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Research article

Optimization of Pheromone Traps for *Coryphodema tristis* (Lepidoptera: Cossidae)

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Abstract

The *Coryphodema tristis* (Drury) is an important pest of *Eucalyptus nitens* (Deane and Maiden) plantations in South Africa. The gregarious larvae of this pest cause damage by feeding on the tree sapwood, and adults emerge in spring each year. The aim of this study was to optimize pheromone traps for operational use in management programs. This was achieved by investigating different pheromone blend combinations and trap types for efficacy under field conditions. Our results confirm that the cross vane bucket funnel trap baited with a 95:2.5:2.5 volumetric blend of Z9-14:OAc, Z9-14:OH, and 14Ac was superior to similarly baited standard bucket funnel and delta traps. We also estimated the release rate and ratios of the pheromone compounds loaded into an artificial permeation dispenser through solid-phase microextraction sampling. Results showed that the released blend of pheromone compounds mirrored the dispensed ratios relatively accurately and that release rates are affected by temperature.

Key words: trap, pheromone, ratio, dispenser, release

Plantation forestry enhances our ability to meet global demands for wood and wood fiber products. This role will increase as demand for products and ecosystem services associated with forests increases because of global population growth (Brockerhoff et al. 2006). One advantage of plantation forests is that they have high productivity levels, which is a consequence of intensive inputs, genetic improvement programs, and relief from pests and pathogens when plantation species are grown outside their native ranges. The absence of these pests and pathogens is unfortunately often short lived, primarily due to increases in global trade that results in higher probabilities that pests and pathogens establish in new areas (Lodge et al. 2006, Wingfield et al. 2010, Keller et al. 2011). In other cases, nonnative plantation trees can also be targets of native insects that can shift host onto an abundant plant food source. Such host shifts can result in serious damage as is found in the case of the *Coryphodema tristis* (Drury) (Lepidoptera: Cossidae) in South Africa (Gebeyehu et al. 2005).

Plantations in South Africa must contend with increasing numbers of pest and disease threats due to accidental introductions and host range expansions of native insects (Wingfield et al. 2008, Hurley et al. 2016). Available land suitable for plantation forestry is also limited, particularly in higher elevation areas where frost-tolerant species need to be planted (Purnell and Lundquist 1986, Little and Gardner 2003). In this regard, *Eucalyptus nitens* (Deane and Maiden) (Myrtales: Myrtaceae) is widely planted as a frost-tolerant species in the Mpumalanga region of South Africa. Plantations of *E. nitens* cover ~12,000 hectares in Mpumalanga and have an estimated standing value of hundreds of millions of Rands for the South African forestry industry (Marcel Verleur, personal communication; Adam et al. 2013).

Coryphodema tristis has been known as a pest of grape vines and quince trees in the Western Cape Provence of South Africa for many years. However, in 2004, it was recorded for the first time damaging *E. nitens* trees in the Mpumalanga Province (Gebeyehu et al. 2005). The larvae of *C. tristis* primarily cause damage through feeding on the xylem of infested trees. Feeding results in resin exudation from the entrance holes and the accumulation of frass at the bases of infested trees. Large numbers of the gregarious larvae can

weaken trees to such an extent that they break at the site of infestation during wind storms.

In response to this new threat to *Eucalyptus* plantations in South Africa, an applied chemical ecology research effort on *C. tristis* was initiated in the Mpumalanga Province. The initial goal of this program was the identification of the *C. tristis* sex pheromone to provide a tool for the detection, sampling, and control of this pest (Witzgall et al. 2010, Howse et al. 2013). The sex pheromone of *C. tristis* has been identified as a combination of Z9-tetradecenol (Z9-14:OH), Z9-tetradecenyl acetate (Z9-14:OAc), and the corresponding saturated acetate (14:Ac) (Bouwer et al. 2015). As with many moth species, specificity appears to be a function (at least in part) of the blend of the compounds emitted (Allison and Cardé 2016a). Preliminary trials showed that a 95:2.5:2.5 volumetric blend ratio of these three pheromone compounds was effective to capture *C. tristis* males in field traps. Trials to test the efficiency of variations of this blend have, however, been limited.

In this study, we report the results of field trapping trials designed to optimize protocols for the operational use of pheromone-baited traps in *C. tristis* management. Specifically, we considered the compound ratios released from artificial pheromone dispensers in detail. The effect of dispensed ratios on the numbers of males captured and the efficacy of three different trap designs was also evaluated.

Materials and Methods

Field Trials

Field trials were conducted during the moth emergence period in three consecutive years (2013–2015). Trials were designed to test the effect of dispensed volumetric pheromone ratio combinations on the numbers of males captured and to subsequently optimize the pheromone trap design. Traps were deployed at two sites each year in the Lothair (Block A31 and Block A19) plantation. An exception was in the 2015 season where both sites were in block A31 (GPS coordinates S26.30642° E030.61562°, S26.30075 E030.61669) due to block A19 having been felled.

Experiment 1

In 2013, a field trial was conducted to test the effect of different volumetrically dispensed pheromone blend ratios on the capture of males. Seven different blends of the three originally identified pheromone compounds and two controls were tested. These included 1) Z9-14:OAc; 2) 94:6% blend of Z9-14:OAc: 14:OAc; 3) 99:1% blend of Z9-14:OAc: 14:OAc; 4) Z9-14:OH; 5) 94:6% blend of Z9-14:OAc: Z9-14:OH; 6) 6:94% blend of Z9-14:OAc: Z9-14:OH; 7) 95:2.5:2.5% blend of Z9-14:OAc: 14:OAc: Z9-14:OH; 8) a negative control without pheromone added to the dispenser; and 9) a positive control including one caged virgin female. All treatments were tested with yellow bucket funnel traps purchased from Insect Science (South Africa, Tzaneen).

Experiment 2

In 2014, a broader range of pheromone blends and the effect of additional compounds (Z9-14:Ald and Z7-14:OAc) was tested. These two compounds were chosen because they are structurally similar but differ in functional groups and double bond position from the originally identified pheromone compounds. The compounds were also shown to be electro-antennographically active on both male and female antennae, with males showing larger responses than females (Bouwer et al. 2015). This experiment tested eight pheromone blends and one negative control as follows: 1) 95:2.5:2.5% blend of Z9-14:OAc: Z9-14:OH: 14:OAc; 2) 94:6% blend of Z9-14:OAc: Z9-14:OH; 3) 50:50% blend of Z9-14:OAc: Z9-14:OH; 4) 80:20% blend of Z9-14:OAc: Z9-14:OH; 5) 92.5:2.5:2.5:2.5% blend of Z9-14:OAc: Z9-14:OH: 14:OAc: Z9-14:Ald; 6) 95:2.5:2.5% blend of Z9-14:OAc: Z9-14:OH: Z9-14:Ald; 7) 65:10:5:20% blend of Z9-14:OAc: Z9-14:OH: 14:OAc: Z9-14:Ald; 8) 92.5:2.5:2.5:2.5% blend of Z9-14:OAc: Z9-14:OH: 14:OAc: Z7-14:OAc; and 9) a negative control with no pheromone added to the dispenser. All treatments were tested with yellow bucket funnel traps purchased from Insect Science (South Africa, Tzaneen).

Experiment 3

The field trial in 2015 investigated the effect of the alcohol component in the pheromone blend and the effect of three different trap designs. The three blends tested were: 1) 95:2.5:2.5% blend of Z9-14:OAc: Z9-14:OH: 14:OAc; 2) 97.4:0.1:2.5% blend of Z9-14:OAc: Z9-14:OH: 14:OAc; and 3) 97.49:0.01:2.5% blend of Z9-14:OAc: Z9-14:OH: 14:OAc. These three blends were tested in the following three trap designs: 1) yellow bucket funnel trap; 2) yellow bucket funnel trap with intercept vane spacer; and 3) delta trap with sticky liner. The intercept vane spacers added an extra 15 cm to the entrance above the funnel of the normal yellow bucket funnel traps. All traps were purchased from Insect Science (South Africa, Tzaneen). An alternative dispenser (re-sealable plastic bag, 40 micrometer thickness) loaded with 1 ml of a 865 ppm (95:2.5:2.5 ratio of Z9-14:OAc: Z9-14:OH: 14:OAc) isopropanol solution was also tested during the 2015 field season (see Tables 1, 2, and 3 for all treatments listed here).

Pheromone compound standards used in all experiments were purchased from Insect Science (South Africa, Tzaneen, purity > 95%). The pheromone compounds were mixed volumetrically in different ratios and dispensed in custom made pheromone permeation devices (Bouwer et al. 2015). All trials were arranged in stratified random block designs with five replicates per treatment per site at two sites ($n = 10$ per treatment, Supp. Fig. 1 [online only]). Traps were attached to trees at a height of 4 m from shelving brackets and the distance between traps was ~10 m. Dispensers were replaced every second week and numbers of male moths captured were recorded.

Because the experimental designs were similar for all three field experiments (randomized complete blocks with multiple collection dates), the analyses for all three experiments were the same. Total captures per trap were analyzed using a blocked permutation procedure (MRBP; McCune and Grace 2002). The catches for all collections were summed by treatment. All analyses were conducted with PC-ORD 6.0 (MjM Software Design, Gleneden Beach, OR) using Euclidean distances to construct the distance matrix with blocks aligned before analysis (McCune and Grace 2002). The multiplicity effect was controlled using step-up FDR (McCune and Grace 2002, Garcia et al. 2004).

To compare results for the three different years, a subset of the data collected was used to visualize a tentative male preference function for the yellow bucket funnel traps. This subset of the data consisted of the results for all the yellow bucket funnel traps baited only with the permeation tube artificial dispenser that contained different pheromone blends of only Z9-14:OAc, Z9-14:OH, and 14:OAc. To construct the plot the volumetric dispensed ratios were displayed on the X and Y axes for the two essential pheromone compounds (Z9-14:OAc, Z9-14:OH). The numbers of males captured for each

Table 1. Treatments and results for pheromone ratio trial during 2013

Trap number	Trap type	Lure type	Replicates	Z9-14:OAc	Z9-14:OH	14:OAc	Mean	SE	Letters
1	Yellow bucket	PT	10	1	0	0	0.2	0.20	BD
2	Yellow bucket	PT	10	0.94	0	0.06	0.6	0.22	AB
3	Yellow bucket	PT	10	0.99	0	0.01	0.3	0.15	BD
4	Yellow bucket	PT	10	0	1	0	0	0	D
5	Yellow bucket	PT	10	0.94	0.06	0	3.9	1.44	AC
6	Yellow bucket	PT	10	0.06	0.94	0	0.1	0.10	BD
7	Yellow bucket	PT	10	0.95	0.025	0.025	6.3	1.09	C
8 (Blank)	Yellow bucket	PT	10	0	0	0	0	0	D
9 (Female)	Yellow bucket	~	10	0	0	0	0.8	0.70	BD

Volumetric ratios of the pheromone compounds are displayed.

PT, permeation tube. Rows with the same letters are not statistically significantly different ($P < 0.05$).

Table 2. Treatments and results for pheromone ratio trial during 2014

Trap number	Trap type	Lure type	Replicates	Z9-14:OAc	Z9-14:OH	14:OAc	Z9-14:Ald	Z7-14:OAc	Mean	SE	Letters
1	Yellow bucket	PT	10	0.95	0.025	0.025	0	0	4.3	1.04	A
2	Yellow bucket	PT	10	0.94	0.06	0	0	0	2.3	0.50	AB
3	Yellow bucket	PT	10	0.5	0.5	0	0	0	1	0.37	B
4	Yellow bucket	PT	10	0.8	0.2	0	0	0	2.5	0.67	AB
5	Yellow bucket	PT	10	0.925	0.025	0.025	0.025	0	1.2	0.99	BC
6	Yellow bucket	PT	10	0.95	0.025	0	0.025	0	0.9	0.48	AB
7	Yellow bucket	PT	10	0.65	0.1	0.05	0.2	0	0	0	C
8	Yellow bucket	PT	10	0.925	0.025	0.025	0	0.025	0	0	C
9 (Blank)	Yellow bucket	PT	10	0	0	0	0	0	0	0	C

Volumetric ratios of the pheromone compounds are displayed.

PT, permeation tube. Rows with the same letters are not statistically significantly different ($P < 0.05$).

Table 3. Treatments and results for the trap optimization trial during 2015

Trap number	Trap type	Lure type	Replicates	Z9-14:OAc	Z9-14:OH	14:OAc	Mean	SE	Letters
1	Yellow bucket	PT	10	0.95	0.025	0.025	2.7	0.45	C
2	Yellow bucket	PT	10	0.974	0.001	0.025	0.1	0.10	AB
3	Yellow bucket	PT	10	0.9749	0.0001	0.025	0	0	A
4	Yellow bucket+ extension	PT	10	0.95	0.025	0.025	35.1	5.96	E
5	Yellow bucket+ extension	PT	10	0.974	0.001	0.025	0.8	0.33	B
6	Yellow bucket+ extension	PT	10	0.9749	0.0001	0.025	1.1	0.99	AB
7	Delta trap	PT	10	0.95	0.025	0.025	6.8	0.94	D
8	Delta trap	PT	10	0.974	0.001	0.025	0.7	0.42	AB
9	Delta trap	PT	10	0.9749	0.0001	0.025	0	0	A
10	Yellow bucket	B	10	0.95	0.025	0.025	2	1.47	ABC

Volumetric ratios of the pheromone compounds are displayed.

PT, permeation tube; B, re-sealable plastic bag. Rows with the same letters are not statistically significantly different ($P < 0.05$).

collection event (scatterplot3d function, R) were displayed on the Z axis.

Chromatographic Determination of Pheromone Dispenser Release Rate

Measurements of pheromone release rates were performed on the permeation dispenser loaded by suspending 1 µl of a 95:2.5:2.5 volumetric mixture of Z9-14:OAc, Z9-14:OH, and 14:OAc in it. This mixture corresponds with the most effective dispensed pheromone blend identified for *C. tristis* (Bouwer et al. 2015). Measurements were made to quantify the absolute and relative amounts released from the dispenser at different temperatures.

During sampling the pheromone permeation device was suspended from a small metal wire stage that was placed inside a 50-ml glass chamber. This was done to prevent the dispenser from physically touching the glass surface. The glass chamber was partially submerged in a temperature controlled water bath, and it was fitted with an inlet sealed with blue-faced (PTFE) white silicone septa (Supelco, product number 27512).

Samples were taken sequentially from the dispenser held at different temperatures inside the sealed glass sampling chamber. This was achieved by placing the dispenser inside the chamber at a set temperature starting at 20 °C. The dispenser was then sampled for a period of 60 min with a solid-phase microextraction (SPME) fiber that was analyzed afterwards. The sampling chamber was opened after the sampling event to release the accumulated volatiles before increasing the temperature of the chamber by 2 °C for the next sampling event. This procedure was repeated seven times up to a temperature of 32 °C. One of these sets of data defined a single

experiment. The sampling chamber was washed with soap and water and baked out overnight to clean it (110 °C) before each experiment and the empty chamber was sampled to verify that the background levels were below our detection limits (lower than three times the FID noise). The experiment was repeated on four separate occasions over a 2-wk period starting on the seventh day after the dispenser was loaded and repeated on day 11, 12, and 18 ($n = 4$). A time window of 6 d was allowed for the lure to stabilize before recordings were made. The dispenser was suspended from a wire placed in open air in a ventilated laboratory at 23 °C when not being sampled. Amounts recorded were taken as the amount of pheromone that absorbed into the fiber after 1 h. This absorption onto the fiber is related to the volatile concentration in the sampling chamber. The volatile concentration in the chamber was defined as the sum of the compounds released from the lure and those partitioning between the glass surface and air inside the chamber. A separate recovery experiment revealed that between 60 and 65% of the three quantified pheromone compounds absorb into the fiber at a temperature of 32 °C (data not shown). Under these sampling conditions the fiber was never overloaded (i.e., saturation did not occur).

During sampling, the sampling chamber inlet septa was pierced with the needle of a conditioned (250 °C, 30 min, 100 ml/min, He) SPME fiber (50/30 µm PDMS/Car/DVB, Supelco, 57328-U) holder. The fiber was exposed inside the chamber and analyzed immediately after sample collection on a GC-FID instrument (Agilent 6890N gas chromatography system, Chemetrix, Midrand, South Africa) equipped with an HP 5 analytical column (J & W scientific, 0.32 mm ID, 0.25 µm). The fiber was desorbed (splitless, 1 min at 250 °C at 7 ml/min, He, vent at 1 min, 100 ml/min) in the GC inlet fitted with an SPME inlet liner (Supelco, 0.75 mm ID, 2-6375). Between sampling events, we verified that the fiber had been reconditioned in the inlet (i.e., no detectable contamination) through redesorbing a desorbed fiber that was used to sample for an extended 20-h sampling period at 18 °C. The GC was operated in constant pressure mode (16 psi, oven: 50 °C for 1 min to 300 °C at 20 °C/min, held 3 min). Standard curves were set up through external calibration with a series of standard mixtures made in dichloromethane at a concentration range spanning 0.87 to 435 ppm (1 µl injection volume). Peak area was recorded and used to quantify unknown quantities adsorbed onto the SPME fiber using univariant linear regression from the chemCal package (R version 3.1.2).

Results

Field Trials

Among the most attractive blend treatments, the binary blend of Z9-14:OAc and Z9-14:OH was the treatment with the fewest number of components (Table 1, treatment 5). The ratio between these two compounds was also shown to be critical for attracting male moths, because changing the ratio from 94 to 6 percent (treatment 5) to the reverse ratio (treatment 6) impacted heavily on the numbers that were captured (39 vs. 1, respectively). Although the tertiary blend of Z9-14:OAc, and Z9-14:OH and 14:Ac captured ca. 60% more males than the binary blend of Z9-14:OAc and Z9-14:OH, there was no statistical difference between the two treatments.

The 2013 trial showed that some of the tested blends were more attractive to males than living female moths (see for example treatment 5 and 7 as compared to treatment 9). This could have been due to limited calling behavior of caged females or the higher pheromone release rates of artificial dispensers (see Allison and Cardé 2016b). Numbers of moths captured in this trial were not relatively high when compared to other similar studies (Dix and Doolittle 1985, Fang et al. 2005, Chen et al. 2006), and additional trials were conducted using the best blend as a benchmark in subsequent years.

The 2014 field trial results showed that changing the ratio of the Z9-14:OAc to Z9-14:OH, did not affect the numbers of moths that were captured (see treatments 2, 3, and 4 in Table 2). This result confirmed that there is a plasticity present in the binary blend combinations for the Z9-14:OAc and the Z9-14:OH because a 80:20 ratio also led to 25 male moths being captured. The data also confirmed that certain pheromone compounds may be antagonistic when present in the pheromone blend. For example, traps baited with the quaternary blend of Z9-14:OAc, Z9-14:OH, 14:OAc, and Z7-14:OAc captured no males, suggesting that Z7-14:OAc is a possible pheromone antagonist of *C. tristis*.

A total of 493 male *C. tristis* moths were captured during the 2015 field season (Table 3). The yellow bucket funnel trap with the intercept vane spacer baited with the 95:2.5:2.5% (Z9-14:OAc: Z9-14:OH: 14:OAc) pheromone blend captured 71% of the moths during the trial (Treatment 4, Fig. 1). This result suggests that the smaller entrance of the yellow bucket funnel traps without the extension prevented many of the attracted moths from falling into the traps. A larger entrance combined with a larger intercept area rather than differences in the lure type enhanced trap captures significantly (see treatment 4 compared to treatment 10). Although less expensive, this field trial revealed that the delta trap is inferior to the bucket funnel trap modified with the intercept vane spacer for capture of *C. tristis*.

The male preference graph for our lures baited with different pheromone combinations in the yellow funnel bucket traps clearly showed that there is an optimum blend of pheromone compounds occurring near the 95% Z9-14:OAc ratio of the blend and that there is considerable variation surrounding this optimum (Supp. Fig. 2 [online only]). Very few moths were captured with the pure compounds used in the blend, which confirms the additive effect of the blends. This graph should be interpreted with caution, as it was constructed from multiple years of field trial data that only tested a limited number of blends.

Chromatographic Release Rate Determination of Pheromone Permeation Device

The release rate of the loaded pheromone compounds from the dispenser was clearly dependent on temperature (Fig. 2). Release rate approximately doubled each time the temperature was increased by 2 °C (Supp. Table 1 [online only]). For Z9-14:OAc the release rates were measured to lie between 9 ng/h at 20 °C up to 570 ng/h at 32 °C. The two minor pheromone compounds were released at rates of between 0.42 ng/h at 20 °C up to 11 ng/h at 32 °C for 14:OAc and 3.5 ng/h at 20 °C up to 18 ng/h at 32 °C for Z9-14:OH. The release rates were also very consistent over the measured period. The pheromone ratios released mirrored the loaded volumetric ratios relatively accurately (Fig. 2B, D, F). This was especially evident at higher sampling temperatures, which resulted in larger peak areas and more accurate quantification for ratio determination.

Discussion

The effects of trap design and pheromone composition on trapping efficiency for the *C. tristis* moth were tested, and the ratios of the pheromones as well as their release from an artificial pheromone dispenser were evaluated in this study. The results showed that bucket funnel with vane extensions and delta traps, were more effective for capturing male moths than the bucket funnel traps. The results also

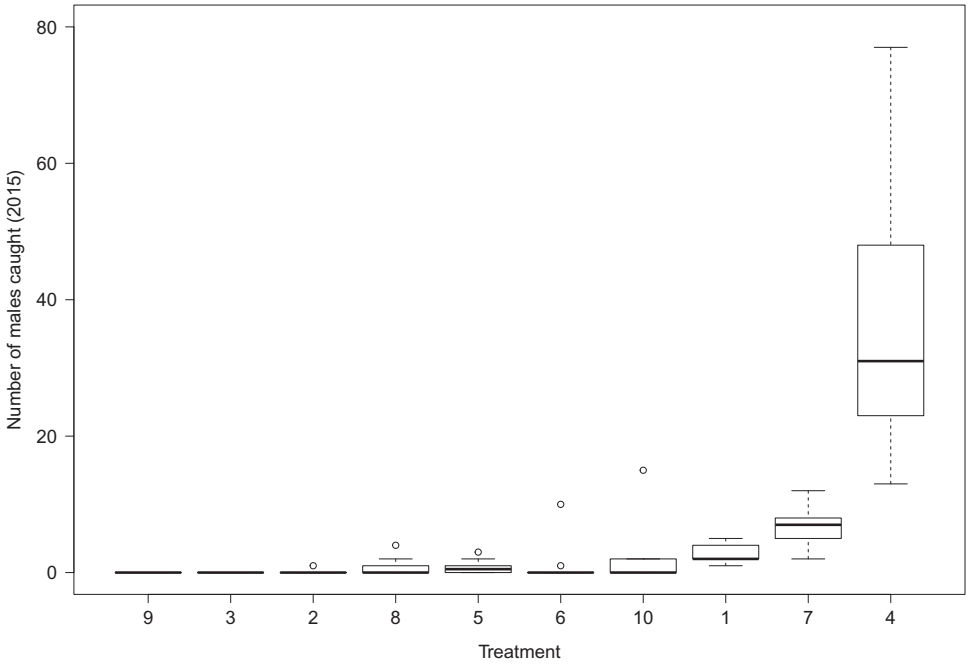

Fig. 1. Numbers of moths captured per trap for the trap optimization experiment during 2015. Treatments and trap designs are given in Table 3.

provided evidence that the released ratios of the dispensed pheromone compounds mirror those loaded into them. Furthermore, that the loaded ratio of pheromone components has a significant effect on the number of male *C. tristis* moths captured.

Trap design was a key factor for optimization of trap captures. Higher numbers were captured when the trap entrance was larger. Trap design is known to play an important part for trap optimization, and there are numerous factors to consider for optimization experiments (Muirhead-Thompson 2012, Allison and Redak 2017). For Lepidoptera, these can include the size of both the trap and its target (Mitchell et al. 1989), the shape and size of the trap entrance (Athanassiou et al. 2004), the pheromone plume shape created by the trap design (Lewis and Macaulay 1976), whether a trap can saturate or not (Ramaswamy and Cardé 1982, Kuenen and Siegel 2016), and trap color (Hashemi 2015). In the moth family Cossidae, the effect of trap designs has been investigated only for the carpenterworm, *Prionoxystus robiniae* (Peck) (Lepidoptera: Cossidae) (Dix et al. 1987) and *Cossus cossus* (L.) (Lepidoptera: Cossidae) (Lapietra et al. 1986). Dix et al. (1987) report no significant differences in numbers captured among the paper cylinder, metal cylinder, and milk carton designs tested. Lapietra et al. (1986) indicated that a plastic funnel trap was suitable for catching *C. cossus* and that trap color had no effect on numbers captured. Other studies on Cossid moths report the pheromone identity and pheromone ratios tested within one trap design (Capizzi et al. 1983, Fang et al. 2005, Chen et al. 2006). It is possible that trapping efficiency could be improved in these systems by modifying trap design, as was evident for *C. tristis*.

For the Cossidae, multicomponent blends are typically more effective than single compounds (Capizzi et al. 1983, Fang et al. 2005, Chen et al. 2006). *Coryphodema tristis* males were shown to rely on a pheromone blend in which both the unsaturated alcohol (Z9-14:OH) and acetate (Z9-14:OAc) have to be present for attraction to occur. The saturated acetate (14:OAc) that was consistently found in pheromone gland extracts (Bouwer et al. 2015) is possibly a pheromone component that augments male attraction, but is not essential for it. A range of effective pheromone blend ratios suggests that the male preference function in *C. tristis* is similarly broad. Pheromone blend composition can vary during the scotophase and as females age. For example, it has been shown that both the pheromone blend composition and release rate changes during the scotophase for the cabbage looper, *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae) (Liu and Haynes 1994). Variation among individuals has also been observed in many species of moths (Allison and Cardé 2006, 2007, 2016b). If this is the case for *C. tristis*, then males should have the ability to respond to variations that occur within their species blend, and might explain their broad preference.

No moths were caught in traps when Z7-14:OAc was added to the blend. Moths utilizing pheromone signals appear to have the ability to monitor for the absence of compounds not present in their pheromone blends (Allison and Cardé 2008). This mechanism could infer a fitness benefit because it prevents the unnecessary investment and predation risk associated with orientation to heterospecific pheromone plumes (Cardé and Haynes 2004). Antagonistic pheromone compounds are often similar in structure to the actual pheromone compounds utilized in a species, and these compounds may function as pheromones in closely related and often sympatric species (Löfstedt et al. 1990). The pheromone compound, Z7-14:OAc, is known as the main pheromone component of *Holcocerus hippophaecolus* Hua (Lepidoptera: Cossidae) (Fang et al. 2005) and minor pheromone component of *Isoceras sibirica* Alpheraky (Lepidoptera: Cossidae) (Zhang et al. 2011). This compound is also the pheromone for other noctuid species such as the soybean looper *Pseudoplusia includens* (Walker) (Lepidoptera: Noctuidae) and cabbage looper, *T. ni* (Grant et al. 1988). It is consequently possible that some species living in sympatry with *C. tristis* in its endemic region produce Z7-14:OAc as a pheromone and that it serves as an antagonist for *C. tristis*.

Field trials are normally used to determine the most effective pheromone blend that should be dispensed within an artificial dispenser, but most studies fail to report the blends that are released from their dispensers. The results of this study show that the

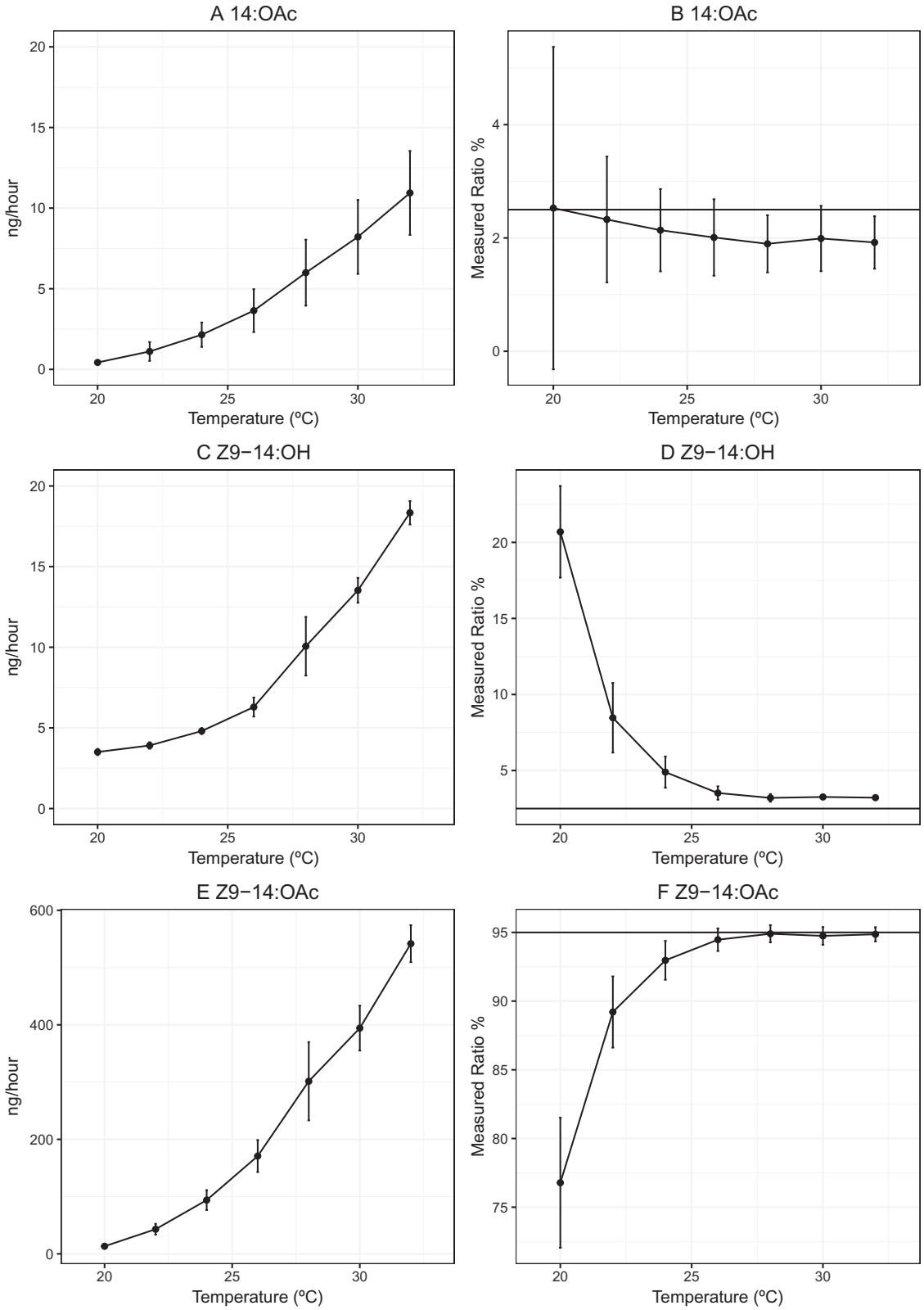

Fig. 2. Pheromone component amounts as measured from an artificial pheromone dispenser with SPME. Graphs on the left display the amount of pheromone absorbed onto the fiber after a 1-h exposure period to a dispenser that contained 1 μl of a 2.5:2.5:95 (14:OAc:Z9-14:OH:Z9-14:OAc) pheromone blend. Repeated measurements were made over 4 d and at different temperatures. Graphs to the right display the calculated relative ratio with the target volumetrically dispensed ratio indicated with a solid horizontal line. Error bars denote standard deviations.

released pheromone ratio mirrors the volumetrically dispensed ratio and that higher temperatures increased the pheromone release rate. Studies on cossid moth pheromones report rubber and polyethylene dispensers that are impregnated with a blend of pheromone dissolved in a solvent matrix (Capizzi et al. 1983, Fang et al. 2005, Chen et al. 2006, Zhang et al. 2011, Herrera et al. 2016). The solvent and its combination with various dispenser materials could have profound effects on the ratios being emitted (Kuenen and Siegel 2015). Moreover, the released quantities of pheromone from rubber septa are known to decrease exponentially in time because the release rate is dependent on the loaded quantity (Butler and McDonough 1979, 1981). The blend ratio being released from such septa can remain constant, but only for a short time period (Showler et al. 2005). Ratios change over extended time periods because there are vapor pressure differences between different pheromone compounds that are dispensed (Heath et al. 1986). Technical difficulties such as these are critically important especially if it is found that moths only respond to a very narrow range of pheromone blends.

Coryphodema tristis larvae feed on the cambium and xylem of trees and are protected inside the trees. The adults are the only phase of the lifecycle that is exposed and could potentially be controlled with cost effective and nondestructive management tactics. In this regard, pheromone-based pest control is currently the only available potentially cost-effective method for *C. tristis* management. This study has shown that *C. tristis* adult males can be trapped more effectively by using traps with a larger entrance that are baited with pheromone lures that release the pheromone components within an optimal ratio of between 50:50:0 and 95:2.5:2.5% blend of Z9-14:OAc, Z9-14:OH, and 14:OAc.

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