

# Sensory Imbalance as Mechanism of Orientation Disruption in the Leafminer *Phyllocnistis citrella*: Elucidation by Multivariate Geometric Designs and Response Surface Models

Stephen L. Lapointe · Lukasz L. Stelinski ·  
Terence J. Evens · Randall P. Niedz · David G. Hall ·  
Agenor Mafra-Neto

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**Abstract** Experimental designs developed to address mixtures are suited ideally to many areas of experimental biology, including pheromone blend studies, because such designs address the confounding of proportionality and concentration intrinsic to factorial and one-factor-at-a-time designs. Geometric multivariate designs coupled with response surface modeling allowed us to identify optimal blends of a two-component pheromone for attraction and trap disruption of the leafminer moth, *Phyllocnistis citrella*, a major pest in citrus growing areas around the world. Field trials confirmed that the natural 3:1 blend of (Z,Z,E)-7,11,13-hexadecatrienal:(Z,Z)-7,11-hexadecadienal was most effective as an attractant for male moths. However, the response surface generated in mating orientation trials revealed that the triene component alone was more effective than the natural blend in disrupting trap catch. Each individual component was

effective at disrupting orientation in field trials, but (Z,Z,E)-7,11,13-hexadecatrienal was approximately 13 times more effective, at the same concentration, compared with (Z,Z)-7,11-hexadecadienal alone. In addition, the application of geometric design and response surface modeling to field studies provided insight into a possible mechanism of mating disruption and supported sensory imbalance as the operating mechanism for this species.

**Keywords** Mating disruption · Pheromone blends · Mixtures · Citrus leafminer · Non-competitive disruption · Citrus canker

## Introduction

Many insects, particularly moths, rely upon pheromonal communication for recognizing and locating potential mates (Cardé and Minks 1995). Although some species use a single compound as a sex pheromone (Novak and Roelofs 1985), most deploy a blend of compounds in species-specific ratios. The production of synthetic sex pheromones to disrupt mating in insect pest species has become a sizeable industry that contributes to a reduced reliance on insecticides in integrated crop protection strategies. Synthetic sex pheromones for mating disruption of crop pests are used on an estimated 662,000 ha of land worldwide (Witzgall et al. 2008).

At least two general models have been proposed to explain the mechanism by which treatment of crops with sex pheromones results in disruption of sexual communication and subsequent reduction in population densities. Competitive models suggest that sources of synthetic

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S. L. Lapointe (✉) · T. J. Evens · R. P. Niedz · D. G. Hall  
United States Department of Agriculture,  
U. S. Horticultural Research Laboratory,  
Agriculture Research Service,  
2001 South Rock Road,  
Fort Pierce, FL 34945, USA  
e-mail: stephen.lapointe@ars.usda.gov

L. L. Stelinski  
Department of Entomology and Nematology,  
Citrus Research and Education Center, University of Florida,  
700 Experiment Station Road,  
Lake Alfred, FL 33850, USA

A. Mafra-Neto  
ISCA Technologies, Inc.,  
1230 Spring Street,  
Riverside, CA 92507, USA

pheromone compete with calling females. Under this scenario, disruption occurs when males follow false plumes and are, therefore, unable to locate females (Miller et al. 2006a). Non-competitive models hold that permeation of the air space with synthetic pheromone interferes with perception of the female's signal by camouflage, desensitization, and/or sensory imbalance (Miller et al. 2006a). Experimental approaches for distinguishing competitive from non-competitive disruption have been proposed based on transformations of moth catch data in traps as a function of pheromone dispenser density per unit area of crop (Miller et al. 2006a, b).

While it is generally understood that proportionalities of multi-component pheromone blends are important in insect attraction, it is not clear whether mating disruption is best achieved by using the natural pheromone blend or some other “unnatural” ratio (Minks and Cardé 1988). The development of mating disruption technology remains largely an empirical process of testing blends and concentrations (Cardé 1990).

Studies of blends, in general, and pheromone blends in particular, often confound the effects of proportionality and amount by allowing them to vary in parallel [cf. Evens and Niedz (2008) for a discussion on confounding in proportion-amount experimental designs]. Distinguishing between proportionality and concentration effects requires a special design known as a “mixture-amount” experiment (Cornell 2002), wherein proportionality and concentration are treated independently. In addition, commonly applied experimental designs such as factorial or one-factor-at-a-time approaches are either inherently confounded or fail to explore the experiment's design space adequately. For rational development of mating disruption technology, an experimental approach is required that distinguishes between proportion and amount, efficiently allocates treatments through a potentially complex multidimensional design space, and accounts for complex interactions between variables in multi-component pheromone systems.

The leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), is a major pest of citrus throughout the world (Heppner 1993). Larvae feed within leaves, producing serpentine mines that result in distortion and loss of photosynthetic capacity, thereby reducing yield (Peña et al. 2000). In addition to direct damage, mining by *P. citrella* increases the susceptibility of citrus leaves to infection by citrus canker, *Xanthomonas axonopodis* pv. *citri*, an important disease of citrus (Gottwald et al. 2007). Our interest was to determine optimal pheromone blends for attraction and mating disruption and to gain insight into the mechanism of disruption for control of *P. citrella* through application of appropriate experimental design. We used mixture-amount designs to quantify optimal pheromone blends and concentrations for both attraction to traps and orientation disruption

in a series of field experiments conducted in Florida citrus groves. We believe the methods employed here to be of wide applicability, within this field and many other fields of biological inquiry, in which responses to proportion and amount of mixture components are measured.

## Methods and Materials

### Pheromone Synthesis

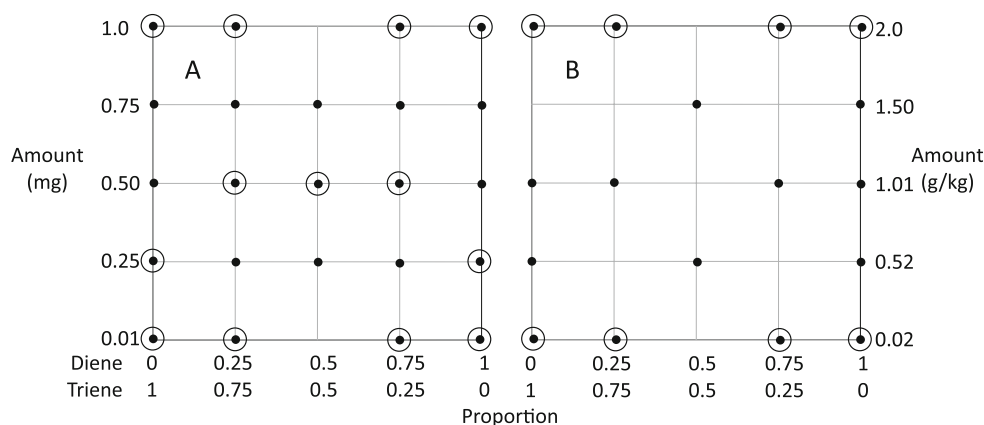
(*Z,Z,E*)-7,11,13-Hexadecatrienal and (*Z,Z*)-7,11-hexadecadienal (referred to also as triene and diene, respectively) were synthesized by ISCA Technologies Inc. Riverside, CA, USA, as previously described (Leal et al. 2006; Moreira et al. 2006), resulting in (*Z,Z,E*)-7,11,13-hexadecatrienal of 94% chemical purity (90% isomeric purity) and (*Z,Z*)-7,11-hexadecadienal of 86% chemical purity (72% isomeric purity).

### Attraction Trial

Red, 11-mm natural rubber septa (West Pharmaceutical Services Co., Lionville, PA, USA) were loaded with hexane solutions (100  $\mu$ l) of test blends of *P. citrella* sex pheromone, to yield 23 treatments consisting of varying proportions and amounts of the triene and diene (Fig. 1a). Thirteen of the treatments were replicated twice to estimate pure error. Blend proportion varied from 100% triene to 100% diene, and the amount of blend loaded onto each septum varied from 0.01 to 1.0 mg. Individual septa containing the pheromone blends were placed in traps (Pherocon VI, Trécé, Inc., Adair, OK, USA), with sticky card liners, and randomly assigned to field locations within a 12 ha mature production block of Duncan grapefruit trees, near Ft. Pierce, FL, USA, on 22 May 2008. Traps were placed at 45 m intervals within rows, with three traps per row. Rows with traps were separated (45 m) by six rows without traps. Adhesive liners were replaced and examined for the presence of *P. citrella* 24 h after deployment and weekly thereafter.

### Disruption Trial

A commercial emulsified-wax matrix designed to provide slow release of semiochemicals (SPLAT<sup>TM</sup>, ISCA Technologies) was formulated with varying proportions and amounts of (*Z,Z,E*)-7,11,13-hexadecatrienal and (*Z,Z*)-7,11-hexadecadienal. Proportions varied from 100% triene to 100% diene, and amount varied from 0.02 to 2.0 g/kg (0.002 to 0.2%) of formulated SPLAT (Fig. 1b) for a total of 17 proportion/amount combinations in separate plots and 3 control plots (no SPLAT). Eight of the treatments



**Fig. 1** A: Mixture/amount experiment design space showing 23 design points, consisting of varying ratios and amounts of (*Z,Z,E*)-7,11,13-hexadecatrienal (triene) and (*Z,Z*)-7,11-hexadecadienal (diene), to assess response of *Phyllocnistis citrella* males to pheromone-baited traps in a Florida citrus grove. The thirteen circled points were replicated twice for a total of 36 proportion-

amount combinations (runs). B: Design space containing 17 design points, of varying ratios and amounts of triene and diene, to assess response of *P. citrella* males to pheromone-baited traps within 0.2 ha plots treated with a slow-release pheromone delivery system (SPLAT®) in a Florida citrus grove. The eight circled points were replicated twice for a total of 28 runs

were replicated twice to estimate pure error. SPLAT treatments and controls were randomly assigned to 0.2 ha plots of 77 trees (7 rows x 11 trees/row), equally spaced within a 24-ha mature production block of Duncan grapefruit trees, near Ft. Pierce, FL. SPLAT treatments were applied on 23 April 2008 using hand-held applicators calibrated to deliver 1 g dollops. Point source density and concentration were based on results previously reported for the 3:1 triene:diene blend (Stelinski et al. 2008). Within plots, each tree received 3 dollops of SPLAT at approximately a 1.5 m height. Perimeter trees received 4 dollops.

Trap shutdown, the disruption of male catch in traps baited with synthetic lures containing the natural blend of sex pheromone, was employed to quantify disruption. To measure trap shutdown, two Pherocon VI traps, each baited with a single rubber septum (West Pharmaceutical Services Co., Lionville, PA, USA) loaded with 130  $\mu$ l of a hexane solution containing 130  $\mu$ g of a 3:1 blend of (*Z,Z,E*)-7,11,13-hexadecatrienal and (*Z,Z*)-7,11-hexadecadienal, were placed in the center row of each plot equidistant from each other and the plot perimeter (trees 4 and 8 in rows of 11 trees each). Lures were deployed in traps on 23 April 2008 and replaced weekly.

### Experimental Design

D-Optimality criteria were used to minimize the number of factor/component combinations necessary to provide accurate estimates of the coefficients of a quadratic x quadratic Scheffé polynomial model (Cornell 2002; Box and Draper 1971). Specifically, sufficient treatments or ‘design points’ (Fig. 1) were selected with Design Expert® software

(v7.0.3, Stat-Ease, Inc. Minneapolis, MN, USA) such that the determinant of the  $(X'X)^{-1}$  matrix was minimized; this has the net result of minimizing the volume of the confidence ellipsoid for the coefficients of the selected model (Piepel 1982). Several design points were added to estimate the lack of fit (LOF) between the response surface models and design points not used to generate the model fits (Weisberg 1985). A number of treatments were duplicated in order to: 1) attain sufficient degrees of freedom (df) to estimate pure error across the design space; 2) provide estimates of block effects; and 3) reduce the potential effect (s) of high leverage points. In all, there were 36 runs (proportion-amount treatments) for the attraction study and 25 runs for the disorientation study.

### Statistical Analyses

A detailed description of the statistical methods used to analyze the data can be found in Lapointe et al. (2008). Briefly, all possible models from the mean to cubic polynomial were calculated with Design Expert® v.7.0.3. Initial model selection was based on a battery of adequacy tests (Anderson and Whitcomb 2005). Normality and constant variance were determined graphically; a Box-Cox plot was used to choose the correct transformations (Box and Cox 1964). Overly influential data points were identified with DFFITS and DFBETAS plots (Belsley et al. 1980). Adequate model precision was determined by comparing the range of predicted values at design points ( $\hat{y}$ ) to the average variance ( $V\text{-bar}$ ) of the prediction (Anderson and Whitcomb 2005). Potential outlier points were checked with externally studentized ‘outlier-t’ (Weisberg 1985; Myers 1990) and Cook’s Distance (Cook and Weisberg

1982) graphical plots.  $R^2$ , adjusted- $R^2$  ( $R_{\text{adj}}^2$ ), and predicted- $R^2$  ( $R_{\text{pred}}^2$ ), were estimated for each selected model [cf. Myers and Montgomery 2002].

#### Test of High Diene SPLAT Disruptant

An estimated relative cost of synthesis of the diene and triene provided by ISCA Technologies was included in a model of results from the disruption trial. The resulting plot suggested that diene alone, if deployed at a much higher concentration, might function as a disruptant of trap catch as well as the triene alone. Therefore, a separate field trial was conducted in October 2008 to test the efficacy of a high concentration of the diene component for disrupting trap catch compared with the triene-only treatment identified as optimal in the disruption trial. Plots (0.2 ha) in a mature citrus grove were as described above for the disruption trial. SPLAT formulations consisted of 2.0 and 20.0 g/kg of diene, and 1.5 g/kg of triene. Four replicates of each of the three SPLAT treatments were applied on 3 October 2008. Four replicates with no SPLAT were included as controls. Two traps baited with the natural 3:1 blend were deployed in the center of the plots to assess trap shutdown as described above. Counts of trap liners were conducted every 3 to 4 d for a period of 26 d. Linear regression was used to describe the percent disruption of trap catch obtained in the treated plots compared with the control plots.

## Results

#### Attraction Trial

There were no significant models that included amount of (*Z,Z,E*)-7,11,13-hexadecatrienal and (*Z,Z*)-7,11-hexadecadienal on capture of male moths (*P*-values ranged from 0.07 to 0.91). Therefore, amount effects were averaged across this dimension, which resulted in a highly significant (*P*-value <0.001) cubic x mean polynomial response surface model (RSM) for the number of male moths captured in pheromone-baited traps (Table 1; Fig. 2). Data were transformed (1/sqrt) based on the best lambda value from the Box-Cox plot [see Lapointe et al. (2008) for complete statistical methods]. The three  $R^2$  statistics ( $R^2$ ,  $R_{\text{adj}}^2$  and  $R_{\text{pred}}^2$ ) were  $\geq 0.92$ . The greatest trap catch was obtained at a 3:1 triene:diene pheromone blend. “Pure” blends consisting of various amounts of the diene component alone were not attractive. While pure blends of triene showed some attraction (6% of the maximum 3:1 blend), trap catch decreased markedly as the lure blend diverged from the 3:1 ratio (Fig. 2) in either direction of the blend ratios.

#### Disruption Trial

The disruption trial produced a highly significant (*P*-value <0.001) linear x linear polynomial RSM (Table 1; Fig. 3) for trap shutdown, calculated as trap catch expressed as a percent of the control (no SPLAT pheromone applied). There was a highly significant interaction between triene proportion and amount, and also a significant interaction between diene proportion and amount (Table 1). No significant blending effect between diene and triene was observed; the highest level of trap shutdown occurred with pure triene (Fig. 3).

#### Test of High Diene SPLAT Disruptant

The high concentration of diene in SPLAT contained 40x the amount of diene in the highest concentration of the natural 3:1 blend tested in the disruption trial. Percent trap shutdown obtained in plots treated with the high concentration of diene (20 g/kg) and triene (1.5 g/kg) was similar (Fig. 4). The high diene treatment resulted in 89% reduction of moth catch in traps relative to untreated control plots over the 26 d duration of the trial. The 0.15% triene treatment resulted in an 83% reduction over the same period.

## Discussion

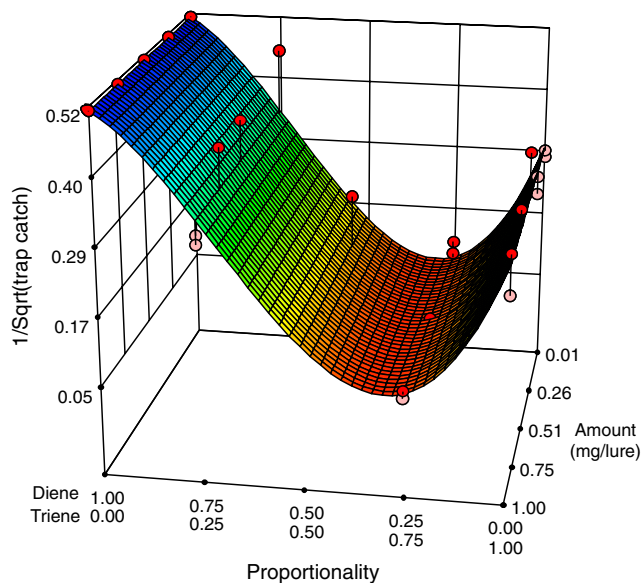
Experimental designs developed to study mixtures (Cornell 2002) are ideally suited for pheromone blend studies. An inherent difficulty in quantifying main effects and interactions of mixture components is the confounding effects of proportionality and concentration, intrinsic to traditional factorial experimental designs. Confounding occurs when two or more quantities are varied together in a manner that makes it mathematically impossible to identify their unique effects (Evens and Niedz 2008). To overcome this, blends can be conceptualized as mixtures in which the sum of the component proportions is one. Because of the proportionality constraint, the resulting experimental design space has  $n-1$  dimensions. The addition of one additional dimension to account for amount completes the design space. For example, the two-component pheromone system of *P. citrella* requires only a two-dimensional design space, one dimension for the mixture (i.e., ratio) of the two components, and one dimension for amount. Current computer hardware and software make it possible to design, execute, and analyze efficient experiments that systematically sample multidimensional experimental design space and generate mathematical equations that describe response variables across the entire design space with a minimum number of runs. Such an approach is more cost effective

**Table 1** ANOVA and model diagnostic data for the cubic x mean and linear x linear Scheffé polynomial models developed for attraction and disruption responses of *Phyllocnistis citrella* in field trials, respectively

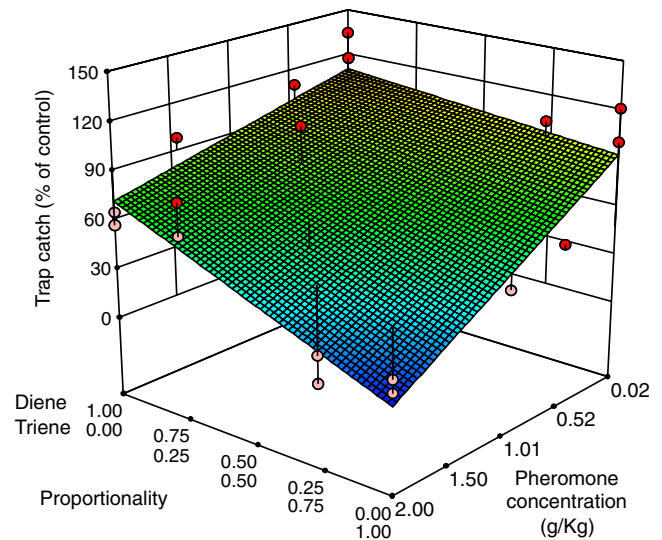
Source	Attraction		Disruption	
	F-value	P-value <sup>a</sup>	F-value	P-value <sup>a</sup>
Model	158.4	< <b>0.001</b>	23.8	< <b>0.001</b>
Linear Mixture	136.0	< <b>0.001</b>	18.1	< <b>0.001</b>
Diene <sup>b</sup> x Amount	n/a		7.0	<b>0.016</b>
Triene <sup>b</sup> x Amount	n/a		36.4	< <b>0.001</b>
Diene x Triene	177.4	< <b>0.001</b>	n/a	
D x T (D - T)	161.7	< <b>0.001</b>	n/a	
Lack of Fit		0.226	3.9	<b>0.031</b>
Std. Dev.	0.05		0.19	
Mean	0.41		0.71	
C.V. %	212.86		6.73	
R <sup>2</sup>	0.94		0.78	
R <sup>2</sup> adj	0.93		0.75	
R <sup>2</sup> pred	0.92		0.69	
Polynomial Coefficient Estimate				
	1/Sqrt =		% of Control =	
Diene	0.60		0.92	
Triene	0.47		0.48	
Diene x Amount	-n/a		0.19	
Triene x Amount	-n/a		0.45	
Diene x Triene	-1.15		n/a	
D x T (D - T)	1.99		n/a	

<sup>a</sup> Significant P-values ( $\alpha=0.05$ ) are indicated in bold

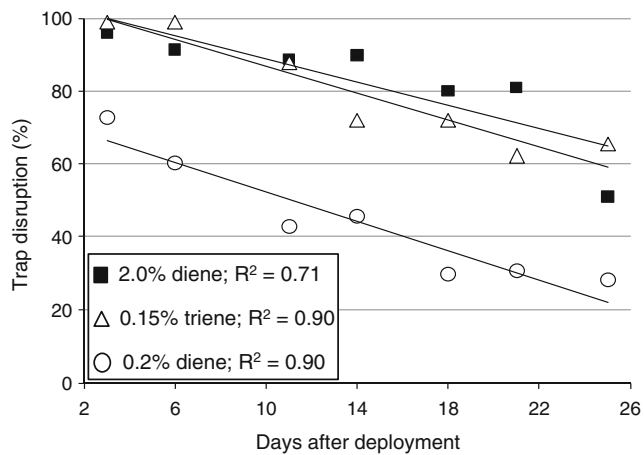
<sup>b</sup> diene = (Z,Z)-7,11-hexadecadienal, triene = (Z,Z,E)-7,11,13-hexadecatrienal



**Fig. 2** Three-dimensional plot of the inverse of the square root of number of male *Phyllocnistis citrella* moths caught in pheromone-baited traps at two weeks after deployment, as a function of pheromone component proportionality and total pheromone amount. Greatest trap catch was achieved with the natural 3:1 ratio of (Z,Z,E)-7,11,13-hexadecatrienal (triene): (Z,Z)-7,11-hexadecadienal (diene)



**Fig. 3** Three-dimensional plot of trap catch within disruptant-treated plots expressed as percent of control trap catch at two weeks after deployment of SPLAT treatments. The lowest number of male *Phyllocnistis citrella* were captured in traps [baited with a 3:1 (Z,Z,E)-7,11,13-hexadecatrienal (triene): (Z,Z)-7,11-hexadecadienal (diene) lure] in plots treated with (Z,Z,E)-7,11,13-hexadecatrienal



**Fig. 4** Decay of trap disruption (%) over time of three SPLAT formulations containing single pheromone components: (*Z,Z*)-7,11-hexadecadienal (diene) or (*Z,Z,E*)-7,11,13-hexadecatrienal (triene). Disruption of trap catch was measured as the number of male *Phyllocnistis citrella* captured in traps baited with a 3:1 triene:diene lure in plots treated with a disruptant (diene or triene) expressed as a percent of the trap catch in the control plots

than traditional factorial designs (Lapointe et al. 2008) and, in the case of pheromone studies, can contribute insight into underlying mechanisms associated with mating disruption. Geometric multivariate approaches to mixture problems have wide applicability for experimental biology, as recently shown for insect diets (Lapointe et al. 2008), studies of ion-specific effects (Evens and Niedz 2008), and plant growth media (Niedz and Evens 2008).

Studies of disruption of moth species using various blends and concentrations of pheromone components, in general, have incompletely sampled experimental space defined by proportionality and amount (Charlton and Cardé 1981; Bellas and Bartell 1983; Flint and Merkle 1983; Moreira et al. 2006). We suggest that a more complete picture of the role of individual pheromone compounds in blends and their interactions, and important insight into mechanisms of mating disruption, can be obtained through use of non-confounded designs. Moreover, this approach minimizes the number of runs required in expensive and labor-intensive field trials that often are limiting for product development.

The natural ratio of two sex pheromone components (3:1 triene:diene) of *P. citrella* (Leal et al. 2006; Moreira et al. 2006) is a potent attractant in the field (Lapointe et al. 2006). In our study, attraction of males to baited traps declined markedly as the blend ratio departed from this natural ratio, with little interaction between proportion and amount (Fig. 2). Our geometric design surveyed the experimental space and produced a response surface that confirmed the work of Moreira et al. (2006) that the 3:1 blend is optimal for attraction at least over the release rates employed in this study.

The response surface generated in the disruption trial, however, was markedly different from that of the attraction trial. The straight line described by the response surface, corresponding to the highest pheromone concentration (0.2%) and X1-X2 axis (Fig. 3), demonstrated that trap shutdown increased in direct proportion to concentration of the triene with no synergistic or antagonistic blending effects between diene and triene. This lack of blending in the disruption trial suggests that an unnatural ratio consisting of one or the other of the single components could be as effective as the natural blend for mating disruption. This, together with the general lack of congruence between response surfaces, supports the conclusion of Stelinski et al. (2008) that disruption in this species is a non-competitive phenomenon.

The linear response of trap catch of *P. citrella* to individual components in binary blends deployed as disruptants may be analogous to the disorientation thresholds that define the “attraction area” according to the threshold hypothesis for pheromone perception proposed by Roelofs (1978). By applying Roelofs’ “attraction area” concept to *P. citrella*, our results suggest that changes in binary proportions, away from the natural blend ratio, would produce a disorientation line with a steep slope for the diene and a much smaller slope for the triene. Considered this way, the amount of diene required to achieve disruption would be much greater than the amount of triene required, as demonstrated. Roelofs’ threshold diagrams predict that, for species that utilize a binary pheromone mixture for upwind orientation, the natural blend should be most effective at low concentrations. Therefore, we might expect mating disruption to be more effective at lower release rates of the binary blend compared with either component alone. Our results with *P. citrella* do not support this extrapolation, at least within the concentrations tested in our investigation. Disruption, as measured in our field tests by reduced trap catch (Fig. 3), did not decline at low pheromone concentrations as the proportionality of the disruptant moved from the natural ratio to pure triene.

Under the competitive theory of mating disruption, males are predicted to follow false plumes associated with pheromone dispensers (Miller et al. 2006a). If disruption is non-competitive, no such orientation occurs. Typically, direct observation of male orientation to pheromone dispensers in the field is required to determine attraction. However, the response surfaces for *P. citrella* for attraction and disruption (Figs. 2, 3) demonstrate visually that the single triene component was equal to or greater in disruptive ability compared to the natural blend, even though males were not attracted to either component (diene or triene) singly. Effective trap shutdown was obtained with the natural blend (0.02%), the triene alone (0.015%), and

the diene alone (0.2%). Males of *P. citrella* were not attracted at close range in the field to SPLAT treatments that contained the natural blend or single component applications of SPLAT (LS, personal observation).

Therefore, our data suggest that disruption of male *P. citrella* occurred by a non-competitive mechanism (Stelinski et al. 2008). The non-competitive mechanisms of mating disruption are camouflage, desensitization, and sensory imbalance, as defined by Bartell (1982). Our data indicate that the 3:1 blend was highly attractive to males at a release rate approximating a female, whereas the triene or diene alone were not attractive. Based on current, as well as previous (Stelinski et al. 2008) research, we know that disruption of orientation by male *P. citrella* to traps by the 3:1 blend, triene alone, or diene alone occurs at certain concentrations of pheromone above the threshold for male activation and orientation. Furthermore, our data revealed that the triene is a superior disruptor of male *P. citrella* orientation compared with the 3:1 blend. Camouflage can be eliminated as a mechanism of disruption in our trials because, if it were operating, its effectiveness (i.e., disruption) should increase as the synthetic pheromone blend approximates the natural blend (Cardé 1990). Our results did not show such an effect (Fig. 3).

Desensitization, as a mechanism of disruption, can occur following prolonged exposure to an unnaturally high stimulation with synthetic pheromone, at the level of the peripheral (adaptation) or central (habituation) nervous system or both (Bartell 1982; Miller et al. 2006a). The two-component blend should be expected to cause greater desensitization of male *P. citrella* than the triene alone, because the blend acts on more receptors in total than does a single component. However, desensitization of a portion of receptors may be highly effective if it interferes with integration in the central nervous system. Thus, although our results indicate that disruption with the triene was superior to the 3:1 blend, desensitization as the mechanism of disruption cannot be ruled out. We believe that our field trapping and disruption results are most consistent with the sensory imbalance mechanism of disruption for *P. citrella*. Sensory imbalance involves males receiving a signal out of balance with the one their sensory system is optimized to perceive (Miller et al. 2006a). We found that the pure triene formulation was superior to the 3:1 blend in the disruption trial, and that we could disrupt trap catch with either pheromone component individually.

Minks and Cardé (1988) explored the question of whether optimal mating disruption of moths could be obtained through deployment of natural or unnatural ratios of pheromone components. They argued that available evidence suggested that natural blends should be most effective at the lowest dose and that, given the constraints of available information, pursuit of unnatural blends was

unwise unless field trials demonstrated otherwise. Cardé and Minks (1995) reported that the majority of documented cases suggested that the natural blend provided best disruption at the lowest dose. As those authors note, none of the cases had been fully substantiated. We argue that with adequate experimental design and execution, optimal blend ratios and dosages for disruption can be identified and, further, may show that the natural blend may not be the most efficacious at low release rates. Furthermore, if the cost differential of synthesis of individual blend components is large, then economic considerations will drive practical decisions regarding product development for mating disruption applications. The case presented here for *P. citrella* demonstrates both of these points. First, the mixture-amount design allowed for exploration of the design space defined by proportionality and concentration of the two pheromone components. Second, the much lower cost of synthesis of the diene, compared to the triene, suggests that use of the diene, at a release rate an order of magnitude greater than that which would generate equivalent trap shutdown by the triene alone, may be warranted on economic grounds.

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## References

- ANDERSON, M. J. and WHITCOMB, P. J. 2005. *RSM Simplified: Optimizing Processes Using Response Surface Methods for Design of Experiments*. Productivity, New York.
- BARTELL, R. J. 1982. Mechanisms of communication disruption by pheromone in the control of Lepidoptera: a review. *Physiol. Entomol.* 7:353–364.
- BELLAS, T. E. and BARTELL, R. J. 1983. Dose-response relationship for two components of the sex pheromone of lightbrown apple moth, *Epiphyas postvittana* (Lepidoptera: Tortricidae). *J. Chem. Ecol.* 9:715–725.
- BELSLEY, D. A., KUH, E., and WELSCH, R. E. 1980. *Regression Diagnostics: Identifying Influential Data and Sources of Collinearity*. Wiley & Sons, New York.
- BOX, G. E. P. and COX, D. R. 1964. An analysis of transformations (with discussion). *J. Royal. Stat. Soc. Ser. B* 26:211–246.
- BOX, M. J. and DRAPER, N. R. 1971. Factorial designs, the X'X criterion, and some related matters. *Technometrics.* 13:731–742.
- CARDÉ, R. T. 1990. Principals of mating disruption, pp. 47–71, in Ridgway, R. L., Silverstein, R. M., and Inscoc, M. N. (eds.). *Behavior-Modifying Chemicals for Pest Management: Applications of Pheromones and Other Attractants*. Marcel Dekker, New York.
- CARDÉ, R. T. and MINKS, A. K. 1995. Control of moth pests by mating disruption: successes and constraints. *Annu. Rev. Entomol.* 40:559–585.

- CHARLTON, R. E. and CARDÉ, R. T. 1981. Comparing the effectiveness of sexual communication disruption in the oriental fruit moth (*Grapholitha molesta*) using different combinations and dosages of its pheromone blend. *J. Chem. Ecol.* 7:501–508.
- COOK, R. D. and WEISBERG, S. 1982. Residuals and Influence in Regression. Chapman and Hall, New York.
- CORNELL, J. A. 2002. Experiments with Mixtures. 3rd edn., Wiley & Sons, New York.
- EVENS, T. J. and NIEDZ, R. P. 2008. Are Hofmeister series relevant to modern ion-specific effects research? *Scholarly Research Exchange* vol. 2008, Article ID 818461, 2008. doi:10.3814/2008/818461
- FLINT, H. M. and MERKLE, J. R. 1983. Pink bollworm (Lepidoptera: Belechiidae): communication disruption by pheromone composition imbalance. *J. Econ. Entomol.* 76:40–46.
- GOTTWALD, T. R., BASSANEZI, R. B., AMORIM, L., and BERGAMIN-FILHO A (2007) Spatial pattern analysis of citrus canker-infected plantings in São Paulo, Brazil, and augmentation of infection elicited by the Asian leafminer. *Phytopathol.* 97:674–683.
- HEPPNER, J. B. 1993. Citrus leafminer, *Phyllocnistis citrella*, in Florida. *Trop. Lepidoptera.* 4:49–64.
- LAPORTE, S. L., EVENS, T. J., and NIEDZ, R. P. 2008. Insect diets as mixtures: optimization for a polyphagous weevil. *J. Insect. Physiol.* 54:1157–1167.
- LAPORTE, S. L., HALL, D. G., MURATA, Y., PARRA-PEDRAZZOLI, A. L., BENTO, J. M. S., VILELA, E., and LEAL, W. S. 2006. Field evaluation of a synthetic female sex pheromone for the leaf-mining moth *Phyllocnistis citrella* (Lepidoptera: Gracillariidae) in Florida citrus. *Fla. Entomol.* 89:274–276.
- LEAL, W. S., PARRA-PEDRAZZOLI, A. L., COSSÉ, A. A., MURATA, Y., BENTO, J. M. S., and VILELA, E. F. 2006. Identification, synthesis, and field evaluation of the sex pheromone from the citrus leafminer, *Phyllocnistis citrella*. *J. Chem. Ecol.* 32:155–168.
- MILLER, J. R., GUT, L. J., DE LAME, F. M., and STELINSKI, L.L. 2006a. Differentiation of competitive vs. non-competitive mechanisms mediating disruption of moth sexual communication by point sources of sex pheromone (Part 1): theory. *J. Chem. Ecol.* 32:2089–2114.
- MILLER, J. R., GUT, L. J., DE LAME, F. M., and STELINSKI, L. L. 2006b. Differentiation of competitive vs. non-competitive mechanisms mediating disruption of moth sexual communication by point sources of sex pheromone (Part 2): case studies. *J. Chem. Ecol.* 32:2115–2143.
- MINKS, A. K. and CARDÉ, R. T. 1988. Disruption of pheromone communication in moths: is the natural blend really most efficacious? *Entomol. exp. appl.* 49:25–36.
- MOREIRA, J. A., MCELFFRESH, S., and MILLAR, J. G. 2006. Identification, synthesis, and field testing of the sex pheromone of the citrus leafminer, *Phyllocnistis citrella*. *J. Chem. Ecol.* 32:169–194.
- MYERS, R. H. 1990. Classical and Modern Regression with Applications. 2nd edn, PWS-KENT, Boston.
- MYERS, R. H. and MONTGOMERY, D. C. 2002. Response Surface Methodology: Process and Product Optimization Using Designed Experiments. 2nd edn., Wiley & Sons, New York.
- NIEDZ, R. P. and EVENS, T. J. 2008. The effects of nitrogen and potassium nutrition on the growth of nonembryogenic and embryogenic tissue of sweet orange (*Citrus sinensis* (L.) Osbeck). *BMC Plant Biology* 8:126.
- NOVAK, M. A. and ROELOFS, W. L. 1985. Behavior of male redbanded leafroller moths, *Argyrotaenia velutinana* (Lepidoptera: Tortricidae), in small disruption plots. *Environ. Entomol.* 14:12–16.
- PEÑA, J. E., HUNSBERGER, A., and SCHAFFER, B. 2000. Citrus leafminer (Lepidoptera: Gracillariidae) density: effect on yield of ‘Tahiti’ Lime. *J. Econ. Entomol.* 93:374–379.
- PIEPEL, G. F. 1982. Measuring component effects in constrained mixture experiments. *Technometrics.* 24:29–39.
- ROELOFS, W. L. 1978. Threshold hypothesis for pheromone perception. *J. Chem. Ecol.* 4: 685–699.
- STELINSKI, L. L., MILLER, J. R., and ROGERS, M. E. 2008. Mating disruption of citrus leafminer mediated by a noncompetitive mechanism at a remarkably low pheromone release rate. *J. Chem. Ecol.* 34:1107–1113.
- WEISBERG, S. 1985. Applied Linear Regression. 2nd edn. Wiley & Sons, New York.
- WITZGALL, P., STELINSKI, L., GUT, L., and THOMSON, D. 2008. Codling moth management and chemical ecology. *Annu. Rev. Entomol.* 53:503–522.