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Research

Development, Life History

Developmental Plasticity of the Flesh Fly *Blaesoxipha plinthopyga* (Diptera: Sarcophagidae) on Different Substrates

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Abstract

Forensic entomologists rely on laboratory growth data to estimate the time of colonization on human remains thus extrapolating a minimum postmortem interval (PMI) if assumptions are satisfied. The flesh fly *Blaesoxipha plinthopyga* (Wiedemann) is one species that occurs in casework in Idaho, Texas, and central California. Because of the few laboratory studies on the development of this fly, the following study was conducted to determine if different substrates impact immature development of the species. In this study, flies were reared on different substrates that are likely to be encountered at indoor and outdoor scenes (Wet Sand, Dry Sand, Clothes [Polyester fibers], and Carpet [Polypropylene fibers]) to determine the influence of substrate on larval, intrapuparial, and total immature development times at 25°C, 50% RH, and 14:10 (L:D) h cycle. The results revealed that substrate significantly affected minimum immature development times without affecting the sexes differently; though a female bias in sex ratio was observed consistently. Average minimum larval developmental times were 160–179 h with a significantly faster development in Carpet than in Clothes. Similarly, average minimum intrapuparial developmental times were 331–352 h; fastest on Carpet and the slowest in Dry Sand. For this species, it may be important to consider the substrates encountered at a death scene as they may affect the development of *B. plinthopyga* (Wiedemann) in casework by up to 29 h at 25°C and 50% humidity. These effects will also be important to consider when planning future development studies with the species.

Key words: development, *B. plinthopyga*, flesh fly, PMI, substrates

Forensic entomologists depend on published development data to estimate the time of colonization on remains, consequently predicting a minimum postmortem interval (PMI) (Erzinclliogˆlu 1983) if assumptions are made (Tarone and Sanford 2017). While there are numerous studies on the development of a variety of commonly encountered species of forensic relevance, there are still gaps in knowledge regarding the development of some important species. Sanford (2017) identified several species that are common in casework in Harris County, TX, but which are not associated with a robust body of development research. One such species was *Blaesoxipha plinthopyga*, which has also been reported in other U.S. casework in Idaho (Wells and Smith 2013) and has been observed on animal remains (Denno and Cothran 1975) and in casework (R. B. Kimsey, personal communication) in central California. There is only one development study on this species (Denlinger et al. 1988), from a population that may not be relevant to U.S. casework given observations of genetic differences in forensically important flies (Tarone et al. 2011, Owings et al. 2014), and it only identifies limited information regarding development periods of specific immature stadia, highlighting

the need for additional information on this species, especially for U.S. casework.

Developmental time, a trait used to estimate insect age in forensic entomology, is a quantitative trait that is anticipated to differ as a result of both genetic and environmental factors (Mackay 2001, Conner and Hartl 2004). Understanding the environmental outcomes on quantitative features is achieved by changing one factor while keeping all others consistent, and such experiments have been performed in forensic entomological casework. For example, Kaneshrajah and Turner (2004) exhibited that *Calliphora vicina* (Diptera: Calliphoridae) developed at different rates when reared on different organs even if they raised under the same conditions. *Blaesoxipha plinthopyga* has been encountered commonly in indoor than outdoor casework in Harris County, TX, but is also reported as occurring on remains outdoors in some conditions (Denno and Cothran 1975). Thus, casework with this species may be in association with a variety of substrates that larvae and intrapuparial individuals may be associated. The potential effects of these substrates on immature development, and thus casework reliant on developmental data from this species, are unknown. Therefore, we conducted a study evaluating the impact of wet and Dry Sand, clothing, and Carpet on the development of this species at a common temperature and humidity encountered when it occurs in casework in Harris County, TX (Sanford 2017). Such information will assist in interpretation of casework associated with *B. plinthopyga* and will allow for planning of future projects evaluating the development of the species.

Materials and Methods

Colony Maintenance

The *B. plinthopyga* used in this study have been reported on previously (Lesne et al. 2020). Briefly, they were collected from the field near Easterwood Airport, in College Station, TX, in April and November 2016 using rotting beef liver as bait or from Harris County casework specimens in June 2016 and reared in the lab for less than 10 generation before completion of the experiment. The colony was resupplied with additional specimens in August/September 2017.

Identification was done using morphological keys based on morphology of adult male genitalia (De Carvalho and De Mello-Patiu 2008, Pimsler et al. 2014, Heo 2016). To maintain genetic variation during this period, three colonies were used in this study. Each one contained 100 individuals, from which 30–40 pupae were transferred to the other cages each generation (Tarone and Foran 2006). Representative collected samples from the colony are vouchered in the Texas A&M University Insect Collection (TAMUIC) under voucher no. 729. The oviposition and larviposition rates of this colony are reported in Lesne et al. (2020).

To confirm species identity, molecular identification was used. DNA was extracted from individual heads of three adults using a lithium chloride DNA extraction method. A ≈650-bp sequence of the barcoding region of the COI gene of the mitochondrial DNA was amplified. The consensus sequences have been submitted to GenBank (accession numbers MN172505, MN172506, and MN172507).

Tissue Source

Beef livers were used in this study. Livers were partitioned into 200 g samples and placed into separate Ziploc sandwich bags, labeled, assigned a substrate treatment, and stored in a –20°C freezer until use. Beef liver was supplied as a protein source 1 d before larviposition.

Experiment Design

Females were allowed to deposit their offspring on the beef liver in plastic cups (Bath Plastic Cups, 3 oz, Great Value). Cages were checked every 1 h until larviposition was observed. Approximately 20–25 larvae were removed 1 h after the first observation of larviposition. Larvae collected within the plastic cups for 24 h, were placed inside (roughly in the middle) of 1-liter jars, half-filled with the substrate: Dry clean sand (100 g), Wet Sand (100 g and 5 ml water), and double layer of (≈8 × 8 cm^2) brown Berber Carpet from an Egyptian market with a level loop pile made of heavily pigmented, trilobal, Polypropylene fibers (unknown brands) and double layer of (≈64 cm^2) clean black knit Clothes from an Egyptian market made from Polyester fiber (unknown brands) (Fig. 1). Old fabrics were examined by Polarized Light Microscope and Fourier-transform infrared spectroscopy to determine their identities. Jars were capped with breathable (Kimberly-Clark Global Sales LLC, Roswell, GA), labeled, and housed in an incubator growth chamber with data loggers at 25°C with 14:10 (L:D) h and 50% RH (modified from Byrd and Butler 1996). They were monitored every 12 h for the formation of puparia and adult emergence.

The minimum time to pupariation and adult emergence were recorded for replicates of each trial. Nine replicates in three trials, with three replicates per treatment in each trial, were examined to study the substrate effect on the developmental time of both immature stages larvae, pupae (we are aware that intra-puparial development differs from pupal development per Martín-Vega et al. 2016, thus references to pupa in this document are for simplicity in language) and on total life cycle.

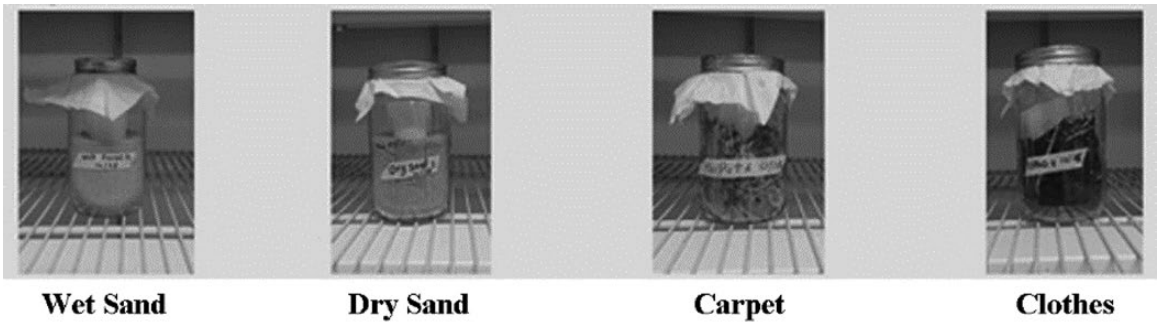

Fig. 1. Different rearing substrates.

The total number of adults emerging after larviposition was reported in counts and as a percentage. Percentages and counts of emerged male and female adults are also reported. As are eclosion times for all members of those cohorts. Three replicates (from the third trial only) were examined to study the effect of substrates on the sex of emerged adults.

All statistics were performed using SAS JMP 15 statistical software (Holmes et al. 2013) and graphs evaluating fly development were produced using the R (Version 3.5.3). The duration of total larval period, pupal period, and larvae to adult period on each substrate were calculated. Analysis of variance (ANOVA) and regression were used to evaluate linear models assessing effects (Substrates, Trials, Temperature, Relative Humidity, Substrate × Temperature, Substrate × Relative Humidity) on development progression. Tukey–Kramer HSD report significant connecting letters for all pairs of substrates.

Results

Fiber Analyses

The PLM work showed that the Carpet consisted of mainly synthetic heavily pigmented (delustered), trilobal, Olefin: Polypropylene fibers. The PLM of Clothes showed that it is consistent with Polyester fiber. The results are confirmed by FTIR analysis (the FTIR work was done with a single bounce ATR accessory) (Fig. 2).

The larvae were feeding continuously through the first 4–5 d on the liver and then stopped feeding, left the liver, and wandered on the substrate. Before pupation, the larvae begin to penetrate the substrate embedding themselves to metamorphose (Fig. 3). Adults started eclosing after ~19–24 d.

Developmental Plasticity

The average minimum development times for each stage and the whole life cycle on the different substrates are shown in Table 1, Fig. 4, and Supp Data (online only) under the conditions recorded by the data loggers in Table 2. The minimal larval period for the *B. plinthopyga* population ranged from 160 to 179 h between different substrates showing a 19-h difference between the fastest development in Carpet and the slowest development on Clothes with a significant difference between them ($P = 0.0227$; Fig. 4). The minimal pupal duration was 331–352 h showing a 21-h difference between the fastest development in Carpet and the slowest in Dry Sand with a significant difference observed between them ($P = 0.0154$; Fig. 4). The minimal total life cycle required from the first larval instar to adult emergence was 491–520 h showing a 29-h difference between the fastest development in Carpet and the slowest in Dry Sand with significant differences observed between them ($P = 0.0031$) and a significant difference between Carpet and Clothes ($P = 0.0076$; Fig. 4).

Regression analysis showed that the total effects (Substrates, Trials, Temperature, Relative Humidity, Substrate × Temperature, Substrate × Relative Humidity) exhibit significance of models evaluating development in all *B. plinthopga* developmental stages: the larval developmental period ($P = 0.0007$) with $R^2 = 0.78$, the pupal developmental period ($P = 0.001$) with $R^2 = 0.77$, and the total life cycle duration ($P = 0.0001$) with $R^2 = 0.91$, respectively, as shown in Table 3.

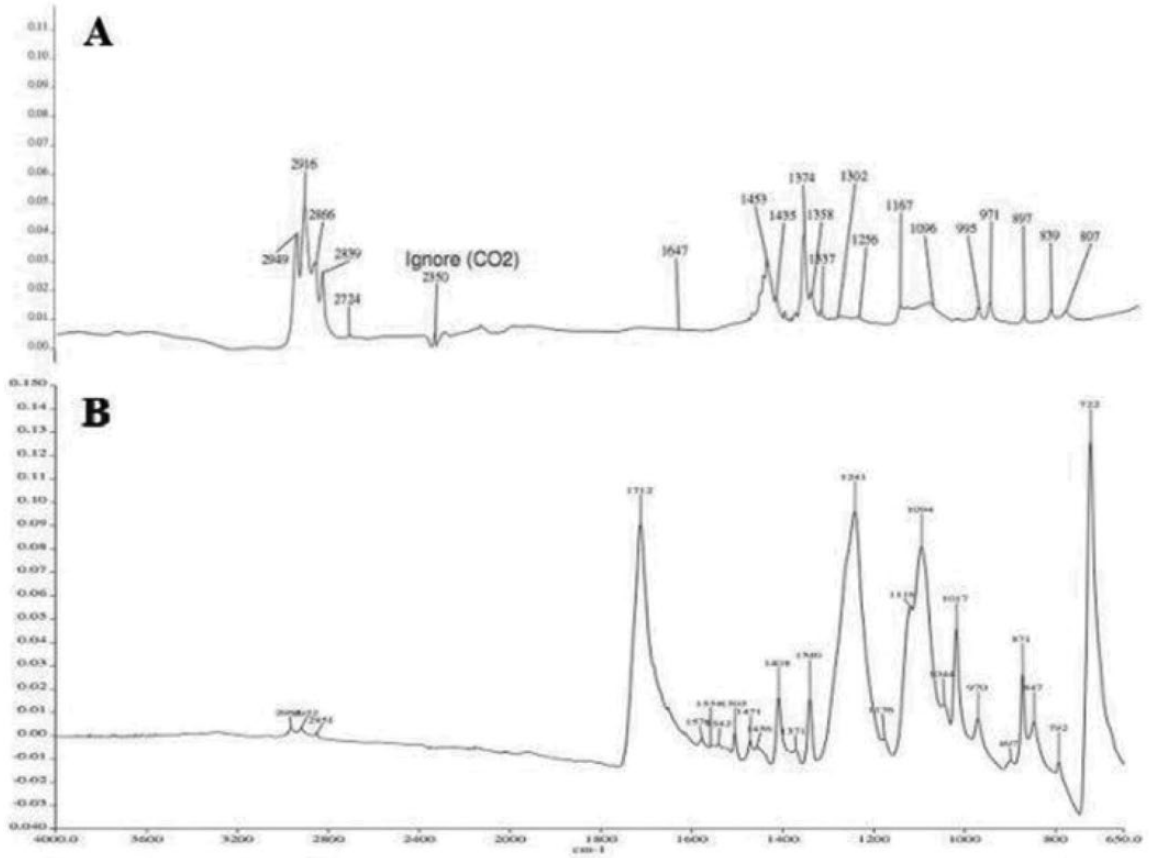

Fig. 2. Fourier-transform infrared spectroscopy (FTIR) (**A**) shows the scan of the tested Clothes (**B**) shows the scan of the tested Carpet.

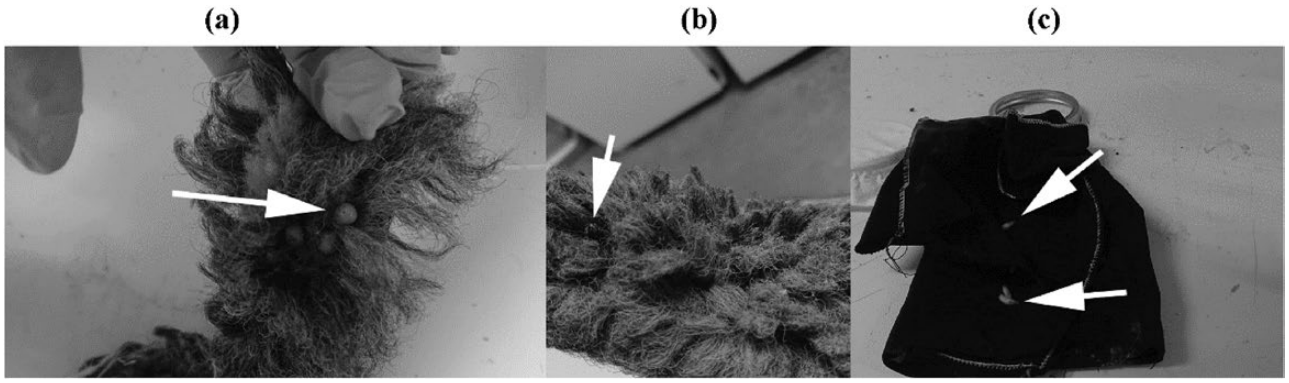

Fig. 3. (A) Larvae penetrate inside the Carpet. (B) Pupae inside the Carpet. (C) Larvae inside the Clothes.

Table 1. Average minimum developmental times ± SDs in hours for rearing of *B. plinthopyga* on different substrates

Substrates	Minimum larval duration (mean ± SDs)	Minimum pupal duration (mean ± SDs)	Minimum total life cycle duration (mean ± SDs)
Wet Sand	162.67 ± 16.00	346.67 ± 29.66	509.34 ± 45.66
Dry Sand	168.00 ± 12.00	352.00 ± 29.39	520.00 ± 41.39
Carpet	160.00 ± 26.83	330.67 ± 28.84	490.67 ± 55.67
Clothes	178.67 ± 12.65	338.67 ± 18.67	517.34 ± 31.32

The substrate showed significant effects on the larval developmental period (P = 0.0124) and on the total life cycle duration (P = 0.0017) as shown in Tables 4 and 6. In addition, temperature variation, resulting from the differences recorded inside the incubators within trials (Table 2), showed a significant influence on the pupal duration and the total life cycle (P = 0.0008 and P = 0.0001) as shown in Tables 5 and 6, respectively. Whereas relative humidity showed a significant influence only on the total life cycle (P = 0.0274) as shown in Table 6. Moreover, the trials showed similar significant effects on the larval developmental period, the pupal duration, and the total life cycle (P = 0.0001) as shown in Tables 4–6 and substrate by trial interaction significantly impacted both the larval developmental period (P = 0.0301) and the total life cycle (P = 0.0383) as shown in Tables 4 and 6, respectively.

The interaction of substrate effects with each trial showed that the 2nd trial with Carpet interaction significantly impacted both the larval developmental period (P = 0.005) and the total life cycle (P = 0.0087), whereas the second trial with Dry Sand interaction significantly impacted only the total life cycle (P = 0.0489) as shown in Table 7.

Among all substrates, Carpet significantly affected the larval developmental period, the pupal developmental period, and the total life cycle period (P = 0.0383, P = 0.0322, and P = 0.0003), respectively. However, Polyester Clothes showed significant effects on the larval developmental period only (P = 0.0027), whereas Dry Sand showed significant effects on the total life cycle period only (P = 0.0205) as shown in Table 8.

For studying the effect of the substrate on the number and sex of adult emerged on each substrate, it was found that flies emerged similarly across substrates. The lowest emergence rate occurred with Wet Sand at 88.98% and the highest percent of immature emergence with Carpet at 100% across all three replicates (Table 9; Supp Data [online only]). For these trials, whole distributions of development time are also observable and generally follow the patterns observed with minimum development time.

In addition, a sex bias in the species was observed, with no difference in emergence between the sexes (the emergence of males and females appear to overlap). The largest difference observed between female and male was recorded in Carpet (58.84:41.16) compared to the lowest in the Clothes with a proportion (54.27:45.73) as shown in Table 9 and Fig. 5.

Discussion

The importance of this species for forensic casework in the United States was recently recognized when Wells and Smith (2013) recorded its first appearance on decomposing human remains in Idaho, United States. In Harris County, TX, Sanford (2017) recorded *B. plinthopyga* as a primary colonizer of indoor human remains. While the species is encountered as a marker of indoor casework in Harris County, Denno and Cothran (1975) found it commonly outdoors in certain seasons; thus, information on the development of the fly is potentially relevant to outdoor casework in some situations. Therefore, there is a need for studying the development of these species from larviposition to adult emergence in regions other than Central America. Such information will facilitate the correct determination of fly age in death investigations. This study evaluated performance of a Texas population under conditions similar to those reported for Texas casework, and with substrates that could be encountered in both indoor and outdoor casework.

It is worth noting the egg stage was ignored from this study as eggs deposited by many in this family are likely to hatch within minutes or eggs are not viable (Pape 1987). Furthermore, most wild adults are generally expected to larviposit (Saloña Bordas et al. 2007). Pimsler et al. (2014) observed that eggs of *B. plinthopyga* hatched 4 h post-oviposition in a lab population, but did not describe them further and it is not clear how relevant such eggs are to wild populations. Recently, Lesne et al. (2020) demonstrated that oviposition varies widely in the lab and oviposited individuals take on average 6 h, but may take as much as 27 h to hatch. Thus, for

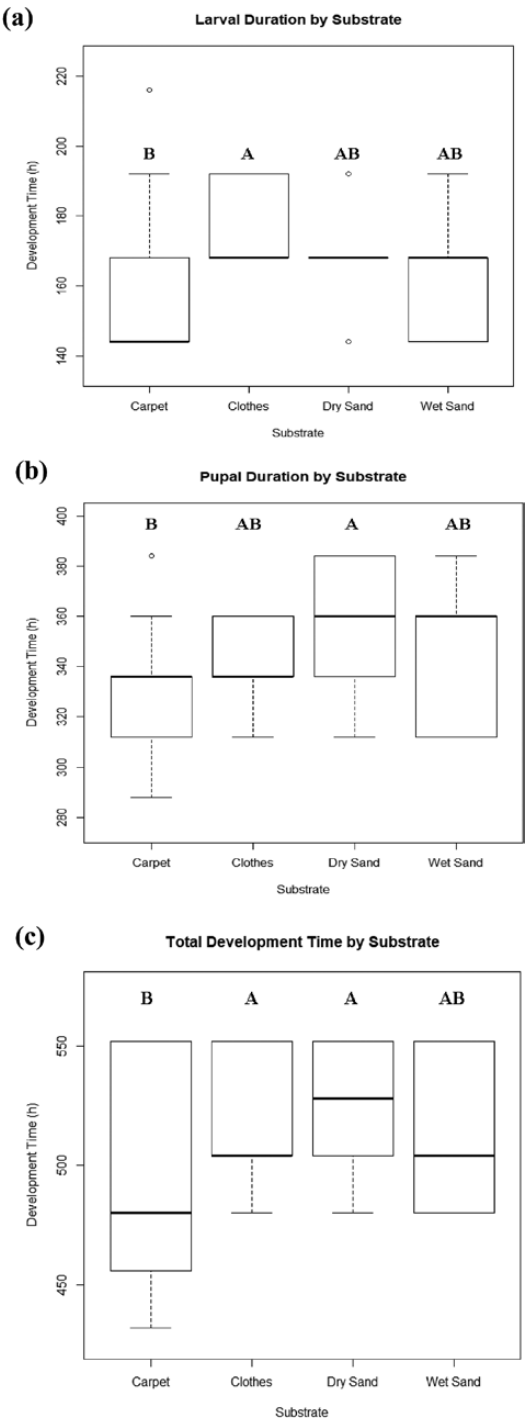

Fig. 4. Variation in minimum development times of the *B. plinthopyga* life cycle by substrate type. Boxplots of developmental time (hours) for each of the three liver-fed trials. The dark black line within the box represents the median developmental hours. The box represents the developmental times between the 25th and 75th percentiles, and the 'whiskers' (outermost lines) represent 1.5 times the interquartile range. (A) Larval developmental period on the different substrates with the Tukey–Kramer representative figure. (B) Pupal developmental period on the different substrates with the Tukey–Kramer representative figure. (C) Total life cycle period on the different substrates with the Tukey–Kramer representative figure. Tukey–Kramer results report significantly different groups.

this study, eggs were not considered, and any variability observed in this study could be added to any potential variability in oviposition rates and hatch times.

Table 2. Average temperature and relative humidity ± SDs per three trials for rearing of *B. plinthopyga* on different substrates

Trials	Temperature (mean ± SDs)	Relative humidity (mean ± SDs)
1	24.39 ± 0.28	50.47 ± 1.09
2	24.82 ± 0.4	53.62 ± 2.98
3	24.51 ± 0.23	50.16 ± 1.03

Table 3. The total parameter effect test results from an ANOVA on larval, pupal, and total life cycle developmental time for *B. plinthopyga*

The total effects	Larval duration	Pupal duration	Total life cycle duration
P-value	0.0007*	0.001*	0.0001*
R^2	0.78	0.77	0.91

*Designates statistical significance ($P < 0.05$).

Table 4. The parameter effect test results from an ANOVA on the *B. plinthopyga* larval developmental time

Parameter	Sum of squares	df	*F*-ratio	*P*-value
Substrates	1,840	3	4.6739	0.0124*
Trials	4,720.3395	1	35.9714	0.0001*
Temp	318.3906	1	2.4263	0.135
RH %	97.1338	1	0.7402	0.3998
Substrates × Trials	1,437.6881	3	3.652	0.0301*
Substrates × Temp	1,140.9922	3	2.8983	0.0604
Substrates × RH %	436.6013	3	1.109	0.3688

*Designates statistical significance ($P < 0.05$).

Table 5. The parameter effect test results from an ANOVA on the *B. plinthopyga* pupal developmental time

Parameter	Sum of squares	df	*F*-ratio	*P*-value
Substrates	2,352	3	2.6975	0.0733
Trials	8,833.2898	1	30.3926	0.0001*
Temp	4,585.7077	1	15.778	0.0008*
RH %	628.701	1	2.1632	0.1569
Substrates × Trials	106.6842	3	0.1224	0.9458
Substrates × Temp	1,962.3646	3	2.2506	0.1137
Substrates × RH %	1,027.3709	3	1.1783	0.343

*Designates statistical significance ($P < 0.05$).

Through these experiments, results show that the substrate can significantly affect the development of *B. plinthopyga*, potentially affecting average minimum total development by as much as 29 h (if development of eggs is discounted) under common casework conditions for Harris County, TX. These outcomes highlight the value of considering the substrate when rearing the fly for subsequent development research or for interpreting developmental data sets developed for the species in casework.

The effects of fibers on development have several potential explanations that could be explored further. The fibers encountered in the study have different thermal conductivity and densities (Abdel-Rehim et al. 2006, Morton and Hearle 2008). These factors could have contributed to changes in development and could be further

investigated. For substrates commonly encountered indoors there were several compelling findings. *Blaesoxipha plinthopyga* showed faster larval development in the Carpet than in the Clothes, which may be due to the low density of the Carpet (0.91 g/cm^3) compared to that of Polyester (1.39 g/cm^3) as low-density substrates are more penetrable, facilitating pupation, whereas high-density substrates are less penetrable for post-feeding larvae and therefore impede pupation; similar to how Holmes et al. (2013) noted impacts of substrate on pupation. Moreover, the thermal conductivity of the Polyester (0.036 W/(mK)) is lower than Polypropylene (0.27 W/(mK)) at room temperature (Weidenfeller et al. 2004, Tilioua et al. 2012) and Polyester samples have higher specific heat resistance than Polypropylene and it is known that the increase of temperature induce the insect to develop more rapidly (Byrd and Butler 1996).

Humidity/moisture may be playing an important role in this study, potentially affecting the development of *B. plinthopyga* during their larval and intrapuparial stages depending on the substrate they are grown on. Moisture defined the difference in Sand treatments. Moisture is known to impact fly development, including carrion flies; thus, it is not surprising that the different treatments in this experiment may differ due to alterations in moisture available to the larvae and pupae (Smith and Jones 1991, Tarone and Foran 2006, Holmes et al. 2012, Holmes et al. 2013, Yee 2013).

Previous reports have suggested that sexual dimorphism may be important to improving forensic analyses of insects (Picard et al. 2013, Frątczak-Łagiewska and Matuszewski 2018). The analysis carried out in this study revealed a sex bias across the experiment, with approximately 10% more females than males. Such a sex ratio may increase the reproductive potential of the species (Tano et al. 2010, Dao et al. 2017), which may be an important detail to consider for the Sarcophagidae, given their relatively low fecundity compared to other competing species, such as blow flies. However, while there was a sex bias, the sexes appeared to develop at similar rates (observations of males coincided with observations of females), suggesting correction for sex in *B. plinthopyga* may not be necessary (at least under the conditions and for the traits tested here).

Table 6. The parameter effect test results from an ANOVA on the *B. plinthopyga* total life cycle developmental time

Parameter	Sum of squares	df	*F*-ratio	*P*-value
Substrates	4,736	3	7.3227	0.0017*
Trials	26,486.137	1	22.7735	0.0001*
Temp	7,320.746	1	33.9576	0.0001*
RH %	1,220.074	1	5.6594	0.0274*
Substrates × Trials	2,189.085	3	3.3847	0.0383*
Substrates × Temp	123.087	3	0.1903	0.9017
Substrates × RH %	230.566	3	0.3565	0.785

*Designates statistical significance ($P < 0.05$).

Table 7. *P*-values for substrate across trials interaction during *B. plinthopyga* development using Tukey–Kramer HSD

Substrates × trials	Larval duration	Pupal duration	Total life cycle duration
Carpet × second trial	0.005*	0.7057	0.0087*
Dry Sand × second trial	0.0639	0.6306	0.0489*

*Designates statistical significance ($P < 0.05$).

Table 8. *P*-values for substrates during *B. plinthopyga* development in the parameter effect test

Substrates	Larval duration	Pupal duration	Total life cycle duration
Carpet	0.0383*	0.0322*	0.0003*
Clothes	0.0027*	0.5060	0.0737
Dry Sand	0.8423	0.0557*	0.0205*

*Designates statistical significance ($P < 0.05$).

Conclusions

Based on these analyses, we can conclude that casework-relevant substrates influence development observed in this study in the larval and intrapuparial stages of *B. plinthopyga*. Such impacts could affect forensic entomology casework estimates by a day or more at common casework conditions. These impacts could be greater (or lesser) depending on conditions and casework details and accentuates the importance of the substrate at a death scene affecting the development of *B. plinthopyga*. For soil substrates potentially

Table 9. The total number of adults emerging after larviposition reported in counts and as a percentage

Substrate	Average of larviposition (mean ± SDs)	Range of development times (mean ± SD) (median)	Average of adult emerged (mean ± SDs)	Percentage of emergence	Male:female
Wet Sand	26 ± 2.65	19–24 (21.44 ± 2.22) (22)	23 ± 2.65	88.98	10:13 (43.93:56.07)
Dry Sand	28 ± 7.64	19–24 (21.56 ± 2.04) (22)	26 ± 6	92.86	11:15 (43.03:56.97)
Carpet	18 ± 1.53	18–22 (20.44 ± 1.9) (21)	18 ± 1.53	100	7:11 (41.16:58.84)
Clothes	18 ± 4.04	20–22 (21.56 ± 0.77) (22)	17 ± 4.51	94.44	8:9 (45.73:54.27)

Percentages and counts of male and female adults are also reported with the range of developmental times per days for each sex.

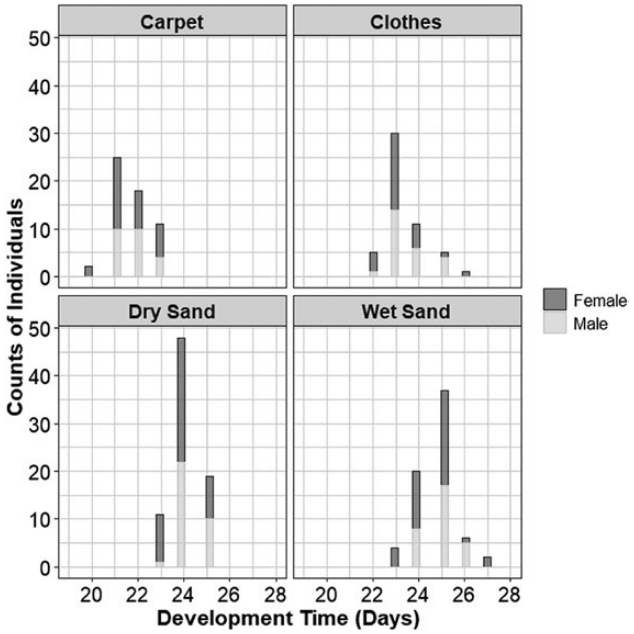

Fig. 5. Number of days that it took the counted adult male (gray) and female (dark) *B. plinthopyga* to emerge after larviposition when reared on different substrates. Development in sand took a few days longer to complete compared to development in fibers. Carpet as a pupariation substrate resulted in 1–3 d faster development at 25°C and 50% humidity than other substrates. Data are from third trial.

encountered outdoors, *B. plinthopyga* developed more rapidly in the Wet Sand compared to Dry Sand, suggesting that recent precipitation and humidity should be considered for casework with this species. For indoor substrates, *B. plinthopyga* showed slower larval and pupal development on the Polyester Clothes than in the Polypropylene Carpet, also suggesting that fibers in the presence of larvae and puparia should be considered when making predictions from immatures of this species sampled during indoor death investigations.

Supplementary Data

Supplementary data are available at *Journal of Medical Entomology* online.

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