Substrate influences mating success and transmission of courtship vibrations for the parasitoid *Cotesia marginiventris*

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Abstract

The influences of artificial and natural rearing substrates on mating success were investigated for the parasitoid wasp *Cotesia marginiventris* (Cresson) (Hymenoptera: Braconidae), a candidate for augmentative biological control of various lepidopteran pests. Five rearing substrates were tested: plastic, glass, chiffon fabric, and leaves of two host plants, bean [*Vigna unguiculata* (L.) (Fabaceae)] and maize [*Zea mays* L. (Poaceae)]. Mating success was highest on chiffon, lowest on plastic and glass, and intermediate on maize and bean. The transmission characteristics of one component (buzz 1) of the courtship vibrations produced by male wing fanning were investigated using laser vibrometry. The duration of buzz 1 was longer on maize, bean, and chiffon than on plastic and glass. The fundamental frequency of buzz 1 (~300 Hz) was lowest on bean and highest on glass, and intermediate among other substrates. The relative amplitude of buzz 1 was higher on chiffon than on plastic, glass, or bean, and intermediate on maize. The relative importance of airborne sound and substrate vibration as courtship signals was also investigated with experiments that manipulated the production of courtship vibrations and the mating substrates. The amplitude of courtship vibrations on chiffon was significantly higher for winged males than for dealated males. The mating success of males was impacted by both the presence of wings and the mating substrate. These findings suggest that mating success and transmission of courtship vibrations are influenced by the rearing substrate, and that courtship vibrations are critical to mating success in *C. marginiventris*. Future efforts to mass rear this parasitoid and other insects should consider the potential influences of rearing substrates on mating.

Introduction

Insect mating is often influenced by chemical and physical environmental factors, such as host plant odors, ambient temperatures, and time of day (van den Assem, 1986; Quicke, 1997), but the influence of the mating substrate and its physical properties on mating success has rarely been investigated. Several studies found that the mating success of some insects and other arthropods that use vibrational communication during courtship can be affected by the mating substrate (Miklas et al., 2001; Elias et al., 2004), while others found that the physical properties of the substrate had little influence on the transmission of courtship vibrations (Henry & Martinez Wells, 2004; Cocroft et al., 2006).

Male parasitoid wasps from several families of Hymenoptera, including those of *Cotesia* spp. Cameron (Hymenoptera: Braconidae), fan their wings during courtship, producing species-specific, low amplitude sounds, and substrate vibrations, which are likely to play an important role in courtship communication (Vinson, 1972; Leonard & Ringo, 1978; van den Assem & Putters, 1980; Sivinski & Webb, 1989; Field & Keller, 1993; Kimani & Overholt, 1995). Transmission of courtship vibrations in the substrate appeared critical for mating in the parasitoid

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Cotesia rubecula (Marshall) (Field & Keller, 1993). Also, replay of courtship sounds to *Nasonia vitripennis* (Walker) (Hymenoptera: Pteromalidae) increased mating for older males, suggesting that courtship acoustics convey information concerning male quality (van den Assem & Putters, 1980). The transmission of parasitoid courtship vibrations may be influenced by host plant substrates and artificial substrates used for mass rearing in the laboratory, which in turn could influence their mating success.

Transmission of courtship vibrations through substrates used in insect mass-rearing programs has not been studied. Courtship vibrations may transmit differently in materials commonly associated with rearing, such as plastic, glass, and fabric, than in natural host plant substrates. A common goal in parasitoid mass rearing for biological control is to produce as many females as possible, because only females attack and kill hosts in the field. Most parasitoid wasps reproduce by arrhenotoky, thus inseminated females produce female and male offspring, while virgin females produce only males. Selecting rearing substrates that facilitate courtship communication and mating could increase the number of female parasitoids produced under mass rearing.

Cotesia marginiventris (Cresson) is a parasitoid wasp whose hosts are moth larvae (Lepidoptera: Noctuidae) and is an important natural control agent of several pest species, including *Spodoptera frugiperda* (J. E. Smith) and *Trichoplusia ni* (Hübner) (McCutcheon & Turnipseed, 1981; Braman & Yeargan, 1991; Novoa & Luna, 1996; Gillespie et al., 1997). *Cotesia marginiventris* is a solitary endoparasitoid that attacks first and second instars (Boling & Pitre, 1970; Kunnalaca & Mueller, 1979; Tillman, 2001) and has been considered for augmentative biological control of *T. ni* in greenhouses (Gillespie et al., 1997; Urbaneja et al., 2002; Riddick, 2006). The sex ratios (% males) of *C. marginiventris* recorded under mass rearing and field conditions vary between 40 and 80% (Jalali et al., 1987; Riggin et al., 1992; Novoa & Luna, 1996; Gillespie et al., 1997; Tillman, 2001). The male-biased sex ratios observed in some studies may have been due to a high frequency of virgin females, although the potential role of mating substrates and transmission of courtship vibrations in rearing materials were not investigated.

The objective of this study was to assess the influence of natural and artificial rearing substrates on the mating success and transmission of courtship vibrations for the parasitoid *C. marginiventris*. In addition, the importance of airborne sound relative to substrate vibrations produced during courtship was determined by experimentally manipulating the production of courtship vibrations on two substrates and quantifying their influence on mating success.

Materials and methods

Insects

A culture of *C. marginiventris* was maintained on larvae of the moth *S. frugiperda*. Parasitoid pupae were originally obtained from a laboratory colony in Tifton, GA, USA. *Spodoptera frugiperda* adults were maintained in 2.4-l white plastic containers with 20% (wt/vol) sucrose solution as food, at 27 ± 2 °C, 60 ± 5% r.h., and a photoperiod of L14:D10. Adult moths oviposited on a paper towel, and emerging *S. frugiperda* larvae were fed with a maize earworm diet (Bio-serv Inc., Frenchtown, NJ, USA). To produce parasitoids, 20 *S. frugiperda* second instars, larval diet, and a streak of honey were placed into a 24-ml glass vial with one adult male and female *C. marginiventris* (0–48 h old) for 48 h. Larvae were then transferred to plastic cups with diet until parasitoids formed cocoons. Each *C. marginiventris* pupa was then isolated in a 1-ml glass vial (30×8 mm; Bioquip, Gardena, CA, USA) so that emerging adults would remain virgin until used in an experiment. All adult *C. marginiventris* were stored overnight at 15 °C before use in mating trials, to increase the likelihood of mating (A Joyce, unpubl.).

Mating success on five substrates

The experiment was designed to assess the effect of rearing materials on the mating success of individual *C. marginiventris* females, and consisted of five treatments: glass shell vials (1 ml, 30×8 mm; Bioquip), plastic Petri dishes (50×9 mm, Falcon®; Becton Dickinson Labware, Franklin Lakes, NJ, USA), white chiffon fabric (Hancock Fabrics; Chiffon Georgette, 100% polyester), and two host plant substrates: maize leaves [*Zea mays* L (Poaceae), Pioneer® seed 34A55; Johnston, IA, USA] and bean leaves [*Vigna unguiculata* (L.) (Fabaceae), California blackeye cowpea no. 5; Gurney's Seed and Nursery Co., Greendale, IN, USA]. Glass shell vials were plugged with cotton to prevent parasitoids from escaping. The chiffon, bean leaf, and maize leaf treatments also consisted of a plastic component, to enclose the insects on the substrate. A chiffon fabric arena was constructed of a plastic Petri dish (50×9 mm) with a circular opening (40 mm diam) cut in the lid, with chiffon fabric glued over the opening, such that the surface was smooth and taut. The maize and bean leaf arenas consisted of a leaf, which remained attached to the plant, covered with a plastic vial (160 ml, 85×50 mm; Bioquip). The five rearing materials varied from thickest to thinnest in the following order: plastic, glass, bean leaf, maize leaf, and chiffon fabric. Although the construction of arenas for leaves and the chiffon material required plastic support material, all matings occurred on the substrate associated with that treatment.
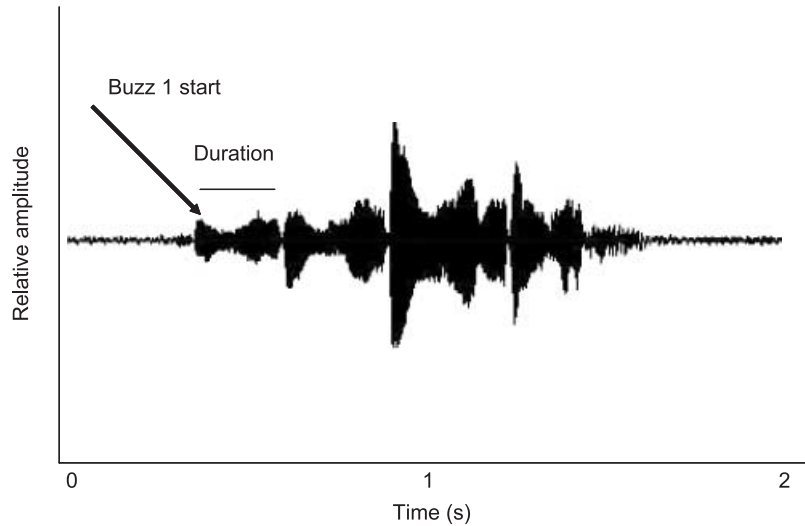
Figure 1 A typical bout of substrate vibrations produced by courting *Cotesia marginiventris* males on bean leaves, showing the buzz 1 component used for statistical comparisons among mating substrates.

Each male–female pair of virgin parasitoids were monitored for 10 min on each of the five treatments at laboratory temperature and humidity (25 ± 2 °C, 50% r.h.), between 08:00 and 10:00 hours, and mating success or failure was recorded. All parasitoids used in this and subsequent experiments were 2–4 days old, and for this experiment, 34 pairs were observed per substrate. Each experimental arena and all parasitoids were used only once and then discarded. A χ^2 goodness-of-fit test was used to determine whether mating frequencies differed among the five treatments. Post-hoc separation of mating frequencies on each treatment was by a modified Tukey test for proportions (Zar, 1999).

Transmission of courtship vibrations on five substrates
Courtship vibrations produced by male wing fanning were recorded on the five substrates described above to determine whether the substrates influenced their transmission. Male courtship primarily consists of wing fanning when in close proximity (1–2 cm) to a female. All parasitoids were used for only one recording. Males were recorded courting a female on each substrate for 10 min. All recordings were made under standardized laboratory conditions (25 ± 2 °C, 50% r.h.) using a randomized block design. Each block consisted of a single replicate (male–female pair) on each of the five substrates and the order of substrates was randomized within each block. Ten pairs were recorded courting on each of the five substrates.

Recordings were made with a laser Doppler vibrometer (Model OFV 353; Polytec Inc., Irvine, CA, USA) at 1 mm s^{-1} volt^{-1} sensitivity in an enclosed, sound reduction chamber (~1 m long × 1 m high × 0.75 m wide), located on a vibration isolation table (TMC™, Model NAF 2000;

Peabody, MA, USA). One male–female pair was placed on one of the substrates. The laser was positioned above the test substrate, and the laser beam was focused on reflective tape (4 mm^2) on the test surface of the substrate of interest. The vibrational signal detected by the laser was digitized and recorded on a Macintosh® computer equipped with an Audiomedia III sound card (16 bit, sampling rate 44.1 kHz). Peak® software (version 3.0; Bias, Petaluma, CA, USA) was used to record the signals. The distance between the laser beam point of contact on the substrate and the location of the courting male was monitored and recorded in 1 cm increments. Only courtship vibrations produced by males within 2 cm of the laser were used for analyses.

Vibrations produced by courting *C. marginiventris* males consist of several long buzzes, which may be preceded and/or followed by shorter pulses (Figure 1). Several parameters of the first buzz component (hereafter buzz 1) were used for comparison of vibration transmission among the five substrates (Figure 1). Cool Edit Pro software (version 2.0, now Adobe Audition 2.0; San Jose, CA, USA) was used to measure the duration, frequency, and relative amplitude of the buzz 1. The buzz 1 duration was measured in ms. The fundamental frequency (Hz) and its relative amplitude (–db, reference 0 db) were measured 10 ms after the beginning of buzz 1. Analyses were based on relative amplitudes rather than absolute amplitudes (i.e., velocity), because it was not practical to calibrate the absolute amplitude of each measurement while maintaining parasitoid age constant for recordings.

Statistical comparison of the fundamental frequency and the relative amplitude among the treatments was done by a one-way analysis of variance (ANOVA), and Tukey's post-hoc test was used to separate means (SPSS, 2001).

A non-parametric Kruskal–Wallis test was used to compare the buzz 1 duration among treatments (Statistix, 2000), with post-hoc comparisons of means as described in Siegel & Castellan (1988), as data did not meet the assumptions of ANOVA.

Role of airborne sound and substrate vibration in mating

Two experiments were conducted to assess how mating success is influenced by airborne sounds relative to substrate vibrations. The first experiment assessed whether wing ablation affected substrate vibrations produced by courting males. This was done by quantitative comparison of the peak velocity (mm s^{-1}) of substrate vibrations produced by courting normal and dealated males on the chiffon arena. This was a calibrated amplitude measurement, which differed from the relative amplitude measurement in the section above. A calibration procedure was performed prior to each recording, following procedures available in SpectraPro (version 3.32c; Sound Technology, Inc., El Cajon, CA, USA). A 200 Hz sine wave was output from a PC to a Tascam (Montello, CA, USA) DA-3-MP digital tape recorder, which served as an amplifier. The output from the tape recorder headphone jack was fed to a Brüel & Kjaer (Naerum, Denmark) Model 4810 minishaker positioned at the center of the arena. The volume was adjusted to within the range typical of wasp signals (A Joyce, unpubl.). Simultaneously, the output of the laser decoder was sent to SpectraPro which detected relative voltage. Actual output voltage was measured directly using a Fluke® 8060A multimeter (Fluke, WA, USA), and was then entered and used by SpectraPro to calibrate subsequent measurements.

Both dealated and normal males were prepared for recording trials by chilling in vials in a freezer for 10 min, and then removing them to a frozen cold pack. Each male was then placed dorsal side up under a dissecting microscope, and the thorax was pressed gently with forceps to spread the wings. Dealated males were prepared by ablating each wing near the base, leaving approximately $^1/_4$ of each wing. Normal males were mock ablated using forceps to touch the wings but not ablate them.

Courting dealated males were recorded on a chiffon arena prior to wing ablation, and then again after wing ablation, to determine the magnitude of any change in courtship vibration amplitude. Normal courting males were similarly recorded prior to and after mock wing ablation. The order of recordings of normal and dealated males was alternated. The first courting bout for each male was used to measure peak velocity of the courtship vibration. Paired t-tests were used to compare the peak velocities for dealated males prior to and after wing ablation, and for normal males prior to and after mock wing ablation. Ten normal males and 10 dealated males were recorded for comparisons.

The second experiment compared the mating success of normal and dealated males on a good (chiffon) and a poor (glass) artificial mating substrate, according to the mating success and transmission characteristics shown for those substrates (see Results). The expectation was that if courtship signaling relied exclusively on airborne (near-field) sound, then wing ablation, but not substrate, would affect mating success. In contrast, if courtship signaling relied exclusively on substrate vibrations, then substrate and not wing ablation would affect the mating success of males.

Each male, normal or dealated, was prepared for trials as described above and was then paired individually with a female for up to 10 min and mating success or failure was recorded. Eighteen normal and 18 dealated males were tested on both chiffon and on glass substrates, with the order of substrates alternated. Logistic regression was used to assess the relative importance of wings and substrate to mating success (Stata, 2005). Mating success was the dependent variable, and substrate (glass = 0, chiffon = 1) and wings (dealated = 0, winged = 1) were independent predictor variables.

Results

Mating success on five substrates

The mating success of females differed significantly among treatments ($\chi^2 = 17.74$, d.f. = 4, and P = 0.001). Mating success was greatest on chiffon, intermediate on maize and bean, and lowest on glass and plastic (Figure 2).

Transmission of courtship vibrations on five substrates

The measurable mean duration of buzz 1 varied significantly among treatments (Kruskal–Wallis: $\chi^2 = 23.22$, d.f. = 4, and P<0.001). The mean duration on plastic was significantly
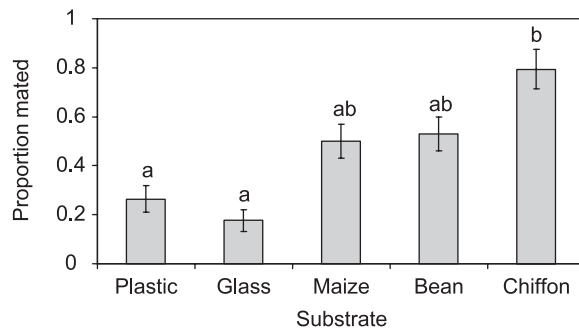
Figure 2 The proportion (mean ± SEM) of *Cotesia marginiventris* females successfully mating on each of five substrates (n = 34 pairs/substrate). Different letters indicate significant differences (P<0.05) (χ^2 test, followed by a Tukey-type comparison for proportions).
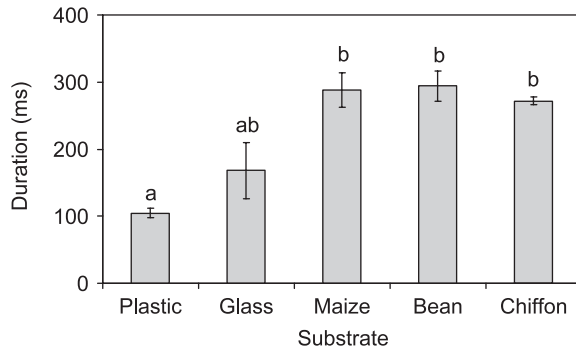
Figure 3 The measurable duration (mean ± SEM) of the buzz 1 component of courtship produced by male *Cotesia marginiventris* on each of five substrates (n = 10 males/substrate). Different letters indicate significant differences (P<0.05) (Kruskal–Wallis test, followed by pairwise comparisons; Siegel & Castellan, 1988).

shorter than on maize, bean, and chiffon, and on glass it was intermediate between plastic and the other substrates (Figure 3).

The mean fundamental frequencies at the beginning of buzz 1 for males on the five treatments ranged from 295 to 319 Hz, and they differed significantly among substrates (ANOVA: $F_{4,45} = 16.24$, P = 0.04; Figure 4). Bean had a significantly lower mean frequency than glass, while the fundamental frequencies on other substrates were intermediate and not significantly different from each other (Figure 4). The mean relative amplitude of the fundamental frequency varied significantly among treatments (ANOVA: $F_{4,45} = 7.43$, P<0.001), and was significantly higher on chiffon than on plastic, glass, and bean, and intermediate on maize (Figure 5).
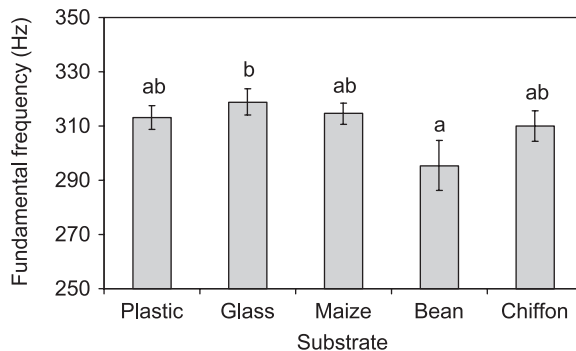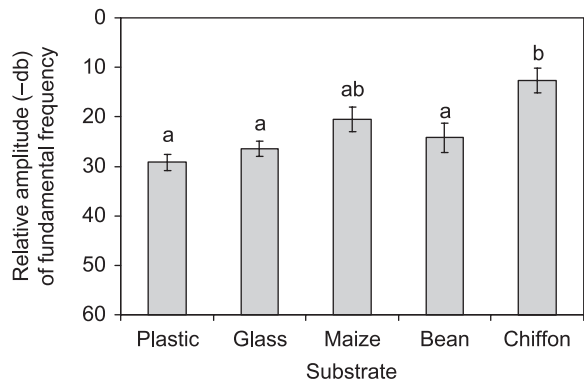

Figure 4 The fundamental frequency (mean ± SEM) at the beginning of buzz 1 (n = 10 males/substrate). Different letters indicate significant differences (P<0.05) [one-way analysis of variance (ANOVA), followed by Tukey's test].

Role of airborne sound and substrate vibration in mating

Both dealated and normal males courted females on chiffon arenas and produced detectable substrate vibrations (Figure 6). The mean peak velocity of courtship vibrations produced by dealated males was reduced by 72% after wing ablation (t = 3.86, d.f. = 8, and P = 0.005), while no change was detected in normal males after mock wing ablation (t = 0.02, d.f. = 7, and P = 0.988; Figure 6).

Both the mating substrate and the presence of wings significantly predicted the probability of mating (Table 1). The odds of mating were 4.3 times greater on chiffon than on glass (P = 0.01), and 9.6 times greater for normal winged males than for dealated males (P<0.001; Table 1). Normal males mated more frequently than dealated males on both substrates, with a higher proportion of normal and dealated males mating on chiffon than on glass, and no mating was observed for dealated males on glass (Figure 7).


Figure 5 The relative amplitude (mean ± SEM) of the fundamental frequency at the beginning of buzz 1 (n = 10 males/substrate). Different letters indicate significant differences (P<0.05) [one-way analysis of variance (ANOVA), followed by Tukey's test].

Discussion

Mating success on five substrates

The mating success of *C. marginiventris* was highest on chiffon, an artificial rearing substrate, followed by the two host plants, bean and maize, and lowest on glass and plastic (Figure 2). Greater mating frequencies on host plants than on artificial substrates might be expected, as plant volatiles are important cues in long range attraction and mate location (McAuslane et al., 1990). However, little is known about how plants and artificial substrates affect close range courtship communication and mating in parasitoids. That
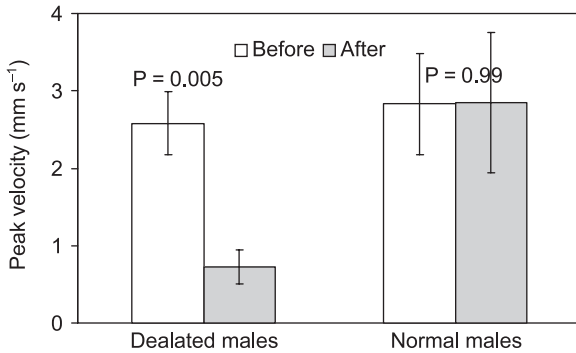
Figure 6 The peak velocity (mean ± SEM) on chiffon arenas from the first courting bout of male *Cotesia marginiventris*, for dealated males before and after wing ablation, and for normal males before and after mock ablation. P-values correspond to paired t-tests (n = 8/dealated males, n = 7/normal males).

mating success was highest on chiffon suggests that physical properties of the substrate are important for mating in *C. marginiventris*. Prior studies showed that vibrations transmit differently through common building materials (Rossing & Fletcher, 2004), and that physical properties of the substrate may affect vibrational signals and mating success in arthropods (Miklas et al., 2001; Elias et al., 2004).

Parasitoid sex ratios that are male biased in laboratory cultures could be partially attributable to a high frequency of virgin parental females. For example, *C. marginiventris* sex ratios were more male biased in several laboratory studies relative to field studies (Novoa & Luna, 1996; Gillespie et al., 1997). *Cotesia melanoscela* (Ratzeburg), a parasitoid of the gypsy moth, had 65% males in mass-rearing programs in which acrylic plastic mating cages were used (Kolodny-Hirsch, 1988), while another rearing program for *C. melanoscela* that used mating cages constructed of paper and cloth, and contained plants, produced 50% males (Webb et al., 1997). In these studies, it is plausible that a high frequency of virgin parental females and consequently male-biased sex ratios could be due to the absence of an appropriate mating substrate.

Table 1 Logistic regression for probability of mating for *Cotesia marginiventris* with two predictor variables, substrate (glass, chiffon) and wings (normal, dealated) (n = 72)

Predictor variable	β coefficient	Odds ratio	Wald's test Z	P
Substrate	1.45	4.29	2.4	0.010
Wings	2.26	9.63	3.56	<0.001

Transmission of courtship vibrations on five substrates

The measurable mean duration of buzz 1 was longer on maize, bean, and chiffon than on plastic, and was intermediate on glass (Figure 3). The durations on maize, bean, and chiffon were similar to the duration of buzz 1 found in a previous study of *C. marginiventris* (Sivinski & Webb, 1989). All *C. marginiventris* males appeared to court similarly on the five substrates tested, although the possibility cannot be eliminated that their courtship behavior changed on plastic or glass. The shorter mean duration of buzz 1 on plastic and glass may have been an underestimate, because its lower amplitude on these substrates was often near the lower detection threshold of the laser vibrometer. Similarly, due to the shorter duration of the courtship vibration on those substrates, female *C. marginiventris* may not detect the entire length of buzz 1 on plastic or glass, leading to reduced mating success. This is consistent with the findings that mating success of *C. marginiventris* was higher on the substrates with longer buzz 1 durations. In a study of the stinkbug *Nezara viridula* L. (Heteroptera: Pentatomidae), longer artificial courtship
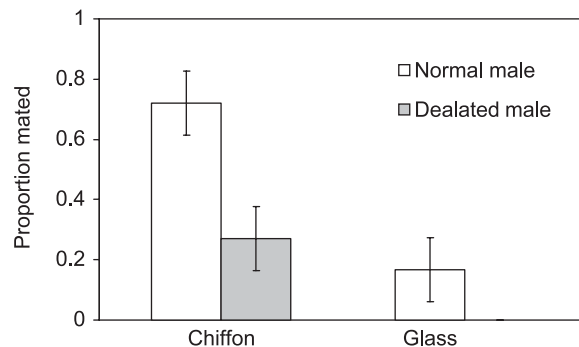
Figure 7 The proportion (mean ± SEM) of normal and dealated *Cotesia marginiventris* males mating on two substrates, glass and chiffon (n = 18 pairs/column). See Table 1 for logistic regression statistics.

signals (600–800 ms) played to males elicited stronger responses relative to shorter signals (<600 ms) (Miklas et al., 2001). Also, species recognition is often influenced by the duration of courtship sounds and vibrations (Greenfield, 2002). For example, *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) lacewings respond more frequently to conspecific relative to heterospecific courtship vibrations, which vary in duration and frequency (Henry et al., 2002).

The mean frequency of courtship vibrations at the beginning of buzz 1 was significantly lower on bean than

on glass (Figure 4). Courtship vibration frequencies may transmit differently through artificial substrates, such as plastic or glass, relative to leaves of host plants, such as maize or bean. Prior studies have shown that leaves can filter signals (Casas & Magal, 2006), allowing transmission of lower frequencies while filtering out higher frequencies. In particular, leaf veins attenuate signals more than leaf lamina (Magal et al., 2000). In our study, the bean leaves had substantially more leaf veins than the maize leaves, which may have contributed to frequency filtering on bean.

The high relative amplitude on chiffon may have been from chiffon being the thinnest substrate evaluated. As noted by others, thicker substrates more than thinner ones can attenuate the amplitude of vibratory signals (Fischer et al., 2003; Cocroft et al., 2006). In this study, *C. marginiventris* mated most frequently on chiffon. Higher courtship vibration amplitudes on some substrates, such as chiffon, may reach a minimum threshold needed to stimulate vibratory receptors sooner (Čokl et al., 2006), and thus be detected more rapidly by females.

Few differences were evident in signal-transmission characteristics between the two host plants that were evaluated. There was no difference in the duration of buzz 1 on bean or corn (Figure 3), and relative amplitudes of the fundamental frequency measured at 0–2 cm from the courting male were similar in both host plants (Figure 5). Cocroft et al. (2006) recorded courtship vibrations of the treehopper *Umbonia crassicornis* (Amyot and Serville) (Homoptera: Membracidae) on a woody host and a woody non-host plant, and did not find significant differences in signal parameters between the two plants at close range (5 cm). However, signal transmission was affected by distance, and by 15 cm there were significant differences in the final frequency and the signal duration. Similarly, few differences in signal transmission between host and non-host plants were observed for courtship vibrations of the lacewings *Chrysoperla downesi* (Smith) and *Chrysoperla plorabunda* (Fitch), although signal amplitude attenuated by a distance of 20 cm (Henry & Martinez Wells, 2004).

Other studies involving arthropods show that substrates influence the transmission of vibrational signals associated with foraging. Substrate density influences the ability of two ichneumonid wasps, *Pimpla turionellae* L. and *Xanthopimpla stemmator* (Thunberg), to locate their hosts (Fischer et al., 2003), perhaps due to the effect of substrate density on signal amplitudes. Substrates also influence the transmission of honeybee waggle dance vibrations. Transmission of artificial vibrations of 250 Hz had greater amplitude in honeybee combs made of beeswax than in artificial plastic combs (Seeley et al., 2005).

Role of airborne sound and substrate vibration in mating

The velocity of courtship vibrations produced by *C. marginiventris* males was within the range of vibrations produced and detected by other species of courting insects. Vibrational courtship signals with velocities as low as 0.1 mm s^{-1} are considered in the range of detection for insects (Michelsen et al., 1982). Courting male *C. marginiventris* produced vibrations with a mean peak velocity of 2.58 mm s^{-1} on chiffon fabric (Figure 6), which is comparable to values of vibrational courtship signals from *N. viridula* males, which vary between 2 and 3.5 mm s^{-1} (Miklas et al., 2001).

Both wings and substrate were important to mating success (Figure 7; Table 1), which suggests that female *C. marginiventris* respond to both substrate vibrations and near-field sound, both detectable by Hymenoptera (Towne & Kirchner, 1989; Meyhöfer & Casas, 1999; Tomov et al., 2003). Some studies that compared the importance of the near-field sound and substrate vibrations potentially used by parasitoids as courtship signals found that mating success was lower in dealated parasitoid males (Miller & Tsao, 1974; Kitano, 1975; Rungrojwanich & Walter, 2000), but the presence of wings appeared more important for some species than others (van den Assem & Putters, 1980; Ruther et al., 2000). A prior study showed that substrate vibrations produced during courtship are essential for mating in *C. rubecula* (Field & Keller, 1993). Vibrational signaling during courtship appears important as well for *C. marginiventris*, and may be used by other species of parasitoids, particularly in the genus *Cotesia* and the family Braconidae (Leonard & Ringo, 1978; van den Assem & Putters, 1980; Sivinski & Webb, 1989).

Courtship signals other than vibrations, such as visual and chemical cues, may have been used by *C. marginiventris*, but it is unlikely that the substrates influenced those signals and impacted female mating success. Chemical cues, such as pheromones, would likely be similar among substrates, although their production or effect may be influenced by the presence of a plant odor (McAuslane et al., 1990). Nevertheless, mating success on chiffon was similar to that on host plants, and significantly higher than on plastic or glass, indicating that physical properties of the substrate and/or physical cues such as courtship vibrations are important for mating success in this parasitoid. As other orders of insects are known to use vibrational communication (Cocroft & Rodriguez, 2005), knowledge of the transmission characteristics of their courtship signals through substrates could be important for efficiently mass rearing them as well.

Acknowledgements

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