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REDUNDANCY IN A CHEMICAL SIGNAL: Behavioral Responses of Male *Trichoplusia ni* to a 6-Component Sex Pheromone Blend

C.E. LINN, JR., L.B. BJOSTAD, J.W. DU, and W.L. ROELOFS

Department of Entomology
New York State Agricultural Experiment Station
Geneva, New York 14456

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Abstract—The flight response of male *Trichoplusia ni* was observed in a flight tunnel to a sex pheromone blend composed of six components: Z7-12:Ac, 12:Ac, Z5-12:Ac, 11-12:Ac, Z7-14:Ac, and Z9-14:Ac. The number of males reaching a 3000-μg source of this blend was $>95\%$, equal to that observed to female glands and significantly greater than with the previously identified two-component blend (Z7-12:Ac + 12:Ac). In subtraction tests, all five-component blends, with the exception of the blend lacking the primary component Z7-12:Ac, and several four-component blends elicited similar peak levels of upwind flight, source contacts, and hairpencil displays to that observed with the six-component blend. We characterize the substitution of certain minor components for one another as a form of redundancy in the chemical signal and suggest that it contributes to response specificity and signal recognition in males. The results also support the concept that the full blend of components acts as a unit to influence male behavior at all phases of the response. Individual minor components were not responsible for eliciting specific behaviors in the sequence.

Key Words—*Trichoplusia ni*, cabbage looper moth, Lepidoptera, Noctuidae, pheromone, redundancy, flight tunnel.

INTRODUCTION

There is now a widespread recognition of the importance in many moth species of sex pheromone compounds that occur in small or trace amounts (Roelofs, 1981; Steck et al., 1982). This has come about largely as a result of advances in instrumentation, principally capillary GLC (Klun et al., 1979) and airborne collections (Baker et al., 1981a; Pope et al., 1982), as well as

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more detailed behavioral tests (Baker and Cardé, 1979; Linn and Roelofs, 1983; Vetter and Baker, 1983).

In the cabbage looper moth, *Trichoplusia ni* (Hubner), the predominant component is (*Z*)-7-dodecenyl acetate (*Z*7–12:Ac), and when first isolated this compound provided a useful tool in many basic studies of pheromone biology that utilized a simple activation bioassay (Shorey, 1974). Subsequently dodecyl acetate (12:Ac) was identified as a secondary component (Bjostad et al., 1980), and behavioral tests, using a more complex bioassay incorporating a sustained flight tunnel, showed that 12:Ac, in combination with *Z*7–12:Ac, enhanced the behaviors exhibited by males close to a source (Linn and Gaston, 1981). The components in this binary mix met the criteria of primary (*Z*7–12:Ac) and secondary (12:Ac) pheromone components as proposed by Roelofs and Cardé (1977).

In a recent report on the sex pheromone of the cabbage looper moth, Bjostad et al. (1984) identified several additional compounds from female gland extracts and airborne collections. Four of these compounds, (*Z*-5-dodecenyl acetate (*Z*5–12:Ac), 11-dodecenyl acetate (11–12:Ac), (*Z*)-7-tetradecenyl acetate (*Z*7–14:Ac), and (*Z*)-9-tetradecenyl acetate (*Z*9–14:Ac), when added to the previously identified two-component blend (*Z*7–12:Ac + 12:Ac) formed a six-component mix that elicited extremely high (95%) levels of male response (completed flights to source and hairpencil display) in a sustained-flight tunnel. The response to the six-component mix was equal to that observed to female glands and significantly greater than the two-component blend or *Z*7–12:Ac alone. An additional compound, (*Z*)-7-dodecenol (*Z*7–12:OH), was identified (Bjostad et al., 1984) from some gland extracts and airborne collections, but when added to the six-component blend resulted in a significant arrestment of the upwind flight response of males. It was suggested that the compound was not a component of the sex pheromone released by females but rather an artifact of the preparations.

Here we present the results of detailed studies designed to demonstrate the importance of each component in the proposed six-component blend for *T. ni*. The results of a series of subtraction assays with five-, four-, three-, and two-component blends showed that whereas individual components exerted differential effects on male behavior, the influence of any compound depended on the presence of certain other compounds, with several blend combinations eliciting peak response.

METHODS AND MATERIALS

Insects. Cabbage looper moths were reared on a semisynthetic medium (Shorey and Hale, 1965). Males were separated from females as pupae, kept as adults on a 14:10 light–dark photoperiod at 25–27°C, and provided with 8% sugar water solution.

Chemicals. The synthetic chemicals used in this study were the same as those in Bjostad et al. (1984). The proportions of each compound in the standard six-component mix were as follows: 12:Ac (6.8%), *Z*5–12:Ac (7.6%), *Z*7–12:Ac (100%), 11–12:Ac (2.3%), *Z*7–14:Ac (0.9%), and *Z*9–14:Ac (0.6%). Solutions of the desired blends (with each compound within 1.5% of the standard) were prepared in Skelly B (predominantly *n*-hexane) and checked on capillary GLC (45-m Carbowax 20 M column) to ensure purity (>99%), with a detection limit of 0.1%. A dilution series was then prepared for each treatment to be applied to a release source, either filter paper, rubber septum, or polyethylene cap.

Release Sources. The filter paper source was a 0.75-cm^2 piece of Whatman No. 1 (qualitative, lot No. 308306) positioned on the end of an insect pin placed in a cork stand. Solutions (1 $\mu g/\mu l$) were added with a disposable pipet and were prepared daily just prior to testing.

The rubber septa (Arthur H. Thomas Co., red, 5×9 mm) were prepared by adding solutions (10 $\mu g/\mu l$) to the wide open end of the septa. Septa were placed individually in 4-dram vials and held at $-10°C$ when not in use.

Polyethylene caps (OS-6 closures, American Scientific Products, McGaw Park, Illinois) were prepared by adding solution (10 $\mu g/\mu l$) to the inside surface of the cap. After 1 hr to allow evaporation of the solvent, the cap was closed and placed in a laboratory exhaust hood for 36 hr. Caps were then placed individually in glass vials and held at $-10°C$ when not in use.

In addition to synthetic mixtures individual female glands were also tested. Individual females (3–4 days old) were prepared as in Bjostad et al. (1984) using intact females and the holder utilized for volatile collections.

Test Procedures: Flight Tunnel. Individual 3- to 4-day old males were tested in the sustained-flight tunnel described by Miller and Roelofs (1978) during the fifth and sixth hours of the 8-hr scotophase period (Linn and Gaston, 1981). Males were placed in the room housing the flight tunnel at the beginning of the scotophase to acclimate to conditions in the tunnel: 0.1–0.3 lux, 50–55 cm/sec air velocity, 21–23°C, 50–70% relative humidity (as in Linn and Gaston, 1981).

Males were allowed 1 min to respond and were scored for the following behaviors: activation wing fanning and walking, taking flight, stationary flight near the release cage with the male oriented in an upwind direction, initiation of upwind anemotactic flight, upwind flight to the midpoint of the tunnel, close range approach and contact with the source, protrusion of hairpencils, and attempted copulations. In the present study, source contact is differentiated from a copulatory attempt by males exhibiting, in the latter case, a bending of the abdomen and eversion of the hairpencils, as described in Gothilf and Shorey (1976). Extension of abdominal claspers without the hairpencil display was difficult to observe in the low light conditions, and this was not recorded as a separate behavior. All behaviors were recorded on cas-

sette tape so that temporal aspects of the flight response could be analyzed at a later time. Males were tested only once and then discarded. At the end of each test all metal plates, release cages, and support stands were thoroughly rinsed in acetone and oven-dried at 100°C overnight.

The cluster analysis of male response for all treatments in the subtraction tests was performed as in Linn and Roelofs (1983). The similarity matrix was calculated using the coefficient described by Gower (1971), and the clustering was done using an average linkage analysis with unweighted means, according to Sneath and Sokal (1974) and was performed on a PRIME 400 computer using a program in the GENSTAT statistical package (Alvey et al., 1977).

RESULTS

Dose Response to Six-Component Blend on Filter Paper, Rubber Septa, and Polyethylene Caps. Peak response for male *T. ni* to the six-component blend was observed to a 1-μg dosage on filter paper and to a 3000-μg dosage on polyethylene caps (Figure 1). In contrast, response levels were significantly lower when rubber septa were used, never reaching the near 100% levels observed with caps or filter paper. In addition, males were observed to locate the filter paper source and hairpencil more easily than with rubber septa or caps when the latter were placed in the usual position in the center of the 15 × 15-cm platform (see Linn and Roelofs, 1981). Males appeared to have difficulty reaching the rubber septum or polyethylene cap by landing and walking to them, preferring to fly up to the source, as with the filter paper, which was elevated several centimeters above the surface of the platform. Further tests showed that males exhibited higher numbers of displays relative to source contacts when the caps were placed at the downwind edge of the platform. In this situation, males made continuous flights up to the source, touching the cap with their forelegs and antennae while hanging and vibrating their wings, then bending the abdomen vertically and everting the hairpencils. As a result of these tests, closed polyethylene caps containing 3000 μg of synthetic compounds were used in the subtraction tests to follow, with the source placed on the downwind edge of the platform.

Tests with Five-Component Blends. Removal of Z7–12:Ac from the six-component blend resulted in significant decreases in male activity, with none of the males initiating upwind flight to the source (Figure 2, treatment 7). In contrast, removal of any other component did not affect male behavior. Further tests with the five-component blends (with the exception of the blend lacking Z7–12:Ac) at two lower concentrations, 300 and 1000 μg (Table 1), showed no significant difference in the number of males reaching the source between the five-component blends and the six-component blend at each concentration.

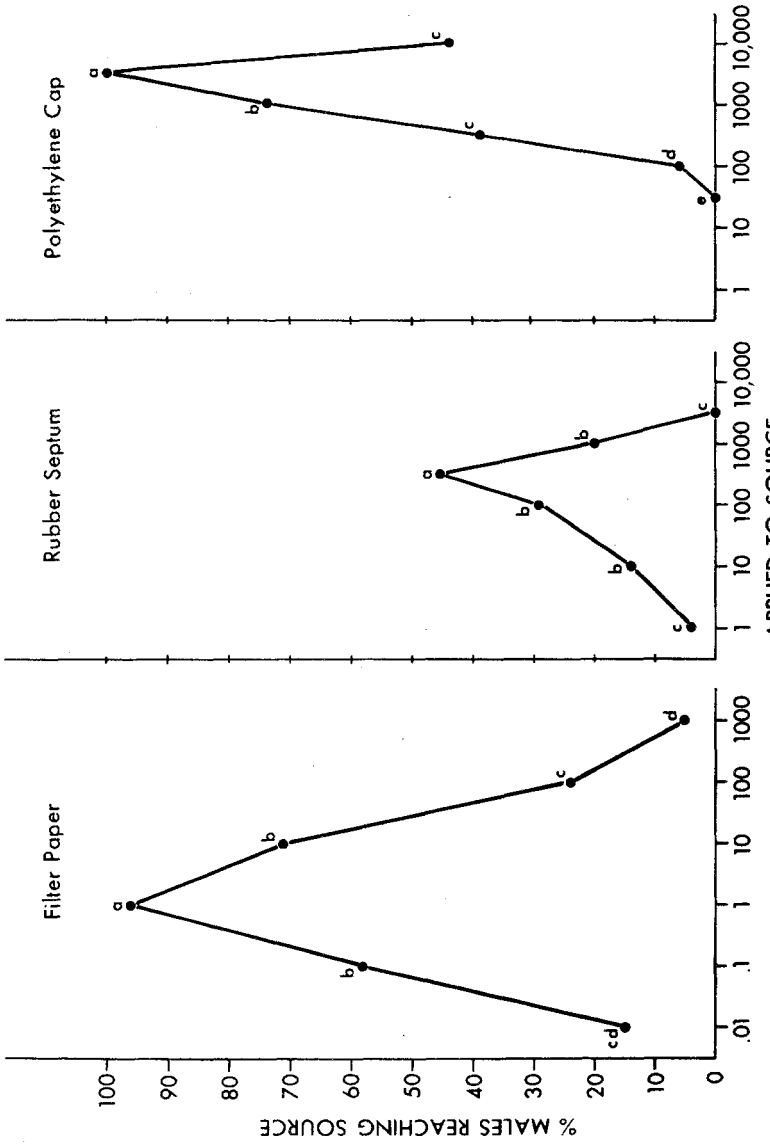

FIG. 1. Percentage of male *T. ni* making source contact with three different pheromone sources containing the six-component blend. For each source type, values having different letters are significantly different ($P < 0.05$) according to the method of adjusted significance levels for proportions (Ryan, 1960). $N = 100$ males for each concentration and source type.

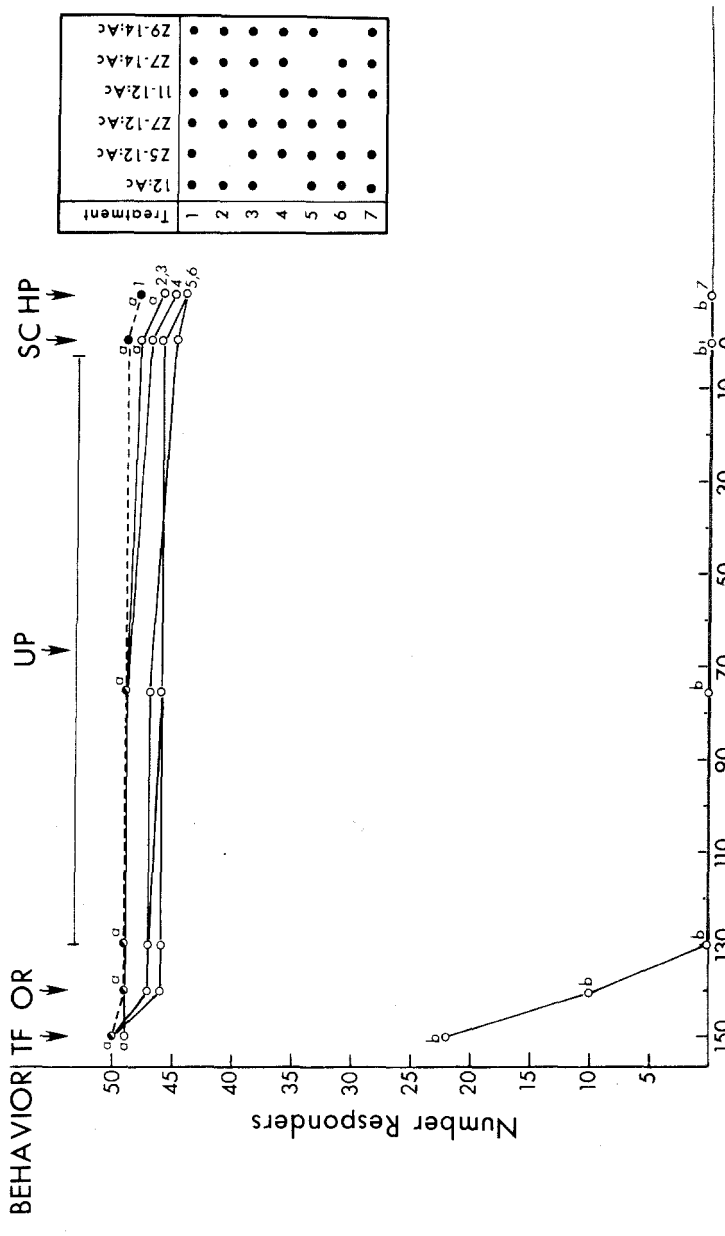

FIG. 2. Response of male *T. ni* to the six-component blend and all five-component blends. Behaviors are taking flight (TF), stationary orientation flight (OR), upwind flight (UP), source contact (SC), and hairpencil display (HP). For all treatments the concentration was 3000 μg. Significant differences for values at each behavior as in Figure 1. $N = 50$ for each treatment.

TABLE 1. PERCENT MALE *T. ni* REACHING SOURCE WITH THREE CONCENTRATIONS OF 6-COMPONENT AND FIVE OF 5-COMPONENT BLENDS[a]

Treatment	Concentration (μg)		
	300	1000	3000
1. 6-component	34a	76a	96a
2. − 12:Ac	38a	70a	90a
3. − Z5-12:Ac	26a	72a	94a
4. − 11-12:Ac	32a	68a	94a
5. − Z7-14:Ac	26a	60a	88a
6. − Z9-14:Ac	30a	78a	86a

[a] $N = 50$ for each concentration. Values in each column with different letters are significantly different ($P < 0.05$) according to the method of adjusted significance levels for proportions (Ryan, 1960).

Tests with Four-Component Blends. The number of males reaching the source was significantly decreased to four of the 10 treatments containing four components when compared to the six-component mix (Figure 3). With the treatment lacking both 14-carbon acetates (treatment 8), male response was most affected in the early part of the upwind flight response. The remaining three treatments (9, 10, and 11) significantly affected the earlier orientation flight to the plume as well as the upwind flight response. In addition, treatments 10 and 11 significantly decreased the level of hairpencil displays when compared to the number of males making contact. Treatments 9, 10, and 11 (Figure 3) all share the common property of lacking two of the following components: Z5-12:Ac, 11-12:Ac, or Z7-14:Ac.

Tests with Three-Component Blends. Male response to all three-component blends was significantly decreased at all stages in the sequence with the exception of the initial response, taking flight, when compared to the six-component blend (Figure 4). The patterns in Figure 4 also show that response to the three-component blends was not significantly different for any blend until the final step, hairpenciling and copulatory attempts. The level of hairpenciling was most affected by treatments lacking combinations of Z7-14:Ac, 11-12:Ac, Z5-12:Ac, or 12:Ac, with no single component or binary combination essential to the success of the display.

Tests with Two-Component Blends. As anticipated from the results in Figure 4, male response was significantly decreased to all two-component combinations when compared to the six-component blend (Figure 5). This figure also shows the response to Z7-12:Ac alone.

Cluster Analysis of All Treatments. Examination of the response patterns in Figures 2, 3, 4, and 5 suggested that certain treatments, which varied

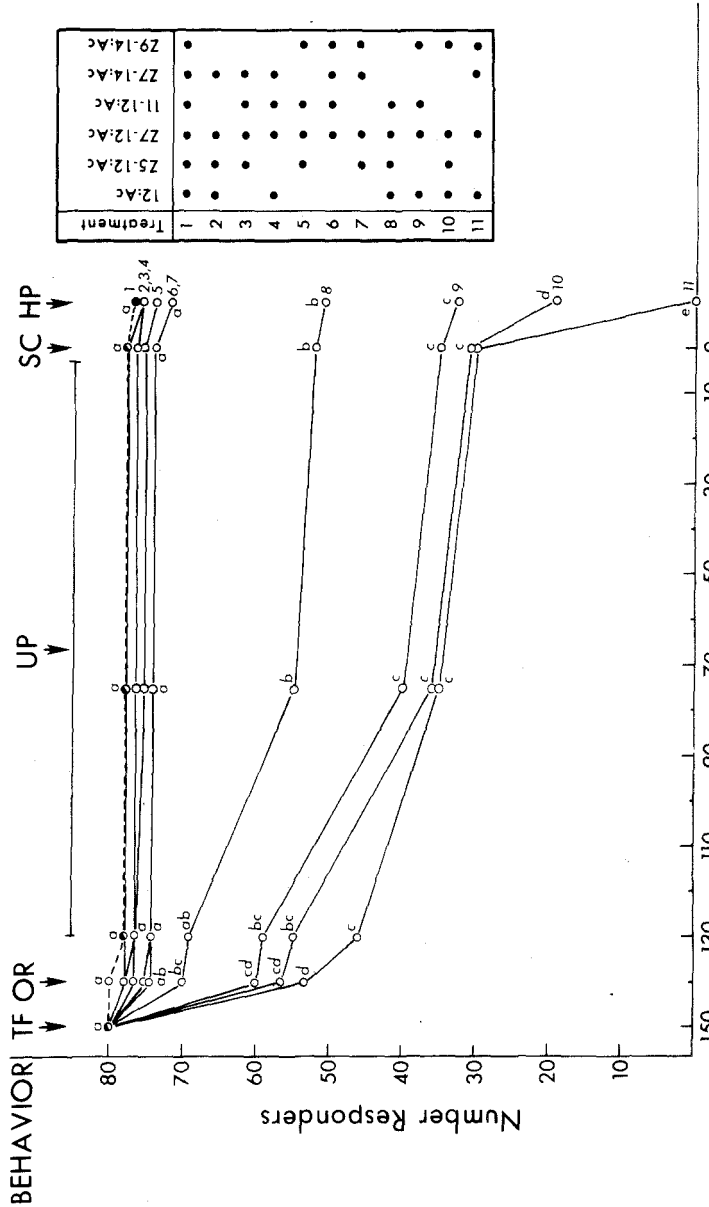

FIG. 3. Response of male *T. ni* to a 3000-μg concentration of the six-component blend and all four-component blends containing Z7–12:Ac. Behaviors and significant differences as in Figure 2. $N = 80$ for each treatment.

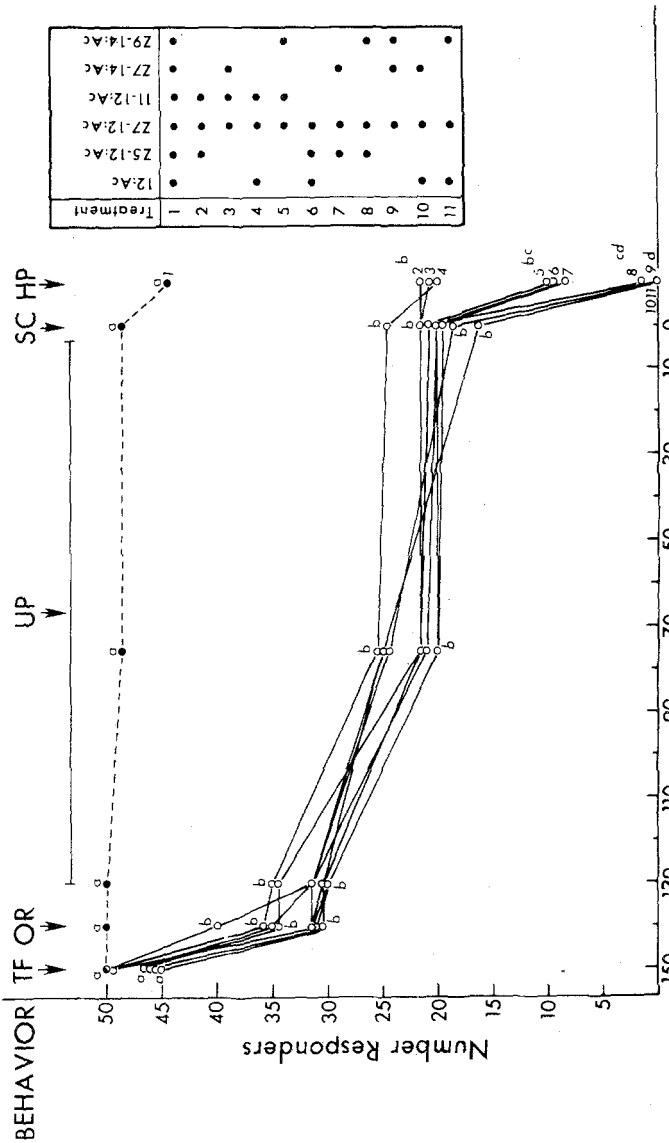

FIG. 4. Response of male *T. ni* to a 3000-μg concentration of the six-component blend and all three-component blends containing Z7–12:Ac. Behaviors and significant differences are in Figure 2. $N = 50$ for each treatment.

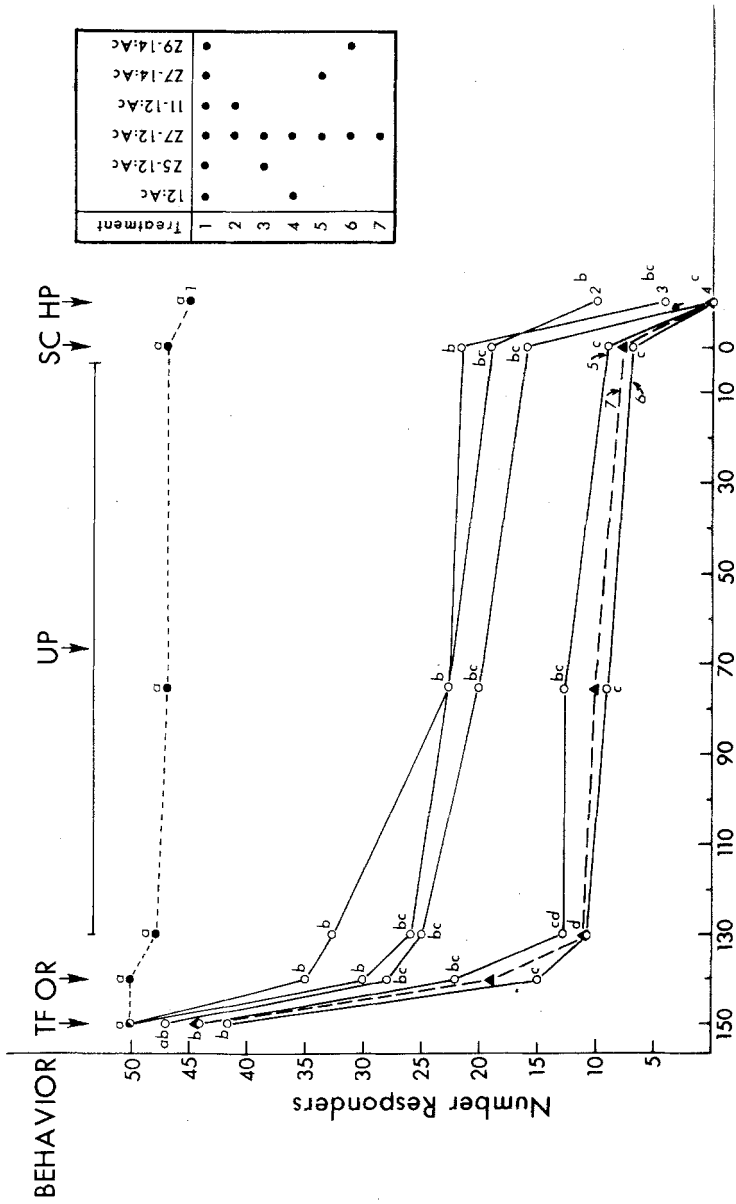

FIG. 5. Response of male *T. ni* to a 3000-μg concentration of the six-component blend, all two-component blends containing Z7–12: Ac, and to Z7–12: Ac alone. Behaviors and significant differences as in Figure 2. $N = 50$ for each treatment.

in the number of components, elicited similar levels and patterns of response. To determine the similarity of treatments, the table of response values for seven observed behaviors to the 33 treatments was subjected to hierarchical cluster analysis. The dendrogram shown in Figure 6 is derived from analysis of four behaviors (taking flight, upwind flight, source contacts, and hairpencil displays), as additional behaviors did not add significant information to the analysis. The response patterns for five identified clusters are shown in Figure 7, and the treatments within each cluster are displayed in Table 2.

From the data presented in Table 2 several important relationships can be noted. Most evident is that *Z*7–12:Ac is essential for any significant level of upwind flight to occur. Cluster III represents a number of treatments containing two, three, or four components, all of which significantly increased the number of males successfully initiating upwind flight and making contact with the source over that observed with treatments in cluster IV. The subdivision of cluster III was made first on the basis of the cluster analysis differentiating increased levels of hairpenciling (levels IIId, c, b, a) and second on the basis of visual inspection of Table 2 indicating the presence of specific combinations of components that were associated with increasing levels of hairpenciling (demarcated by the dashed lines). Treatments in cluster IIId, for example, lack *Z*5–12:Ac and 11–12:Ac and represent those for which no hairpenciling was observed. Treatments in clusters IIIc, b, and a show the effect on hairpencil displays of adding *Z*5–12:Ac or 11–12:Ac to the treatments in IIId.

Cluster II represents a single four-component blend resulting from addition of 12:Ac to *Z*7–12:Ac + *Z*5–12:Ac + 11–12:Ac, and lacking both of the 14-carbon acetates. Significant increases in source contacts compared to treatments in cluster III occurred as a result of an increase in the number of males successfully initiating upwind flight.

Peak levels of response occurred to treatments in cluster I, comprising the six-component blend, five of the five-component blends, and several four-component blends. Examination of treatments in cluster I leads to a set of associations, shown in Figure 8, describing all possible signals eliciting peak response.

Analysis was also made of several other quantitative aspects of the flight response and the hairpencil display, using the treatment clusters in Table 2. Males were observed to take significantly longer to fly upwind to the source to treatments in clusters III and IV compared to I and II (Figure 9). There was no difference in this temporal measure for the subdivided treatment groups within cluster III. With respect to the hairpencil display, males spent significantly longer periods at the source and exhibited greater numbers of displays to treatments in clusters I, II, and IIIa, compared to those in IIIb, c, d; IV; or V (Figure 10).

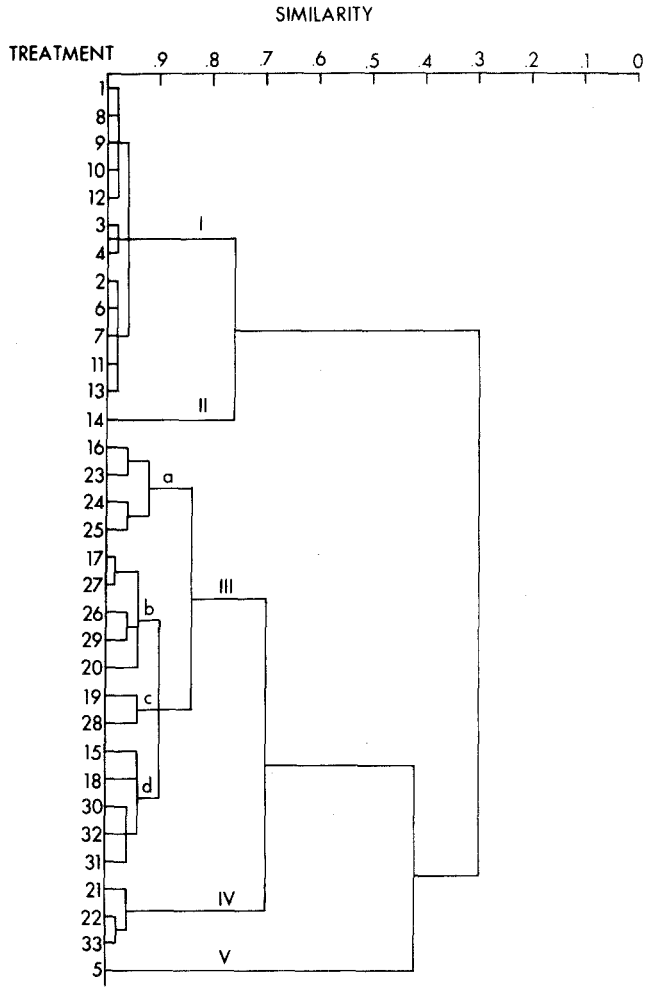

FIG. 6. Dendrogram resulting from hierarchical cluster analysis of response values for four behaviors (TF, UP, SC, HP; see Figure 2) to the 33 treatments tested in subtraction assays.

Z7–12:OH and Male Behavior. Response levels to the six-component blend on filter paper were found to be greater at all dosages tested when compared to the six-component blend with 0.5% Z7–12:OH added (Figure 11). Male response to the six-component blend was also found to be significantly affected during the upwind flight phase of the sequence as increasing amounts of Z7–12:OH were added to the 1-μg dosage (on filter paper) (Figure 12).

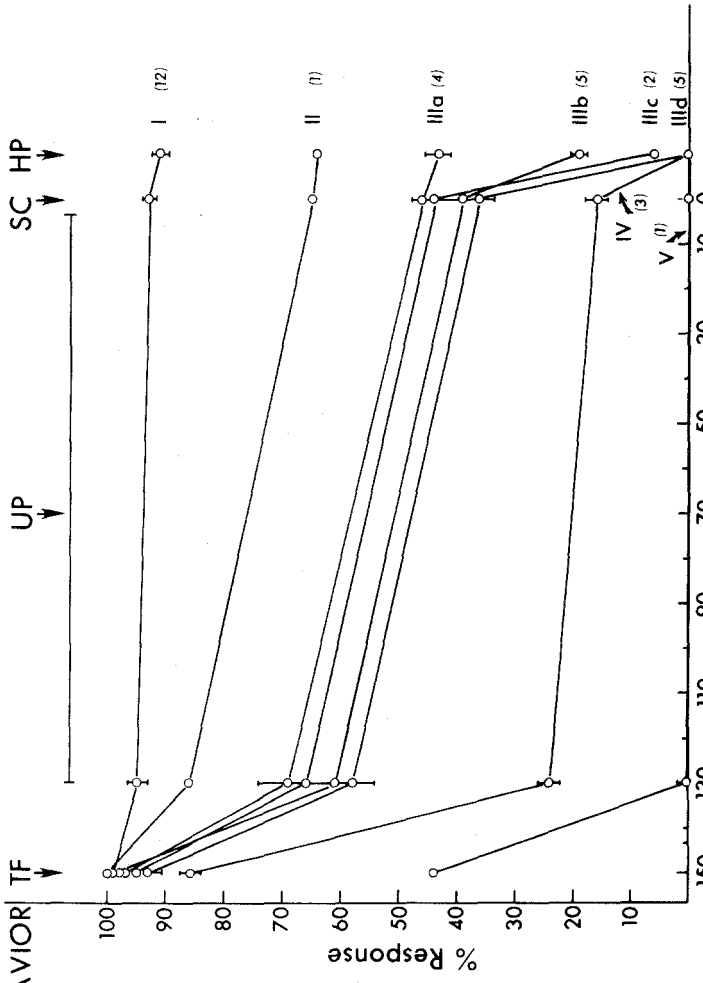

FIG. 7. Response patterns for clusters of treatments from dendrogram in Figure 6. Response values are the mean (±SD) for each behavior (as in Figure 2) in the sequence. Values next to numerals indicate the number of treatments in each cluster.

TABLE 2. PERCENT RESPONSE OF MALE *T. ni* TO 33 TREATMENTS SHOWN IN FIGURE 6[a]

Treatment	Taking flight (%)	Upwind flight (%)	Source contacts (%)	Hairpencil displays (%)	12:Ac	Z5-12:Ac	Z7-12:Ac	11-12:Ac	Z7-14:Ac	Z9-14:Ac		Cluster
1	100	98	98	96	*	*	*	*	*	*	6	
3	100	98	94	96		*	*	*	*	*	5	
2	100	94	92	92	*	*	*	*	*	*	5	
4	100	98	96	92	*	*	*		*	*	5	
6	100	94	90	88	*	*	*	*		*	5	
7	100	92	92	88	*	*	*	*	*		5	
11	100	93	93	90			*	*	*	*	4	I
13	100	92	92	90		*	*		*	*	4	
12	100	96	95	92		*	*	*		*	4	
9	100	96	96	95		*	*	*	*		4	
10	100	96	96	95	*		*	*	*		4	
8	100	98	98	95	*	*	*		*		4	
14	100	86	65	64	×	×	×	×			4	II
25	94	62	46	46		●	●	●			3	
16	100	74	44	41	◒		◒	◒		◒	4	IIIa
23	100	70	48	40	◒		◒	◒			3	
24	92	68	46	44			◒	◒	◒		3	
26	96	60	40	20			◒	◒		◒	3	
20	100	62	38	16			◒	◒			2	
17	100	69	39	19	○	○	○			○	4	IIIb
27	100	66	40	18	○	○	○				3	
29	98	60	38	16		○	○		○		3	
28	90	66	44	6		○	○			○	3	IIIC
19	100	66	44	7		○	○				2	
32	90	62	34	0	◒		◒			◒	3	
31	94	66	36	0	◒		◒		◒		3	
15	100	57	37	0	◒		◒		◒	◒	4	IIId
18	94	50	32	0	◒		◒				2	
30	90	60	40	0			◒		◒	◒	3	
21	88	26	18	0			+		+		2	
22	84	22	14	0			+			+	2	IV
33	87	23	15	0			+				1	
5	44	0	0	0	●	●		●	●	●	5	V

[a]Treatments are arranged in clusters as characterized in Figure 6, and listed within each cluster to show relationships between blend composition and response level. See text for details. $N = 80$ for the four-component blends, and 50 for any others.

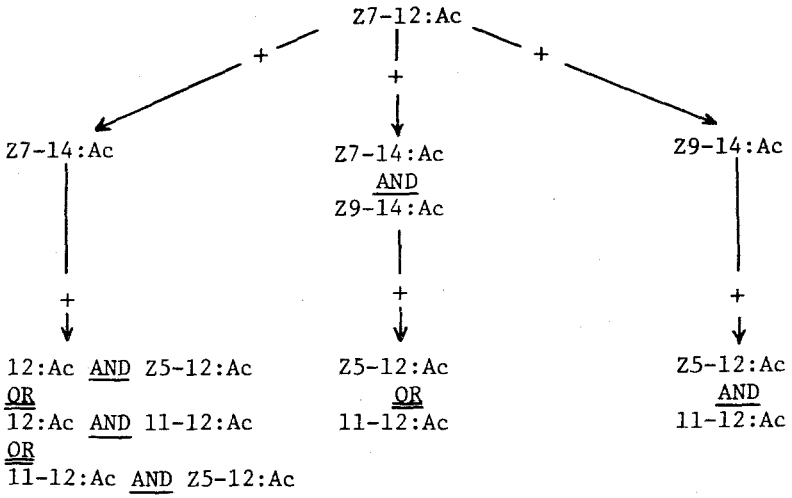

FIG. 8. Flow chart showing the association of components into blends that will elicit peak response.

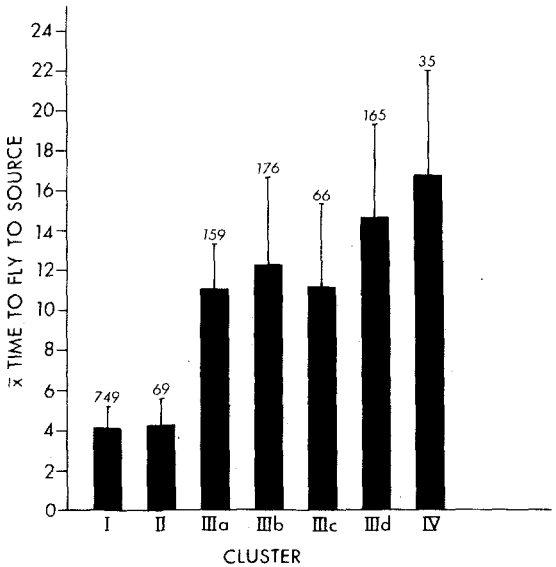

FIG. 9. Time (sec) for male *T. ni* to fly upwind 130 cm to the source to treatments within each cluster identified in Figure 7. Values are the mean (±SD), with the number of responders shown above each column.

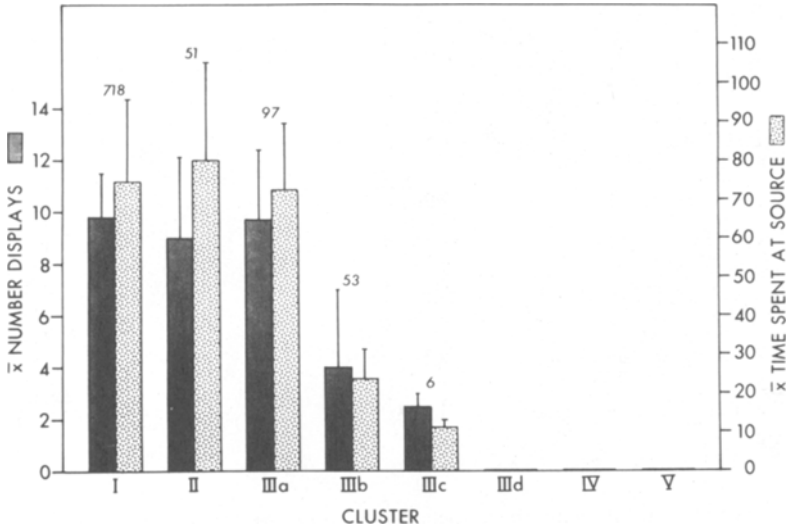

FIG. 10. Time (sec) spent by male *T. ni* at the source, and the number of hairpencil displays exhibited. Values are means (±SD), with the number of responders shown above each column.

Increasing levels of Z7–12:OH resulted in a significant number of males that initiated upwind flight exhibiting arrested flight patterns in the first half of the flight downwind in the tunnel. This arrestment was assessed as an abrupt slowing of upwind flight to the stationary zig-zag pattern with no net upwind movement.

DISCUSSION

Pheromone Component Functions and Redundancy in a Chemical Signal. We propose, on the basis of the present study, that all six components are involved in the sex pheromone communication system of *T. ni* and that the observed redundancy with respect to component substitutions and interactions comprises a new and important element of pheromone studies. All of the five-component blends (except the one lacking Z7–12:Ac) and several four-component blends elicited peak response levels as high as those observed with the six-component blend. The evidence implies that none of the five secondary components is absolutely required for peak response. It appears instead that the pheromone is polythetic in character, in that a combination of any four minor components can compensate for the lack of the fifth, and in some cases combinations of the three minor components can compensate for the lack of the other two. The results also show that as re-

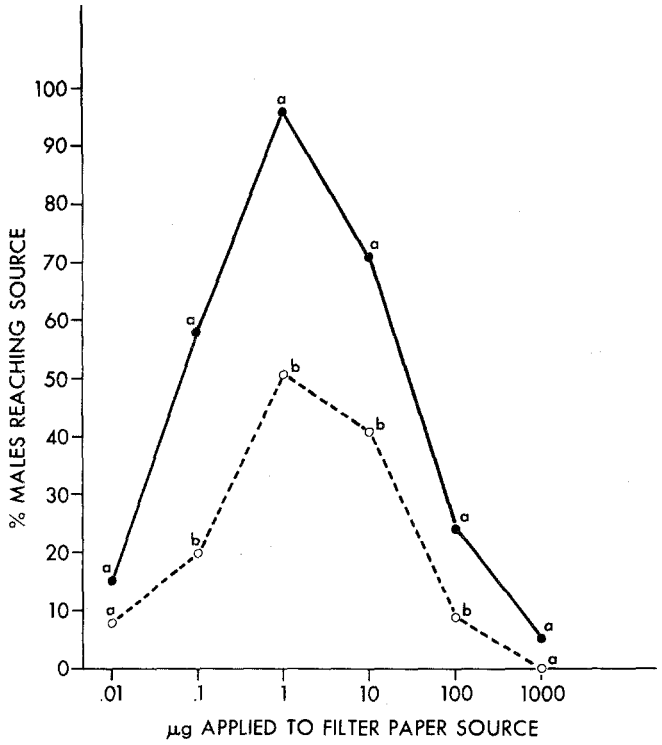

FIG. 11. Response of male *T. ni* to the six-component blend alone (solid line) and with 0.5% Z7-12:OH added (dashed line). Significant differences as in Figure 2. $N = 100$ for each concentration and treatment.

sponse levels increased with increasing blend complexity, the importance of individual components as functional elements associated with specific behaviors diminished dramatically. The conclusion is that it was not possible to assign specific behavioral functions to any of the individual compounds.

Our results raise some important points concerning the classification of a given compound as a pheromone component. In the classic view of a pheromone complement, if subtraction of a compound from a candidate blend fails to diminish the response to the blend, the compound is not considered a pheromone component. Our conclusion that all six acetates are pheromone components in *T. ni*, despite the fact that any of the five minor components can be omitted without diminishing male response, is a significant departure from this view. In drawing this conclusion, we were careful to satisfy two criteria that we propose are essential: that male responses to females or female extracts should be high, indicating that the assay conditions are appropriate, and that the male response to the best synthetic blend should be equally high.

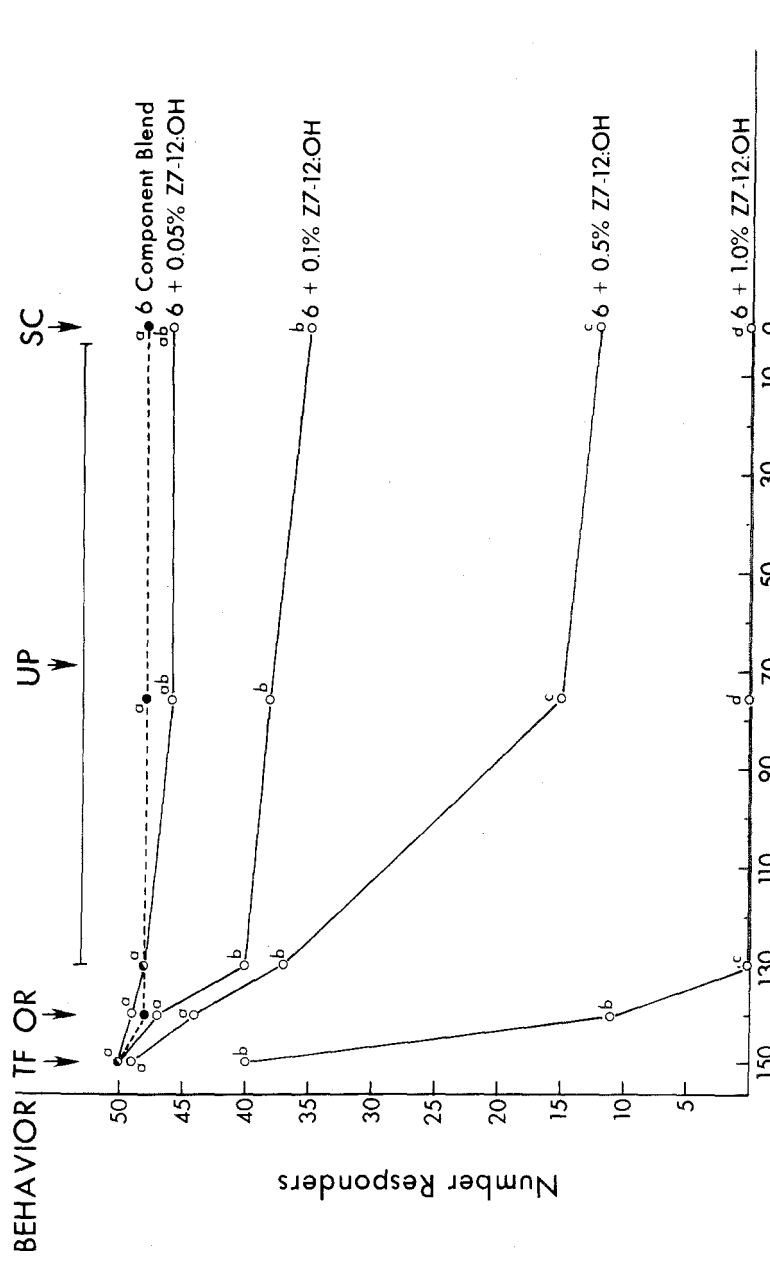

FIG. 12. Response of male *T. ni* to a 1-μg dosage of the six-component blend on filter paper with four added proportions of Z7-12:OH. Behaviors and significant differences as in Figure 2. $N = 50$ for each treatment.

Our analysis supports the concept that it is the blend of components acting as a unit that is critical in effecting optimal behavior in males (Linn and Roelofs, 1982, 1983; Baker and Cardé, 1979; Baker et al., 1981b). Studies with *Grapholitha molesta* have shown that males are more sensitive to the natural blend of components compared to single components (Linn and Roelofs, 1983) and that they are very sensitive to slight changes in blend. The resulting sensitivity is presumably adaptive since it enhances the males ability to detect, recognize, and respond rapidly to the airborne signal, thus optimizing the males' likelihood of locating a mate.

Recognition involves an assessment by the male of the chemical signal and a decision as to whether the signal is being released by a female of his species. Inherent in the decision process is a possibility that the male will make a wrong choice (Wiley and Richards, 1982), either by not responding to the appropriate signal or perhaps by responding to a similar signal of a closely related species. The uncertainty involved is greatly reduced by utilizing complex blends of chemicals, because the decision to respond can be made dependent on perception of a specific blend of components.

While complex blends aid in achieving response specificity and increased mating success, the presence of redundant elements in the signal is not a necessary feature. In the present study redundancy was evidenced by the fact that certain minor components could substitute for each other without any observed change in male behavior. We would propose that the specific substitutions observed for the minor components reflect interactions, at the level of the peripheral antennal receptors, which significantly influence neural processing of the chemical signal. The presence of redundant elements could add to the efficiency of the processing by allowing some minor components to interact at several receptor sites, resulting in an amplification of the stimulus induced by specific minor components, or the major component.

Examples from neurophysiological studies indicate that there are a number of possibilities for component–receptor site interactions at the peripheral level. It has been established for a number of species that individual senilla trichodea contain from two to five receptor types (Preisner, 1983). In the *Yponomeuta* complex of species, for instance, response spectra of sensilla trichodea cells showed that two or three types of sensilla containing two to three different cells could be distinguished. Up to six different sensilla could thus occur in one species, and this did not take into account the added variability in sensitivity as a function of concentration (Van Der Pers, 1982). There can also be considerable variability in response across receptor neurons to any one compound, and across compounds to any neuron (O'Connell, 1975), presumably due to intrinsic properties of the neuron, such as kind, number, and location of the different receptor sites. In general, while the receptors are specific for individual compounds (Preisner, 1979), interactions can occur, and it is not always certain whether one is dealing

with distinct sites or a quantitative difference in binding strength between different chemicals at a common site (O'Connell, 1975). In some cases there appears to be a competition between compounds for the same receptor site, as in *Adoxophyes orana* (Den Otter, 1977). In this species one cell (the B cell) is excited by Z9–14:Ac alone, whereas another cell (the B cell) is excited by Z11–14:Ac and to much lesser extent Z9–14:Ac. The A cell shows a decrease in response to Z11–14:Ac when Z9–14:Ac is present, and it was concluded that the two compounds compete for the same sites in the A cell membrane, with the binding of Z9–14:Ac resulting in a smaller potential change than occurs with Z11–14:Ac. In other cases, an interaction between compounds can occur at the same receptors, as appears to be the case in *Spodoptera litura* (Aihara and Shiboya, 1979). In both *S. litura* and *S. exempta* (Steinbrecht, 1982), minor components do not elicit strong responses unless the major components are present. This is also the case with *Argyrotaenia velutinana*, in which dodecyl acetate gives a weak response when presented alone, but strongly potentiates the response to Z11–14:Ac (O'Connell, 1972).

While our hypothesis is speculative in nature, it is testable. We would propose, for example, that differential adaptation studies as well as further behavioral tests involving preexposure of males to individual components (as in Linn and Roelofs, 1981), as well as specific combinations of components illustrated in Figure 8, would help elucidate receptor–component interactions.

With respect to the female, the releaser in the system, studies on the biosynthesis of the *T. ni* sex pheromone (Bjostad and Roelofs, 1983) suggest that all of the secondary components are products of a common pathway and thus the probable expense of producing and releasing a six-component vs. a two-component blend is slight. At the same time, producing a complex blend provides the female with a mechanism for selecting mates that preferentially respond to her and also avoiding hybridizing with closely related species.

Complex pheromone blends that include redundant elements are one end of a spectrum of possibilities that insects might use in their communication systems. We do not propose that all species will have complex pheromone blends, and we recognize that this will depend on several factors. One involves consideration of the biosynthetic route for the primary component(s) and the potential for additional components from precursors in the pathway (Bjostad and Roelofs, 1983; Roelofs an Brown, 1982). Another is the inter- and intraspecific pressure on males to utilize a highly specific blend. It is hoped that future studies will address the importance of additional components in optimizing male behavior and that greater consideration will be given to ecological questions concerning male competition for mates (as in Parker, 1978) and the importance of interspecific interactions (see Tumlinson, 1982).

Experimental Design and Peak Response. The success of the flight tunnel as a sensitive assay for this complex blend was not anticipated based on previous experience with this insect (Linn and Gaston, 1981). *T. ni* males are very sensitive to movement as well as several environmental stimuli during the scotophase activity period, and it was found in the previous study that an acclimation period to flight-tunnel conditions was essential for male flight to the pheromone. In the present study, a longer acclimation period (4 hr vs. 1 hr) was incorporated to aid in handling of the insects, and yet even with all precautions, male response levels to the two-component blend, Z7–12:Ac + 12:Ac, were highly variable from day to day when tested over a one-month period, as was done in preliminary tests. This variability (which was not observed with the six-component mix) had also been observed in previous work (Linn and Gaston, 1981) and necessitated establishing in that study a daily response criterion of at least 50% of the moths taking flight for the results to be considered in the final analysis. This resulted in assay data for many days not being included, and in what now can be recognized as an artificially high response value reported to the two-component blend [>70% in Linn and Gaston (1981) compared to 30–40% in the present study, Figure 5].

We propose that the poor response to rubber septa was the result of an inappropriate ratio of compounds being released from this substrate. Analysis of two airborne collections of the six-component mix on rubber septa showed that Z7–14:Ac and Z9–14:Ac were released in much lower proportions than was applied to the source (0.02 and 0.01% vs. 1.0 and 0.6%, respectively). Polyethylene caps were not tested due to physical limitations of the airborne collection apparatus.

Close Range Behavior and Hairpencil Displays. The hairpencil display observed in the present study was very similar to that reported by Gothilf and Shorey (1975). As reported by them and discussed in Colwell et al. (1978), females of several species characteristically release pheromone while wing fanning and hanging from a substrate. Males typically fly up to the female and initiate courtship while flying in a hovering position. Examples include *I. ni*, *Lymantria dispar* (L.), and *Pectinophora gossypiella* (Saunders) and are in sharp contrast with species with as *A. velutinana* (Walker) and *Grapholitha molesta* (Busck), which typically land and walk to the female. This basic difference in close range flight behavior proved critical in the hairpencil display observed here with *T. ni.* Males performed the display more often and with more intensity when they could fly up to the source and hang on it while wing-fanning.

The hairpencil display was the behavior most affected by subtle changes in blend composition. Males did not hairpencil to the two-component blend containing Z7–12:Ac and 12:Ac, and the minimum blend for optimal display was found to depend on the presence of 11–12:Ac and either 12:Ac,

Z5-12:Ac, or Z7-14:Ac (cluster IIIa, Table 2). The compound 11-12:Ac, however, was not essential since within cluster I treatments containing both Z5-12:Ac and Z7-14:Ac were equal to any containing 11-12:Ac. As noted earlier, the conclusion is that no single component can be labeled as a close range or hairpenciling component, when evaluated in the context of the response to the full blend.

Z7-12:OH and Male Behavior. The identification of a sex pheromone component has typically involved some behavioral test, either field testing or a laboratory bioassay. In all cases, confirmation of the compound's status has depended on a positive or enhancing effect on male behavior (Cardé, 1979). At the same time, several studies have demonstrated that males of certain species are very sensitive to compounds that are related to known pheromone components but are not released by females. Such is the case with Z7-12:OH in *T. ni*. Field tests (Tumlinson et al., 1974) and flight-tunnel studies (McLaughlin et al., 1974) provided evidence that Z7-12:OH significantly decreased the level of upwind flight in male *T. ni*. Electrophysiological studies (O'Connell et al., 1983) also support the fact that male *T. ni* can perceive this compound.

It was thus of some interest to us to observe this compound in some of the female airborne collections (Bjostad et al., 1983). Data from the present study confirm that, under all conditions tested, addition of Z7-12:OH resulted in decreased male response. The weight of evidence suggests that its occasional presence of about 0.5-1.0% in gland extracts and airborne collections is an artifact, perhaps due to a small amount of hydrolysis of Z7-12:Ac during handling. While we have concluded that the presence of this compound in the airborne collections is an artifact, the question concerning the biological relevance of the observed activity remains. It most likely has biological significance in interspecific interactions, wherein *T. ni* males have reduced behavioral response to other cohabitating species utilizing Z7-12:OH.

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