

## RESEARCH

## Livestock Bedding Effects on Two Species of Parasitoid Wasps of Filth Flies

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Subject Editor: Seth Barribeau

J. Insect Sci. 14(185): 2014; DOI: 10.1093/jisesa/ieu047

**ABSTRACT.** Choice of livestock bedding has been shown to affect density of filth fly maggots. Here, laboratory experiments indicate that bedding type can also affect natural enemies of the flies, specifically the parasitoid wasps *Spalangia endius* Walker and *Urolepis rufipes* (Ashmead) (Hymenoptera: Pteromalidae) parasitizing a natural host, the house fly *Musca domestica* L. (Diptera: Muscidae). For both parasitoid species, when females parasitized hosts under bedding, cedar shavings resulted in fewer parasitoids compared with pine shavings, but pine shavings did not differ from wood pellets and corn cob pellets. In the absence of exposure to hosts, longevity of adult females was reduced in cedar shavings compared with pine shavings and pellets. In contrast to the effects on parasitization and on adult survival, shavings treatment had no significant effect on the number of parasitoids or flies that emerged when hosts were not exposed to shavings until after parasitization.

**Key Words:** parasitism, *Spalangia*, *Urolepis*, *Musca domestica*, substrate

The control of filth flies such as house flies and stable flies is a major concern for livestock rearing facilities. Such flies are nuisances to both livestock and humans and may spread pathogens (Alam and Zurek 2004, Talley et al. 2009). They decrease feed efficiency in beef cattle and the production of milk in dairy cattle (Campbell et al. 1987), with significant economic consequences (Taylor et al. 2012). In addition, as human populations grow, the distances between livestock rearing facilities and housing developments often decrease, raising concerns that large populations of adult flies may lead to lawsuits against livestock producers (Thomas and Skoda 1993).

Control of pest filth flies has traditionally relied heavily on insecticides (Agricultural Statistics Board N, United States Department of Agriculture 2007). However, use of natural enemies such as parasitoid wasps to control flies is also important because flies continue to develop resistance to insecticides (Scott et al. 2000, Rinkevitch et al. 2012) and because of public concern about health and environmental risks associated with insecticides. Parasitoid wasps provide some natural control of filth flies, and augmentative releases of such parasitoids are an effective means of biological control in some situations (Weinzierl and Jones 1998, Skovgård and Nachman 2004). This study examines livestock bedding, one aspect of the parasitoids' habitat that may affect their ability to parasitize, and therefore control, filth fly populations.

Laboratory experiments were used to demonstrate effects of livestock bedding on two species of parasitoid wasp that have been found parasitizing the pupal stage of filth flies in livestock facilities, *Spalangia endius* Walker and *Urolepis rufipes* (Ashmead) (Pteromalidae) (Rueda and Axtell 1985b, Floate et al. 1999). Adult parasitoids, particularly females, have significant contact with bedding as they burrow in search of hosts. *Spalangia* spp. are well known for burrowing (Legner 1977, Rueda and Axtell 1985a, Geden 2002), and hosts parasitized by *Spalangia* spp. have been found even 10 cm under manure (Rueda and Axtell 1985a). *U. rufipes* can burrow at least 4 cm through house fly media for hosts (Floate and Spooner 2002). Parasitoids of filth flies are technically ectoparasitoids because the female oviposits on the surface of the fly pupa; however, that pupa is within a puparium.

Choice of bedding is known to affect fly larvae density and development (Schmidtman et al. 1989, Schmidtman 1991, Watson et al. 1996) and livestock health (Kirkegaard et al. 2003, Norring et al. 2008, Hill et al. 2011). Sawdust, woodchips, or ground corncobs suppressed house fly and stable fly development in calf hutches on dairy farms (Schmidtman et al. 1989, Schmidtman 1991). Sawdust bedding resulted in lower numbers of house fly and stable fly larvae than straw bedding in dairy calf hutches that had been treated with an insecticidal fungus (Watson et al. 1996).

However, controlled studies directly comparing the effects of bedding-type alone on parasitoid wasps have been lacking. Such effects seem likely given that previous laboratory studies have shown an effect on parasitism for substrates such as fly rearing media versus poultry manure versus sandy soil (Geden 2002) and soiled equine bedding substrate versus wood chips (Pitzer et al. 2011). In addition, collections of fly pupae from farms have shown an effect of substrate on parasitism rates (Petersen and Meyer 1983, Greene et al. 1989, Smith et al. 1989, Smith and Rutz 1991a,b). This study addressed the effect of bedding type on the ability of female parasitoid wasps to reach and parasitize hosts, the longevity of adult parasitoid females, and the development of parasitoid offspring within hosts.

## Materials and Methods

**General Methods.** The *S. endius* utilized in this study were from a colony established with parasitoids from Zephyr Hills, FL, and vouchers are at the Illinois Natural History Survey Center for Biodiversity, catalog numbers "Insect Collection 6035 through 6054." The *U. rufipes* that was utilized was a strain originally collected from cattle feedlots in southern Alberta. House flies, *Musca domestica* L., were used in the pupal stage as hosts, both for the experiments and for maintaining the parasitoid colonies. Cages of adult house flies were fed sugar water ad libitum, and cotton dental wicks in the milk containers provided oviposition sites (King 1988). Pupae were produced by placing about 800 mm<sup>3</sup> of house fly eggs in a plastic box (about 31 by 16 by 9 cm) containing 1,030 ml of medium (10 parts pine shavings: 8 parts commercial fly larvae media: 1 part fish meal) mixed with 660 ml of water (modified from King 1988). The box was kept tilted at about 30° and

slightly stirred as needed to prevent a crust from forming. The commercial fly larvae medium consisted of ground oat hulls, ground barley, wheat bran, and dehydrated alfalfa meal (Lab Diet, St. Louis, MO). The pine shavings were kiln-dried large flakes with low dust. Prior to pupation, the fly larvae crawled out of the box containing the mixture and into a larger empty box below it. The parasitoid colonies had no direct contact with the shavings.

To generate parasitoids for experiments, open Petri dishes (10 cm in diameter, 1.3 cm in height) of hosts that were 0- to 1-d-old postpupariation were exposed for about 3 d to colonies of adult parasitoids within plastic containers with cloth windows for ventilation. The parasitoids were reared at  $\sim 25^{\circ}\text{C}$  with a photoperiod of 12:12 (L:D) h. Adult females ranging from 0- to 1-d old were used in experiments, except where noted. Age was known because females were removed daily from dishes of parasitized hosts; males began emerging before females and were not removed in order to allow mating. Each experiment herein included females from more than one such dish of parasitized hosts. Four types of bedding were tested: corncob pellets (horse bedding of commercial ear corn, Best Cob, Rock Falls, IL), compressed-wood pellets (American Wood Fibers, Columbia, MD), red cedar shavings (kiln-dried large flakes, low dust), and pine shavings (kiln-dried large flakes, low dust). Depth of bedding was controlled within shavings and within pellets, but not between because pellets are denser than shavings so less volume may be applied. Although the same protocol was used regardless of bedding type or of parasitoid species, degree of experimental control was strongest within each species and within pellets or within shavings, e.g., in terms of exact age of females and batch of hosts used.

**Oviposition and Development.** This experiment tested whether bedding type affected parasitoid and fly production. Such effects could be through effects on oviposition or development. Adult parasitoid females were individually exposed to hosts buried in one of the four bedding types: corncob pellets, wood pellets, red cedar shavings, or pine shavings. Used fly media was spread to a depth of 1.5 cm at the bottom of 1,000 ml glass jars (8 cm in diameter, 16.5 cm in height). One hundred hosts that were 0- to 1-d-old postpupariation were then placed on top of the media and at least 1 cm away from the edges of the jar. Thus, females had to navigate through the bedding not just move along the glass side (Floate and Spooner 2002). One of the four bedding types was then placed on top of the pupae. Shavings were not packed and were at a depth of 5 cm. For pellets, a single layer of pellets just covering the media surface was used, which corresponded to the pellet manufacturer's recommendations. Pellet depth was 6 mm; pellets are 6 mm in diameter and of variable length. Bedding livestock facilities will frequently become mixed with urine and manure, but fresh bedding is often thrown on top of concrete, manure, or old bedding (Olbrich and King 2003); it is this latter sort of situation that these experiments address.

A female was released onto the surface of the bedding, and the jar was covered securely with cloth. After 4 d, the hosts were transferred to a glass vial (20 mm in diameter, 70 mm in height), which was fitted with a cotton plug; and parasitoid offspring were allowed to complete their development. This experiment was repeated eight different times with *S. endius* and nine different times with *U. rufipes*. The goal was to detect any relatively large effects because on farms any small effects will likely be swamped by other environmental factors and by other considerations, such as cost. At least 2 mo after parasitization, flies and adult male and female parasitoids had finished emerging and died; the number of each was recorded, and the proportion of male parasitoids was computed.

**Adult Longevity.** This experiment tested whether bedding type affected the survival of adult female parasitoids. A female was placed in a plastic Petri dish (10 cm in diameter, 1.3 cm in height) that had been filled with one of the four types of bedding. In the center of each dish was a glass test tube (12 mm in diameter, 75 mm in height) kept filled with water and with a wet cotton plug from which the female drank; no honey was provided. The dish was closed and then sealed with Parafilm. Every 24 h, survival was checked, and water was added if needed. A female was considered dead when she could no longer walk.

Sample sizes were as follows: for the *S. endius* shavings experiment,  $N=23$  for cedar and  $N=20$  for pine; for the *S. endius* pellet experiment,  $N=35$  per treatment; for the *U. rufipes* shavings experiment,  $N=13$  per treatment; and for the *U. rufipes* pellet experiment,  $N=35$  per treatment.

**Development.** This experiment tested whether bedding affected development of parasitoids within hosts. To create parasitized hosts, adult female parasitoids were individually provided with 15 0- to 1-d-old hosts for 24 h in a glass vial (20 mm in diameter, 70 mm in height) that lacked bedding. The hosts were then transferred into a larger glass jar (5.5 cm in diameter, 6.5 cm in height) that was filled with cedar shavings, pine shavings, or no bedding. The jar was filled one-third full, then the hosts were added, and then additional bedding was added to fill the jar to the top. The jar was covered securely with cloth. The jar with no bedding acted as a control. Adult parasitoids were allowed to emerge and were counted after death. For each species, sample sizes were  $N=15$  for each of the three treatments. Pellets were not tested because their effect was not different from that of pine shavings in the first experiment (Fig. 1).

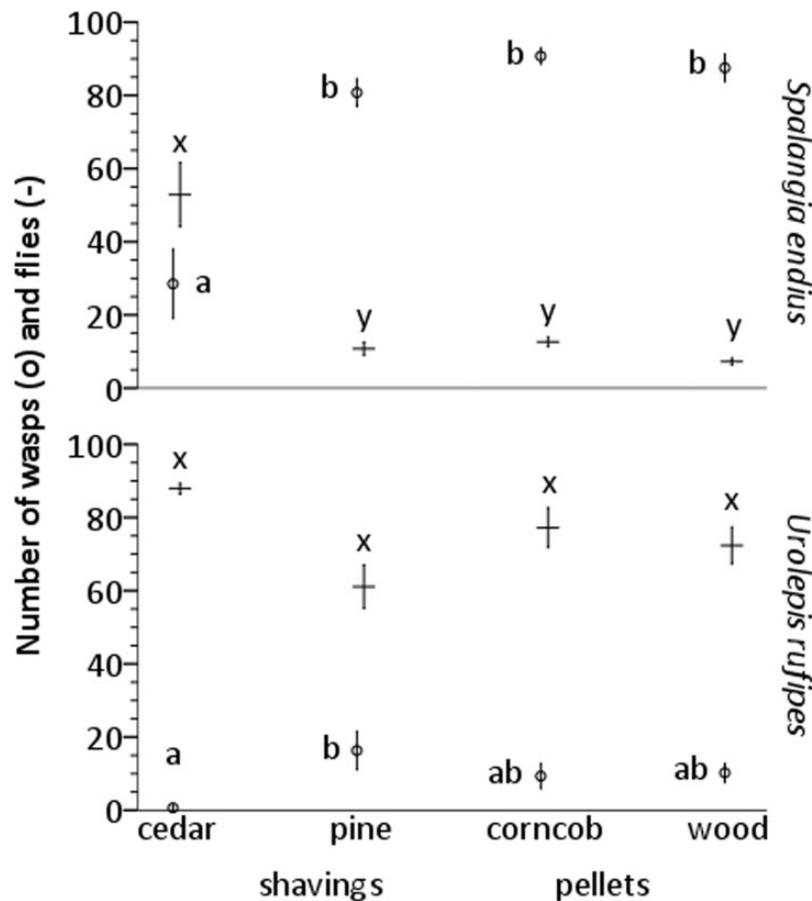
**Statistical Analyses.** Statistical analyses were performed with Statistical Package for Social Scientists (SPSS 2010). In the first and third experiment, effect of bedding on number of flies and parasitoids that emerged was tested with generalized linear model (GLM) or analyses of variance (ANOVA). GLMs with a negative binomial distribution and a log link function (SPSS 19.0) were used where data exhibited a Poisson distribution. Negative binomial distribution was chosen because of overdispersion. Post hoc comparisons were by least significant difference. For comparisons where distributions differed significantly from a Poisson distribution, ANOVA was used because ANOVAs are robust to assumptions of normality and homogeneity, particularly when sample sizes are very similar (Zar 2010, Kikvidze and Moya-Laraño 2008). Post hoc comparisons after ANOVAs were by Tukey's test. In the first experiment, effect of bedding on sex ratio of parasitoids that emerged was tested with GLM with a binomial distribution and a logit link function. In the second experiment, the effect of bedding on longevity was analyzed by life table analysis, a type of survival analysis. The overall effects of bedding, as well as pair-wise comparisons, were with Wilcoxon (Gehan) tests (SPSS 2010). Two-tailed  $P$  values are presented.

## Results

**Oviposition and Development.** The number of parasitoids that emerged was affected by bedding type for both parasitoid species (Fig. 1; *S. endius*:  $F_{3,28}=27.93$ ,  $P<0.001$ ; *U. rufipes*:  $F_{3,32}=3.59$ ,  $P=0.02$ ). The number of flies that emerged was affected by bedding type for *S. endius* but not for *U. rufipes* (Fig. 1; *S. endius*: Wald  $\chi^2=17.17$ ,  $df=3$ ,  $P=0.001$ ; *U. rufipes*: Wald  $\chi^2=0.61$ ,  $df=3$ ,  $P=0.90$ ). For *S. endius*, the cedar shavings resulted in fewer parasitoids and more flies than any of the other bedding types, whereas there were no significant differences among the pine shavings and the two types of pellets. For *U. rufipes*, the cedar shavings resulted in fewer parasitoids than the pine shavings, whereas neither type of shavings differed significantly from the two types of pellets.

The proportion of male parasitoids that emerged differed between bedding type for both species (Fig. 2; *S. endius*: Wald  $\chi^2=9.69$ ,  $df=3$ ,  $P=0.021$ ; *U. rufipes*: Wald  $\chi^2=21.01$ ,  $df=2$ ,  $P<0.001$ ). (Sex ratio data for *U. rufipes* from cedar shavings was excluded because *U. rufipes* emerged only from one cedar shavings replicate.) For *S. endius*, the proportion of sons from wood pellets was significantly greater than from either type of shavings but not significantly greater than from corn pellets. For *U. rufipes*, the proportion of sons from pine shavings was significantly less than from either type of pellets, which did not differ significantly.

**Adult Longevity.** Longevity was affected by bedding type for both species of parasitoid (Fig. 3; *S. endius* Wilcoxon (Gehan) statistic = 33.32,  $df=3$ ,  $P<0.001$ ; *U. rufipes* Wilcoxon (Gehan) statistic = 31.95,  $df=3$ ,  $P<0.001$ ). Specifically, cedar shavings resulted



**Fig. 1.** Mean  $\pm$  SE number of parasitoid wasps (●) and of house flies (○) that emerged after *S. endius* and *U. rufipes* parasitized hosts that were buried under 5 cm of cedar versus pine shavings or under a single layer of corncob pellets versus wood pellets. *U. rufipes* emerged from only one of the cedar shavings replicates. Within each species, different letters (a, b for parasitoids; x, y for flies) indicate differences at  $P < 0.05$ .

in the lowest longevity for both *S. endius* and *U. rufipes* ( $P < 0.001$  for all pairwise comparisons to other bedding types). The type of pellet did not influence longevity of either *S. endius* ( $P = 0.86$ ) or *U. rufipes* ( $P = 0.46$ ). Pine shavings resulted in higher longevity than all the other bedding types for *U. rufipes* ( $P < 0.05$  for all pairwise comparisons to other bedding types), whereas for *S. endius* pine shavings did not differ from the pellets ( $P > 0.98$  for both pairwise comparisons).

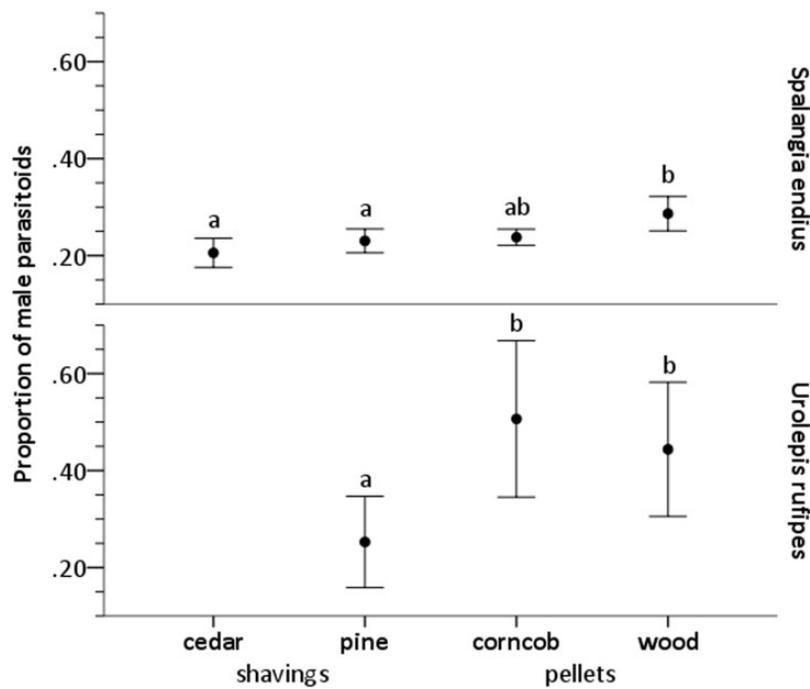
**Development.** No significant differences were found among treatments for the number of flies or parasitoids that emerged for either parasitoid species (Fig. 4; *S. endius*: flies Wald  $\chi^2 = 2.35$ ,  $df = 2$ ,  $P = 0.31$ ; parasitoids Wald  $\chi^2 = 0.50$ ,  $df = 2$ ,  $P = 0.78$ ; *U. rufipes*: flies Wald  $\chi^2 = 0.015$ ,  $df = 2$ ,  $P = 0.99$ ; parasitoids  $F_{2,42} = 0.80$ ,  $P = 0.46$ ). (Parasitism was unexpectedly low regardless of treatment in the *U. rufipes* experiment on two dates representing 6 of the 15 replicates. However, conclusions are unaffected by excluding those dates.)

## Discussion

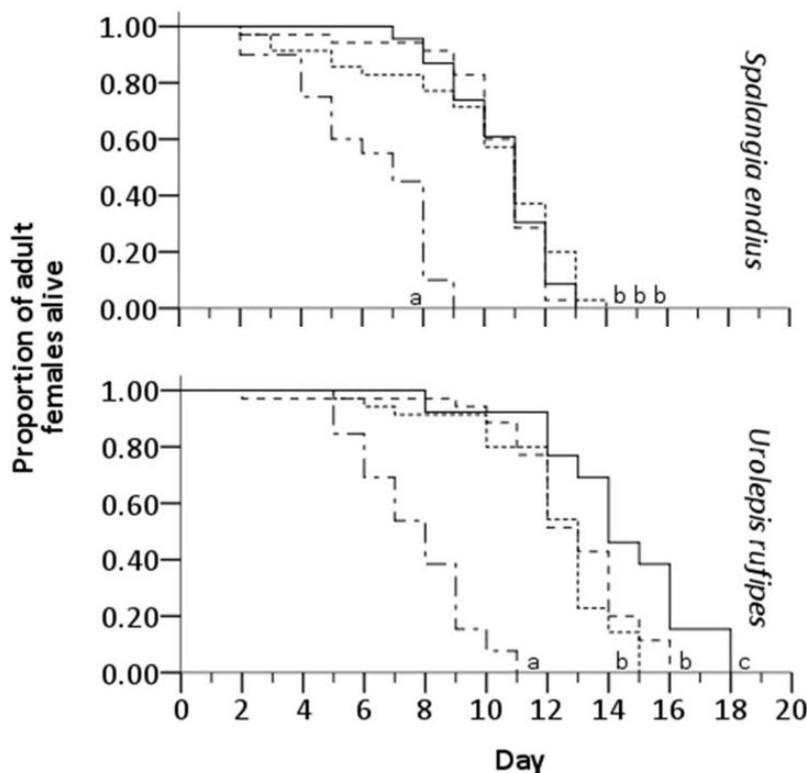
From the perspective of fly control, results of our study suggest that choice of bedding can influence the effectiveness of control by parasitoid wasps. The effects of bedding type appear to be through effects on the adult female parasitoid's ability to parasitize rather than through effects on the parasitoid offspring within their hosts. When hosts were not exposed to bedding until after exposure to parasitization, type of shavings had no significant effect on the number of adult parasitoids or flies that emerged. In contrast, when adult parasitoids had to move through bedding to parasitize hosts, bedding type had an effect. When females were exposed to bedding but were not ovipositing, bedding type affected the females' longevity. Generally, the effects of cedar shavings differed most from the other bedding types. For *S. endius*,

cedar shavings resulted in more flies, fewer parasitoids, and lower adult parasitoid longevity than the other bedding types, whereas there were no detectable differences among the effects of pine shavings and the two types of pellets except on sex ratio. For *U. rufipes*, cedar shavings were again worse than pine shavings in producing fewer parasitoids and lower adult parasitoid longevity. However, for *U. rufipes* neither type of shavings differed significantly from the pellets in its effects on parasitoid and fly production, although pine resulted in somewhat greater longevity of adult female parasitoids. Thus, for both parasitoid species, pine shavings were better than cedar shavings, but type of pellets did not matter; however, pine shavings were not significantly better than pellets, except in causing greater longevity of adult *U. rufipes*. The parasitoid colonies in this study were reared on hosts that had developed as larvae in media containing pine shavings, and this may have affected the results. However, the parasitoid colonies had no direct contact with the shavings. Regardless, the results show that bedding type is important under certain conditions.

It is well established that depth of bedding affects rates of parasitism and does so differently for different species (King 1997, Rueda and Axtell 1985a, Floate and Spooner 2002, Geden 2002). Thus, the comparisons between shavings and pellets should be viewed with this in mind. In this study, effects of shavings on the parasitoids were not strictly due to differences in depth of bedding because the depth of pine shavings was equal to that of the cedar shavings, yet pine shavings produced results more similar to those of the pellet treatments. Size and texture of the two types of shavings were intentionally similar, although effects of minor differences cannot be ruled out. Alternatively, the differences between the effects of cedar shavings versus pine shavings may have been due to effects of cedar oils or their volatiles. Cedar is well known



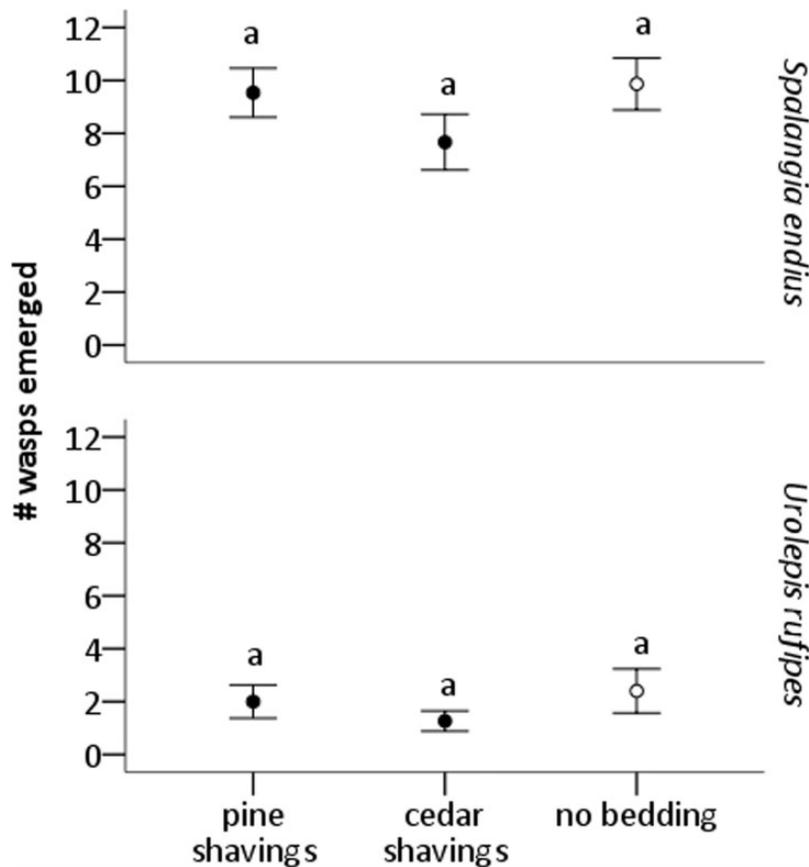
**Fig. 2.** Mean  $\pm$  SE proportion of male parasitoids that emerged after *S. endius* and *U. rufipes* parasitized hosts that were buried under 5 cm of cedar versus pine shavings or under a single layer of corncob pellets versus wood pellets. *U. rufipes* emerged from only one of the cedar shavings replicates, so that treatment was excluded. Within each species, different letters (a, b) indicate differences at  $P < 0.05$ .



**Fig. 3.** Survival of adult female *S. endius* and *U. rufipes* in cedar shavings — — —, pine shavings ———, corncob pellets - - - - -, and wood pellets — · — ·. Within each species, different letters (a, b, c) indicate differences at  $P < 0.05$ .

for being harmful and repellent to certain arthropods (Dolan et al. 2007). There are wasp species that can tolerate cedar, although these tend to be cedar specialists (Wickman 1967), species that occasionally use cedar for nesting material (Hagiwara and Kojima 1994), and parasitoids whose hosts live in or on cedar (Fortier and Sherman 2008).

Cedar is sold and recommended as livestock bedding (Gay 2009). Even if such bedding is detrimental to filth fly parasitoids, the net effect of using cedar bedding may still be positive if cedar interferes with larval development of pest flies. Larval fly development was not examined in this study. Bedding type is known to affect maggot density



**Fig. 4.** Mean  $\pm$  SE number of wasps that completed development and emerged when parasitized hosts were buried in different shavings types (cedar, pine, and none). Within each species, different letters indicate means of those treatments differed significantly ( $P < 0.05$ ).

(Schmidtman et al. 1989, Schmidtman 1991); but the specific effect of cedar remains to be tested. A further consideration with cedar materials is the effect on the growth of microorganisms. Bacteria that are harmful to humans can grow on cedar bedding (Davis et al. 2005), but whether it does so less than on other bedding types has not been examined. Bewley (2009) and Janni (2010) hypothesize that cedar oils may also inhibit the growth of microorganisms that are necessary for the decomposition that is used to create compost-pack bedding.

Many questions remain about how bedding affects natural enemies. The relative advantage of different bedding types may differ when they become wet or soiled, e.g., due to differences in absorbency and suitability for growth of mold and other microorganisms. Furthermore, proposed bedding materials for livestock include not only wood shavings and pellets but also straw, sawdust, wood chips, sand, ground limestone, separated manure solids, shredded newspaper, corn stalks, bark, peanut hulls, sunflower hulls, and rice hulls or mixtures of these (Spiehs et al. 2011). Direct comparisons of these latter types of bedding on filth fly natural enemies have not been done.

Studies of microhabitat usage are consistent with our general conclusion that bedding type can affect parasitoids of filth flies (Smith et al. 1989, Smith and Rutz 1991a,b). Microhabitat has been shown to influence both which parasitoid species are present and parasitism rates. *Spalangia* spp. and *Urolepis* spp. may be more effective than *Muscidifurax* spp. in wetter areas (Smith and Rutz 1991c, Geden 1999), and beddings may vary in their absorbency. If *Spalangia* spp. burrow more than *Muscidifurax* spp., as is often the case, then *Spalangia* spp. may be more effective when deep bedding is used (Legner 1977, Rueda and Axtell 1985a, King 1997, Geden 2002). Pitzer et al. (2011) suggested that in equine habitats in Florida, *Spalangia* spp. are better suited than *Muscidifurax* spp.; *Muscidifurax raptorellus* attacked significantly

more stable fly pupae in 120-ml plastic cups containing wood chips than in 4.8-liter chambers containing soiled equine bedding, whereas parasitism rates by *Spalangia cameroni* and *S. endius* were not different between the treatments. Geden (2002) found that no parasitoid wasps penetrated sand even though flies frequently pupated in it, although sand bedding has some positive effects on livestock (LeJeune and Kaufmann 2005, Norring et al. 2008, Hill et al. 2011). Bedding type may affect not only the ability of parasitoids to reach hosts but also their ability to locate fly pupae by olfaction. Parasitoids of filth flies use odors to locate host habitats, and substrate odors, which are attractive, vary among parasitoid species (Pawson 1989). If parasitoids learn host habitat characteristics, or over longer periods of time adapt to bedding features, then changing bedding type might impact parasitization.

Other important considerations in bedding choice besides effects on livestock, insects, and microorganisms include cost; availability; disposal (Zdanowicz et al. 2004, Norring et al. 2008, Hill et al. 2011); and even effects on air quality in livestock facilities (Spiehs et al. 2011). The relative merit of different bedding types will vary with the specific application.

#### Acknowledgments

This study was supported by the Institute for the Study of Environment, Sustainability and Energy at Northern Illinois University (NIU), NIU's Department of Biological Sciences, and NIU's Undergraduate Research Assistantship program. We are grateful to K. Kuban and S. Broski for considerable assistance with fly and parasitoid colony maintenance, to H. Olson, T. Schulmeister, M. Hyatt, H. Hildebrand, B. T. King, and T. Caar for laboratory assistance, and to C. Geden at the United States Department of Agriculture in Florida and K. Floate at Lethbridge Research Center for starter parasitoids.

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