ABSTRACT

Composting plant waste is a beneficial practice commonly used by American gardeners, but disposal of infested fruit directly into the environment creates a potential pathway for introduction of insect pests. This study estimates the likelihood of adult emergence for exotic fruit flies (Tephritidae) from residential composting in south Florida. Ripe grapefruits, *Citrus × paradisi* Macfad., were infested with the Caribbean fruit fly, *Anastrepha suspensa* (Loew). Half of the infested fruit was placed onto outdoor compost piles and half was maintained under controlled laboratory conditions. Adult fly emergence was recorded daily for 30 d from both the compost piles and control bins. Compost temperature, air temperature, relative humidity, and precipitation were monitored, and the study was repeated 4 times under different seasonal conditions. Despite high mortality of flies from the composted fruit relative to control fruit, the overall risk of a potentially mated female emerging from composted fruit was calculated to be ~10%. Of the environmental factors evaluated, compost temperature was found to have a significant effect on adult emergence. Mortality approached 100% in piles with maximum compost temperatures ≥48°C. This report provides experimental data in support of quantitative risk analysis for a tephritid-compost pathway.

Key Words: Caribbean fruit fly, risk assessment, pathway analysis, quarantine pest

In pest risk analysis, a pathway is any means that allows the entry or spread of a pest species. Pathway risk analysis entails identification of viable pathways, assignment of probabilities to the sequence of events involved (e.g., entry, reproduction, establishment, etc.), and assessment of the consequences of pest introduction (Hennessey 2004). Invasive fruit flies, family Tephritidae, constitute a serious threat to U.S. agriculture, as evidenced by a congressional appropriation of $57 million to fruit fly risk management programs in 2005 (USDA-APHIS 2006a). This threat is pronounced in Florida due to a favorable climate, availability of hosts, and the large volume of foreign produce entering the state’s ports. From Dec 2003 to Sep 2006, there were over 1200 interceptions of live tephritids at the Miami International Airport, primarily from larval-infested fruit concealed within baggage.
(2048 larvae, 40 puparia, 15 adults; USDA-APHIS 2006b). The Mediterranean fruit fly, Ceratitis capitata (Wiedemann), is one of the most destructive pests worldwide. Several outbreaks of C. capitata have occurred in Florida, with the most recent invasion in 1997-1998 (Silva et al. 2003). Of the 198 recognized species of Anastrepha fruit flies (USDA-ARS 2007), only 1 species of economic importance is established in Florida, the Caribbean fruit fly, A. suspensa (Loew). Restricted to the Bahamas and Greater Antilles until the 1960s (reviewed in Weems et al. 2001), A. suspensa is now common in the southern half of the peninsula where it impacts production of citrus, guava (Psidium guajava L.), and other subtropical fruits (Greany & Riherd 1993). Several other Anastrepha species pose a threat to Florida, including A. ludens (Loew), A. obliqua (Macquart), A. fraterculus (Wiedemann), A. striata (Scherer), and A. grandis (Macquart). Anastrepha ludens represents a special concern because it has an affinity for grapefruit (Citrus × paradisi Macfad.) and Florida is one of the world’s leading producers (Weems et al. 2004).

Anastrepha females have well-developed ovipositors, inserting their eggs beneath the skin of host fruits, where larvae feed and develop within the flesh (White & Elson-Harris 1992). Consequently, larval infestations are difficult to detect through visual inspections of intact fruit. At ports of entry, quarantine inspectors check incoming shipments by cutting open a small sample of fruit (typically no more than 2 percent) and searching for eggs or larvae (USDA-APHIS 2006c). The efficacy of this procedure has been evaluated in laboratory tests, and Gould (1995), using trained agricultural inspectors, concluded that only about 35% of grapefruits infested with A. suspensa could be detected through manual dissection. A comparison of 6 different inspectors revealed considerable variability, with the percentage of larvae detected ranging from 8% to 49% (Gould 1995). Thus, it is highly likely that some infested fruit may evade detection and, if not subjected to appropriate quarantine treatments, contain viable eggs or larvae when distributed to consumers. Subsequent to entry as immature stages within host fruit, pest Anastrepha may spread if that fruit is discarded directly into the environment.

Two risk assessment studies have estimated the amount of infested fruit discarded by consumers into the environment. Wearing et al. (2001), reviewing potential spread of coding moth (Cydia pomonella L.) via imported cherries, determined that up to 5% was discarded in suburban New Zealand. For urban Japan, Roberts et al. (1998) calculated 0.5% disposal of apples infected with Erwinia amylovora (Burr.), the pathogen of fire blight. Although no data are available for the amount of fruit infested with fruit flies that is discarded by Americans, the USDA-APHIS (2004) has estimated it to be 5% for avocados imported from Mexico, choosing the higher proportion from the 2 former studies so as not to underestimate the risk.

With today’s emphasis on environmentally-friendly practices there is increased interest in organic gardening, including composting of plant waste. A quick internet search will yield much information on residential composting, disseminated to the public by federal, state, and local agencies (e.g., EPA 2006, USDA-NRCS 2004, Sarasota County 2006, UF-IFAS 2004). But unlike commercial organic recycling facilities which are regulated in Florida, composting in backyard, micro-scale, and farm sites is exempt from state regulation, Florida Administrative Code 62-709.300(10). As a result, compost practices vary widely among consumers, creating a potential means for spread of pests into susceptible areas.

This study simulated disposal of Anastrepha-infested grapefruit on backyard compost piles in south Florida to estimate the likelihood of emergence of mated females. The results provide experimental data to facilitate quantitative risk analysis for a tephritid-compost pathway, information currently lacking in the scientific literature.

**MATERIALS AND METHODS**

Infestation

Anastrepha suspensa were obtained from a laboratory colony maintained at the USDA-ARS, Subtropical Horticulture Research Station, Miami, FL. Insects were reared at 25°C (±2), 70% RH, and a photoperiod of 12:12 h (L:D), by methods previously described (Kendra et al. 2006). Approximately 3500 sexually mature (10-12 d old), mated females were placed in each of 2 infestation cages (94 × 51 × 51 cm) constructed from PVC frames covered with mesh pollination bags (Delstar Technologies, Middletown, DE). Each cage contained 60 ripe, Florida-grown grapefruit (Citrus × paradisi Macfad., cv White Marsh), arranged in a single layer, and oviposition was allowed for 7 d under the same environmental conditions used for rearing. Infested fruit (120 total) was then removed from the cages and randomly divided into 3 groups: Compost fruit (50), Control fruit (50), and Inspection fruit (20). All grapefruits were held in the laboratory while the Inspection fruits were cut open at 2-3 d intervals (5 fruits per sample) to monitor progress of larval development and to approximate the level of infestation. When the majority of the larvae had reached the third (final) instar, typically 9 d after oviposition, field tests were initiated.

Field Tests

Ten replicate compost piles (~1.5 m³ each) were constructed for each field test. Piles were contained within square wooden frames (1.2 × 1.2 m), spaced 5 m apart, located under partial shade.
Compost material consisted of a 1:1 mixture of wood chips and fresh grass clippings, applied in alternating layers, according to the guidelines provided by the USDA Natural Resources Conservation Service (USDA-NRCS 2004). Piles were turned weekly to promote active decomposition, then left undisturbed once grapefruit had been added. Compost piles received no manual watering during the study, only natural rainfall. For each of the 10 compost replicates, 5 infested fruit were placed on top of the pile, nestled 4-5 cm deep, and left in place for 30 d. For the first 5 d, the piles were left exposed to allow access of potential predators and competitors. Then each pile was covered with a pyramidal screen cage (modified from Raney & Eikenbary 1969) fitted on top with the upper assembly from a boll weevil trap (Great Lakes IPM, Vestaburg, MI). The cages were sealed with hook-and-loop tape (Velcro Industries, Manchester, NH), and the screen at the base of each cage was pulled tightly over the wooden frame and secured with bricks and soil, creating a closed system for the remainder of the field test. Adult fly emergence (number and sex) was recorded daily for the next 25 d. Temperature of compost beneath the fruit (15 cm depth) was monitored throughout the 30-d period, using compost thermometers (Reotemp, San Diego, CA). Air temperature, relative humidity, and rainfall data were obtained from the SHRS weather station. At the completion of the tests, the upper layer of compost and any remaining fruit was collected for sampling with Berlese funnels.

To accompany each field test, 10 replicate controls were set up in polyethylene bins (51 × 31 × 15 cm; U.S. Plastic Corp., Lima, OH) maintained in the laboratory under the same conditions used for rearing the _Anastrepha_ colony. Each bin contained 5 infested fruit placed above vermiculite (8 cm deep), and bins were enclosed in clear plastic cages (Bug Dorm-2; BioQuip Products, Rancho Domínguez, CA). Vermiculite was sifted weekly to collect wandering larvae and puparia, which were held for adult emergence for the same 30-d period monitored in the field. The controls showed that 30 d was sufficient to allow all larvae to complete development. The study consisted of 4 field tests (and controls), conducted from the late summer through early spring of 2004-2005. The 30-d monitoring periods were as follows: Summer test from 23 Aug to 22 Sep 2004; Early Fall test from 26 Sep to 26 Oct 2004; Late Fall test from 29 Oct to 28 Nov 2004; and Spring test from 28 Feb to 30 Mar 2005. This time period allowed us to evaluate composting of infested fruit under a variety of environmental conditions, with the first 2 tests conducted during the wet season, and the second 2 tests conducted during the dry season in south Florida.

Statistical Analysis

The levels of infestation (number of larvae per fruit) varied among the 4 field tests, therefore adult emergence counts were converted to percent emergence from compost fruit relative to control fruit prior to statistical analysis. Differences in percent emergence among the tests were analyzed by one-way analysis of variance (ANOVA) followed by Tukey-Kramer HSD test for mean separation (P = 0.05), with JMP (SAS Institute 2006). To evaluate environmental factors during the 4 tests, ANOVA followed by mean separation was performed initially. If significant differences were found among tests, then regression analysis was used to explore the relationship with percent fly emergence, which was then graphed with Sigmaplot 10 (Systat Software, Inc. 2006). Additionally, two-way ANOVA was used to assess potential interaction effects of environmental factors on percent emergence. Actual fly counts were analyzed in 1 instance, to determine if there was a difference in adult emergence based on sex. Two-way ANOVA with interaction, followed by Student’s t-test, was performed separately on compost and control treatments to evaluate the number of males and females emerging in each test.

**RESULTS AND DISCUSSION**

Manual grapefruit dissections indicated the following levels of infestation at the start of the 4 field tests: 19.0, 22.4, 34.8, and 8.2 larvae per fruit, respectively. In light of Gould’s (1995) conclusions regarding the reliability of this detection procedure, these values were used only as relative indicators of the degree of infestation among the tests. Infestation was considered to be moderate for the Summer and Early Fall tests, high for the Late Fall test, and low for the Spring test. Differences in levels of infestation were not unexpected since Marsh grapefruit susceptibility to _A. suspensa_ infestation has been shown to vary seasonally in relation to fruit senescence (Greany et al. 1985). All 3 instars were represented in the infested fruit of each test, but on average, 62.4% of the larvae were in the third instar when field tests were initiated. The percentages for the 4 tests were 75.8, 50.9, 66.8, and 56.1, respectively. This developmental stage was chosen to provide conditions comparable to those used in previous investigations of _Anastrepha_-infested grapefruits (e.g., Hallman et al. 1990; Hallman 1994; Gould 1995), but more significantly, use of fruit infested with third instars represented a scenario that would maximize the likelihood of adult emergence and thereby estimate the greatest potential risk of pest escape and establishment via a compost pathway. Shortly after infested fruit was placed on the piles, third instars would exit the fruit, burrow into the compost, and pupate. By restricting the field tests to this time window, any reduction in adult emergence would result primarily from conditions in the compost environment. Also, a third instar/wandering larva scenario would...
represent the point at which consumers would most likely notice an insect and discard the fruit.

Overall, the mean emergence of adult flies (males and females) from composted fruit was 11% of that observed emerging from the control treatments (Table 1). In terms of risk assessment, the critical component is the percentage of females that survive the composting process, successfully mate, and escape into the environment. For the purposes of this study, 2 criteria were adopted to address that component. First, risk was defined as the presence of a single mated female emerging from compost. Second, a female was considered potentially mated if at least 1 male emerged from the same compost pile. These are valid assumptions since a single A. suspensa female (wild strain) has been shown to lay an average of 1.9 (±0.3) eggs/day over a lifespan of 73.6 (±4.9) days (Sivinski 1993), and males of A. suspensa have been documented to engage in multiple matings (Teal et al. 2000). Based on these criteria, percent emergence of potentially mated females was calculated for each field test, and presented in Table 2. The probability of a mated female emerging from compost was estimated to be 10% for the study, with the greatest risk (22% emergence) observed during the Spring 2005 test. The lowest risk was seen with the Early Fall 2004 test, when no mated females emerged. In addition, the compost treatments had a significant difference in mean number of adults emerging based on sex (F = 6.27; df = 1, 79; P = 0.014). The average number of females emerging from compost piles was 1.15 times greater than the number of males (t = 2.50; df = 75; P = 0.014), suggesting differential survival of females within the composting environment. This bias was not observed in fly emergence from the control fruits (F = 3.43; df = 1, 79; P = 0.068).

Adult emergence data indicated approximately 90% mortality of Anastrepha in the late larval and pupal stages when exposed to composting conditions. Factors in the physical environment during the 4 seasonal tests (Table 3) were assessed for potential effects on fly emergence. The Spring 2005 test, which had significantly higher percent emergence than the other replicates (F = 6.40; df = 3, 39; P = 0.002, Table 1), was characterized by having the lowest compost temperatures and the lowest precipitation. Conversely, the test conducted in Early Fall 2004 had the lowest fly emergence, and the highest values for both compost temperature and precipitation. Therefore, these 2 environmental factors were analyzed further. For compost temperature, the mean maximum values were used for analysis, since maximum temperatures were more likely to contribute to fruit fly mortality. The interaction between maximum compost temperature and precipitation had no effect on the percentage of flies that emerged (F = 1.66; df = 1, 39; P = 0.205), but there was a significant effect due to maximum compost temperature (F = 6.52;
The mean maximum temperature of compost piles was significantly different for each of the 4 seasons (F = 137.6; df = 3, 39; P < 0.0001; Table 3). The relationship between maximum compost temperature and percent emergence (Fig. 1) showed a marked clustering of the compost treatments: the Spring 2005 test with the highest emergence was at the lower end of the temperature scale, and the Early Fall test with the lowest emergence was at the upper end. Adult emergence decreased with increasing compost temperature, predicting mortality to approach 100% as the temperature rises above 48°C. Since the host fruits were placed 5 cm into the compost, and A. suspensa larvae typically pupate at a depth of 1-3 cm into the substrate (Hennessey 1994), the actual temperatures experienced by the insects were probably slightly less than that recorded by the compost thermometers inserted to 15 cm. It should also be noted that the heat of the compost, since generated by biologically active decomposing organic matter, is relatively stable and not subject to rapid changes due to fluctuations in weather conditions. Thus, the insects within an active compost environment are exposed to long periods of sustained high temperatures. Under “hot composting” conditions, as defined by the USDA-NRCS (2004), internal compost can reach 43-71°C, temperatures sufficient to kill most insects, weed seeds, and plant pathogens. The compost temperature of 48°C estimated for fly mortality in this study is consistent with the range of temperatures used in quarantine heat treatments of commercial fruit, 43-48°C (Hallman 1994), and documented in A. suspensa laboratory studies for mortality of isolated third instars and pupae, 43°C (Hallman 1996), or for mortality of third instars in host grapefruits, 43-46°C (Hallman et al. 1990).

In addition to the abiotic conditions of the compost system, several factors contributing to mortality were identified from Berlese sampling of the arthropod community. Competitors on the host fruit included adult and larval sap beetles, Lobopha insularis (Cast.) and Carpophilus spp., which were often quite abundant. Predators and parasitoids detected in the compost included species known to feed on dipteran larvae, such as a macrochelid mite, Glypholaspis fimicola (Sellnick) (Krantz 1998) and a rove beetle, Belonuchus pallidus Casey (Frank 2004); plus several species which have been documented to attack A. suspensa larvae and pupae, including a parasitoid wasp, Diachasmimorpha longicaudata (Ashmead) (Lawrence et al. 1976), the ringlegged earwig, Euborellia annulipes (Lucas) (Hennessey 1997), and the red imported fire ant, Solenopsis invicta Buren (Hennessey 1997). Predation by ants has been shown to be an important biotic mortality factor for Anastrepha larvae during the wandering prepupal stage (Aluja et al. 2005). Fungal growth, common on the surface of host fruits, was another potential mortality factor since it contributed to rapid breakdown of fruit on compost piles, especially in tests conducted during the wet season.

### Table 2. Emergence of Potentially Mated Female A. suspensa from Infested Grapefruits Placed on Outdoor Compost Piles in Miami, Florida.

| Season    | Number emerged | Compost pile replicate | Percent emergence of mated females (Mean ± SE) |
|-----------|----------------|------------------------|-----------------------------------------------|
|           |                | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |                           |
| Summer    | Male           | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 ± 0.0 a |
|           | Female         | 0 | 0 | 2 | 2 | 5 | 3 | 2 | 1 | 3 | 4 | 5.7 ± 1.3 ab |
|           | Mated female² | 0 | 0 | 3 | 3 | 4 | 4 | 6 | 5 | 5 | 6 | 1 |
| Early Fall| Male           | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 ± 0.0 a |
|           | Female         | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
|           | Mated female² | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Late Fall | Male           | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 2 | 0 | 0 | 3 |
|           | Female         | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 4 | 2 | 0 | 8 |
|           | Mated female² | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 4 | 0 | 0 | 8 |
| Spring    | Male           | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 ± 0.0 a |
|           | Female         | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 1 | 8 | 3 | 4 |
|           | Mated female² | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 8 | 3 | 4 | 0 |
| Overall   |                |   |   |   |   |   |   |   |   |   | 10.1 ± 2.9 |

¹Percent emergence from compost relative to controls. Means followed by the same letter are not significantly different (Tukey-Kramer HSD test, P = 0.05).

²Females were considered potentially mated if at least 1 male emerged from the same compost pile.
In summary, this study estimates a 10% likelihood for emergence of a mated female *Anastrepha* through a residential composting pathway. This risk applies to the spread of the established exotic, *A. suspensa*, into areas of Florida or other states that are currently fly-free. It applies equally to spread of exotic *Anastrepha* species should infested fruit evade detection and quarantine measures, as was the case in 2003 with manzano peppers imported from Mexico (Thomas 2004). Peppers infested with *A. ludens* cleared customs in Texas in Apr, were distributed to several U.S. locations, including 2 in Florida, and an adult *A. ludens* was captured in Orlando in May (Thomas 2004). Should pest entry occur despite current safeguards, the risk of spread via composting can be reduced by burying produce deep and promoting high internal compost temperatures (e.g., keeping piles moist and turning them often). Gould & Maldonado (2006) recently reported data on the likelihood of escape of *Copitar-sia decolora* (Lepidoptera: Noctuidae) larvae from disposal of infested asparagus, estimating that ~1.2% of first instars were able to escape from a commercial garbage dumpster during a 1-week period. These data were critical components of a pathway risk assessment estimating the likelihood of establishment of *C. decolora*. To our knowledge, this is the only other experimental study which quantifies risk of insect pest escape as a result of discarded produce. Comparable studies assessing tephritid emergence from fruit discarded in dumpsters or landfills are needed to support comprehensive pathway risk analysis for invasive fruit flies in Florida.

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