spores	12	0.53	0.62*	0.51	0.61*	0.43
	3	Uninfected	15	0.32	0.38	0.36	0.38	0.42
		Infected	36	0.22	0.36*	0.22	0.39*	0.41*
		<15 spores	37	0.37*	0.41*	0.42**	0.48**	0.37*
		$\geq 15$ spores	14	0.14	0.49	0.05	0.38	0.46

\* $P \leq 0.05$ , \*\* $P \leq 0.01$ , \*\*\* $P \leq 0.001$ .

as well as with total distance and duration of flights (table 3). Only total duration of flights was significantly correlated with forewing length in 3-day-old uninfected males, and no flight parameters were correlated with forewing length for 1- or 3-day-old uninfected females. When data from uninfected moths were pooled with those of moths lightly infected with *Nosema* (<15 spores/mg tissue), all flight parameters of uninfected males of both ages were significantly correlated with forewing length (table 3). In the case of 1-day-old females with <15 spores/mg, no correlations were significant, but all flight parameters were significantly positively correlated with forewing length among 3-day-old females with <15 spores/mg.

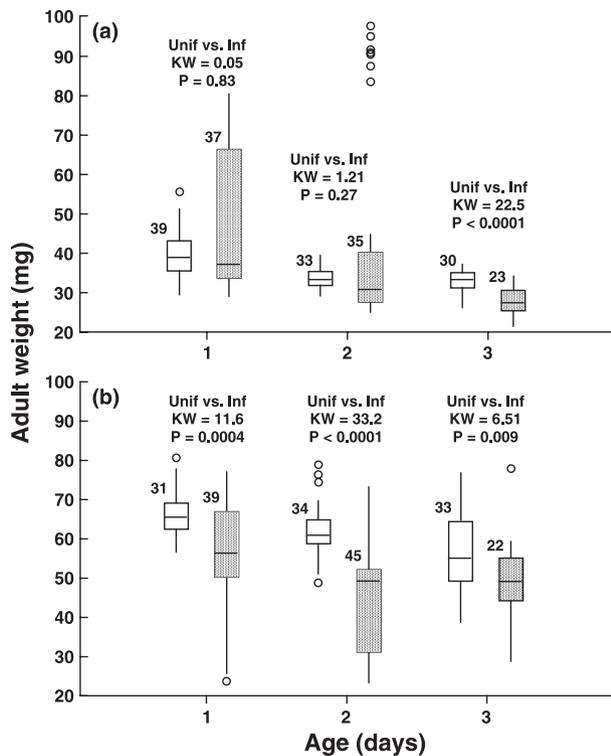
In 1-day-old males infected with *Nosema*, all flight parameters tested were significantly positively correlated with forewing length, but none were significantly correlated with forewing length in 3-day-old infected males (table 3). When the data were regrouped as moderate + high infection ( $\geq 15$  spores/mg), only distance and duration of the longest uninterrupted flight were significantly correlated with forewing length in 1-day-old males, and no correlations were significant in 3-day-old males. In females, only total duration of all flights in infected 1-day-olds were significantly correlated with forewing length, but total distance, total duration, and speed

of the longest uninterrupted flight in 3-day-olds were significantly correlated with forewing length. When regrouped as moderate + high infection, total distance and duration of flight in 1-day-old females were correlated with forewing length, while none of the correlations were significant for 3-day-old females.

#### *Nosema* infection and adult size

Weights of 1- and 2-day-old males infected with *Nosema* were not significantly different than those of uninfected males, but by the third day infected males weighed significantly less ( $P < 0.05$ ) (fig. 3a). Females weighed significantly more than males within each age and infection category (fig. 3b). Infected females weighed less than uninfected females at all ages tested. Greater variability in weights among infected 1 to 2-day-old males and females than among their uninfected counterparts was striking (fig. 3). Adult weight was significantly negatively correlated with intensity of infection in 1- and 2-day-old males and 2- and 3-day-old females (table 4).

Because spore count did not differ significantly between 1- and 3-day-old females (data not shown) and because wing morphology does not change with age, the relationship between forewing length and



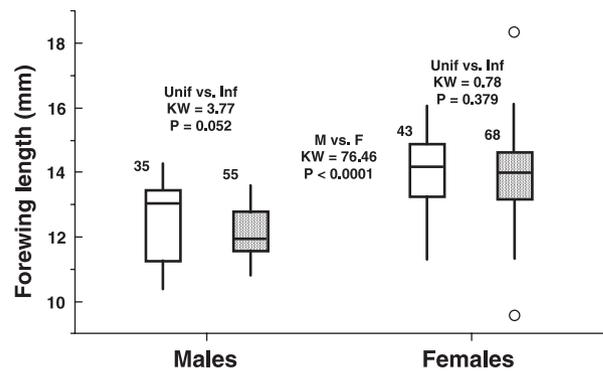
**Fig. 3** Box and whisker plots (Analytical Software 2000) for the weight (mg) of male (a) and female (b) European corn borers of different ages uninfected (open box) or infected (shaded box) with *Nosema pyrausta*. Box encompasses middle half of data and is bisected by the median. Vertical lines (whiskers) indicate data within 1.5× the length of the box, and circles indicate potential outliers outside that range (Velleman and Hoaglin 1981). Numbers at top of each box indicate sample size.

**Table 4** Pearson correlation coefficients (*r*) of *Nosema pyrausta* spore count with dry weight of male and female adult European corn borers at indicated ages, and with forewing length of males and females of combined ages. Weight and forewing length data sets were taken from different moths

Sex	Dry weight			Forewing length		
	Age (day)	n	r	Age (day)	n	r
M	1	37	-0.56***	1 + 3	55	-0.09
	2	35	-0.46**			
	3	23	-0.01			
F	1	39	-0.11	1 + 3	68	-0.02
	2	45	-0.47**			
	3	22	-0.52*			

\**P* ≤ 0.05, \*\**P* ≤ 0.01, \*\*\**P* ≤ 0.001.

infection status was compared within sexes with ages combined. Forewing length of females was significantly greater than that of males, but did not



**Fig. 4** Box and whisker plots (Analytical Software 2000) of forewing length (mm) of male and female European corn borers uninfected (open box) or infected (shaded box) with *Nosema pyrausta*. Box encompasses middle half of data and is bisected by the median. Vertical lines (whiskers) indicate data within 1.5× the length of the box, and circles indicate potential outliers outside that range (Velleman and Hoaglin 1981). Numbers at top of each box indicate sample size. Infection status was combined within sex for Kruskal–Wallis test between males and females.

differ significantly between *Nosema*-infected or uninfected moths within either sex (fig. 4).

### Discussion

Infection by *Nosema pyrausta* negatively affected various measures of flight performance of European corn borer adults depending on sex, age and intensity of infection. The effects were most pronounced in 1-day-old males and when infections were ≥15 spores/mg tissue in both sexes at 1-day of age. The total number of independent flights per night were similar between infected and uninfected moths, but the flights were slower and ended sooner for the former. The consequence was that infected 1-day-old moths covered 4–6 fold less total distance than uninfected moths. Monarch, *Danaus plexippus* (L.) (Lepidoptera: Nymphalidae), butterflies parasitized by the protozoan *Ophryocystis elektroscirrha* McLaughlin & Myers likewise flew for shorter times, shorter distances, and at slower speeds on flight mills than unparasitized butterflies (Bradley and Altizer 2005). In flight mill studies of male obliquebanded leafrollers, *Choristoneura rosaceana* (Harris) (Lepidoptera: Tortricidae), Gardiner (1990) found that moths infected with *Nosema fumiferanae* flew at slower speeds than uninfected moths. However, the reductions of 14–19% observed in these studies were modest compared to the 65–85% reductions reported here for *Nosema*-infected European corn borers. Results more similar to ours were observed for female woodwasps,

*Sirex noctilio* Fabricius (Hymenoptera: Siricidae), in that those infected with the nematode *Beddingia siri-cidicola* (Bedding) flew 32% slower and only about half as far as uninfected females on flight mills (Villacide and Corley 2008). In the case of the red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae), some alate females infected with the microsporidian *Thelohania solenopsae* Knell, Allen & Hazard did not even attempt to fly (Overton et al. 2006). This effect is mirrored in the reduction of the proportion of *Nosema*-infected European corn borer males and females that engaged in flights of at least 0.5 km (fig. 1).

It is interesting that none of the flight parameters in 3-day-old moths of either sex were significantly affected by *Nosema* infection, although a larger sample of moderate-heavily infected adults would be desirable before concluding there are no negative effects. Nevertheless, the differences in effects of infection on flight performance between 1- and 3-day-old females are striking. Previous studies (Reardon et al. 2006; Dorhout et al. 2008) suggest that 1-day-old females engage in an obligate migratory flight. The threefold greater median distance of the longest uninterrupted flight by uninfected 1-day-old than 3-day-old females in this study is consistent with this conclusion and with previous results (Dorhout et al. 2008). The implication is that a *Nosema* infection has the effect of reducing migratory capacity of 1-day-old females, creating correspondingly large differences between them and uninfected adults engaged in long migratory flights. In contrast, any differences between the shorter flights characterizing the normal non-migratory flight activity of uninfected 3-day-old females (Dorhout et al. 2008) and those of infected females would be more subtle. That there is an effect is suggested by the observation that 24% of uninfected or lightly infected 3-day-old females flew at least 9 km, whereas only 7% of those with a moderate-heavy infection flew even as far as 3 km (fig. 2), but again the sample size of the latter group was small.

Environmental stress during development can cause changes in wing shape or body size (Sappington and Showers 1992; Hoffmann et al. 2002; Coll and Yuval 2004; Sarvary et al. 2008), and parasitization can result in size reductions of adults (Villacide and Corley 2008). *Nosema fumiferanae*-infected *C. fumiferana* females have smaller bodies, including shorter forewing lengths, than uninfected females (Sanders and Wilson 1990; Eveleigh et al. 2007), but wing area and wing load of *C. rosaceana* infected by the same pathogen were not affected (Gardiner 1990).

Variation in body size and wing morphology can affect flight performance or propensity (Hammond and Fescemyer 1987; Sappington et al. 1994; Srygley and Kingsolver 2000; Coll and Yuval 2004; Sakamoto et al. 2004), and negative morphological effects caused by parasites and pathogens are thus sometimes associated with decreased flight performance (Eveleigh et al. 2007; Villacide and Corley 2008).

The mechanism underlying the negative affects of *Nosema* infection on European corn borer seems not to be related to a reduction in overall body-size as indexed by forewing length. Although forewing length was positively correlated with most parameters of flight performance of 1-day-old male European corn borers and with several parameters in 3-day-old females, we found no evidence that *Nosema* infection resulted in adults with shorter wings. In the case of 3-day-old females, the correlation coefficient values are similar among infection categories, but the pattern of significance seems to be related to sample size (table 3). However, the positive correlations of total distance and duration for heavily infected 1-day-old females are significant despite small sample size, and suggest that long wings may help mitigate stress on flight performance caused by *Nosema* infection during the migratory flight period of young females (Dorhout et al. 2008).

Rather than a reduction in size, it is more likely that the observed negative effects of *N. pyrausta* on European corn borer flight performance is related to energetic stress imposed by the parasite on the host with the consequence that flight muscle, the most energetically demanding tissue in insects (Canavoso et al. 2001), is starved for fuel. Oxygen consumption by *Nosema*-infected European corn borers is higher than that of uninfected insects during all life stages (Lewis et al. 1971), probably due in part to the metabolic requirements of the parasite, but also to physiological stresses imposed by the infection such as immune response (Seyoum et al. 1994; Mayack and Naug 2009). In European corn borer, adult weight of both sexes, and therefore energy reserves, was severely negatively affected by *Nosema* infection. The greater prevalence of negative effects of *Nosema* infection on male than female flight performance observed in our study may reflect lesser energy reserves in the smaller males (Williams and Robertson 2008; Elliott and Evenden 2009). Even in the presence of adequate energy reserves, the parasite may interfere with the host's physiological ability to utilize them (Seyoum et al. 1994), as demonstrated for the dragonfly, *Libellula pulchella* Drury (Odonata: Libellulidae), parasitized by the gregarine protozoan,

*Hoplorhynchus* sp. (Marden and Cobb 2004; Schilder and Marden 2006). Short-duration flight in insects depends mainly on carbohydrates for fuel, while mobilization of lipid reserves is required to maintain flight activity beyond a few minutes (Van Handel 1974; Teo et al. 1987; Sappington et al. 1995; Candy et al. 1997; Haunerland 1997). *Hoplorhynchus* appears to interfere with lipid utilization resulting in poorer flight performance of infected dragonflies (Schilder and Marden 2006, 2007). Depletion or interference with utilization of lipids by *Nosema* in European corn borer would be consistent with the observation that infected and uninfected moths initiate a similar number of flights during the night, but the flights of infected moths are of shorter duration.

As adoption of Bt corn increases and European corn borer populations decline regionally, it will be of great interest to observe the effects on incidence and intensity of *Nosema* in the ecosystem. The future rate of decline in *Nosema* incidence may not be symmetrical with the rate of decline in the host population, because of the former's density dependent nature. Apart from the direct interest in the fate of this important regulator of European corn borer populations, the dynamics of the parasite–host relationship will serve as a model for other systems. For example, momentary escape from a pathogen or other natural enemy may be associated with invasive potential of an introduced exotic species or of a native species expanding its range (Aliabadi and Juliano 2002; Torchin et al. 2003; Prenter et al. 2004; Allen et al. 2007), but the dynamics of this important process are poorly understood (White et al. 2000; Hierro et al. 2005). Loss of *Nosema* from a declining corn borer population would essentially represent an escape from the pathogen *in situ*, and could affect the dispersal dynamics of the moths in that community (French and Travis 2001). Population and metapopulation-level effects of potential intermittent loss of *Nosema* from local areas (Cross et al. 2007; Driscoll 2007) on European corn borer dispersal and gene flow will be important in modeling resistance evolution, European corn borer population dynamics under high fragmentation of suitable (i.e., non-Bt corn) habitat, and of *Nosema* maintenance in this new transgenic-dominated agricultural landscape.

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