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MALE MOTH SENSITIVITY TO MULTICOMPONENT PHEROMONES: Critical Role of Female-Released Blend in Determining the Functional Role of Components and Active Space of the Pheromone

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Abstract—In the present study male redbanded leafroller (*Argyrotaenia velutinana*), cabbage looper (*Trichoplusia ni*), and Oriental fruit moths, (*Grapholita molesta*), were tested in a flight tunnel to (1) the major pheromone component, (2) the *Z/E* pheromone component mixtures for Oriental fruit moth and redbanded leafroller, (3) and the female-released blends, over a series of dosages. Experiments were designed to test the hypothesis that male response downwind of a female is initiated by the major component and that minor components function only to elicit behaviors close to the female during close-range approach and courtship. The results did not support this hypothesis, but rather showed that males initiated upwind flight in significantly higher percentages to the complete blends of components, at all dosages, compared to single components or partial blends. Addition of minor components also signficantly enhanced male perception of the major component at lower dosages, resulting in completed flights to dosages of the major component that alone did not elicit any upwind flight. Our results support the concept that minor components function to enhance male sensitivity to the pheromone, and the specificity of the signal. Our results also support the hypothesis that the active space of the pheromone is a function of the upper and lower concentration thresholds for the blend of components, and not simply for the major component.

Key Words—Sex pheromone, active space, *Trichopulsia ni*, Lepidoptera, Noctuidae, *Argyrotaenia velutinana*, *Grapholita molesta*, Tortricidae, multicomponent pheromones, sustained-flight tunnel.

INTRODUCTION

Recently, in our lab, the sex pheromone of the cabbage looper (CL), *Trichoplusia ni* (Hubner), and the redbanded leafroller (RBLR), *Argyrotaenia velutinana* (Walker), were reinvestigated (Bjostad et al., 1984a,b; Linn et al., 1984), as part of a series of studies concerned with sex pheromone biosynthesis (Roelofs and Brown, 1982; Bjostad and Roelofs, 1983; Roelofs and Bjostad, 1984). Several new compounds were identified for each species from sex pheromone gland extracts and airborne collections, as predicted from the proposed biosynthetic routes. Subsequent behavioral tests in a sustained-flight tunnel revealed two important points: the female-released blend was superior to the previously identified pheromones (Roelofs et al., 1975; Bjostad et al., 1980), and the blend enhanced all aspects of the male response, both quantitatively and qualitatively. The results of these tests indicated that previously characterized behavioral functions of the components were artifacts (Baker et al., 1976; Linn and Gaston, 1981a,b), the result of analysis based on observations of male behavior with partial or incorrectly identified blends. Our results were also in agreement with those reported previously for the Oriental fruit moth (OFM), *Grapholita molesta* (Busck) (Baker and Cardé, 1979).

The present work was initiated to expand on the results of the studies with OFM, CL, and RBLR. We tested male moths in the sustained-flight tunnel to a dosage series of the major (or most abundant) pheromone component, of the *Z/E* mixture for OFM and RBLR, and of the full blend for each species. Our objective was to determine if males are significantly more responsive to the female-released blend of components, and if they are more sensitive to the full blend compared to only the major component(s) at low dosages, simulating conditions downwind from a female. The results are discussed with respect to current ideas concerning male perception and the active space of the pheromone.

METHODS AND MATERIALS

Insects. RBLR and CL were reared on semisynthetic diet (Shorey and Hale, 1965), and OFM were reared on small green thinning apples, at 25°C, 16:8 light–dark photoperiod. The sexes were separated as pupae, and adult males were segregated daily by age and kept under conditions similar to those during rearing, in chambers separated from females.

Chemicals. For RBLR, the (*Z*)-11-tetradecenyl acetate (*Z*11–14:OAc) isomer (Farchan) was collected by GLC [2m × 2mm glass column packed with 10% XF-1150 (50% cyanoethyl methyl silicone) on 100–120 mesh chromosorb W-AW-DMCS], to ensure purity and was found to be > 99.9% pure. The *E*11–14:OAc isomer (Farchan) was added to the *Z* isomer to make a 92:8 *Z/E* mix,

as shown by capillary GLC (45-m Carbowax 20 M column). The ratio of components in the seven-component blend (Bjostad et al., 1984a) was as follows: 12:OAc (7.5), Z9-12:OAc (1.2), E9-12:OAc (2.5), 11-12:OAc (3.6), 14:OAc (4.6), Z11-14:OAc (100), E11-14:OAc (8.1). The ratio was checked by capillary GLC with all components $\pm 1\%$ of the desired blend.

For OFM the Z8-12:OAc isomer (Farchan) was purified by HPLC (Baker et al., 1981) and shown by capillary GLC to be $> 99.9\%$ pure. The mixture of Z8- and E8-12:OAc (6% E) was prepared and checked by capillary GLC, as was the three-component blend (6% E in Z8-12:OAc with 10% Z8-12:OH added, Cardé et al., 1979; Linn and Roelofs, 1983).

For CL the Z7-12:OAc isomer (Farchan) was shown by capillary GLC to be $> 98\%$ pure with no detectable E7-12:OAc or Z7-12:OH present (Linn et al., 1984). The synthetic chemicals for the six-component mix were the same as in Linn et al. (1984). The proportions of each compound were as follows: 12:OAc (5.6), Z5-12:OAc (7.7), Z7-12:OAc (100), 11-12:OAc (1.9), Z7-14:OAc (0.8), and Z9-14:OAc (0.6). A solution of this blend was prepared in Skelly B and checked on capillary GLC.

Chemical Sources. For RBLR and OFM, the single components Z11-14:OAc and Z8-12:OAc, the respective Z/E mixes, and the seven- and three-component blends were prepared in Skelly B (predominantly *n*-hexanes) and applied in 100-μl amounts to rubber septa (red, 5 × 9 mm, A.H. Thomas Co., Philadelphia, Pennsylvania) to achieve dosages of 3, 10, 30, 100, and 300 μg/septum for RBLR, and 0.001, 0.01, 0.1, 1, and 10 μg/septum for OFM.

For the CL, Z7-12:OAc and the six-component solution in Skelly B were applied to polyethylene caps (OS-6 closures, American Scientific Products, McGaw Park, Illinois) from a 1, 10, or 100 μg/μl solution to achieve dosages of 0.1, 0.3, 1, 3, and 10 mg/cap.

Test Procedures. Moth behavior was observed in the flight tunnel of Miller and Roelofs (1978). Each species was tested independently, in the order CL, OFM, RBLR. For RBLR and OFM, males were placed in the room housing the tunnel 1 h prior to testing (2 h prior to the initiation of scotophase), to acclimate to photophase temperature and light intensity: 21–22°C, 350 lux. Male CL were placed in the tunnel room at the initiation of scotophase, 4 h prior to testing (the fifth and sixth hours of the 8-h scotophase). Flight tunnel temperature and light intensity were 25°C, 0.3 lux. Relative humidity was 50–70% and wind speed was 50 cm/sec for all three species.

The procedures and apparatus for handling and testing males were as previously described (Linn and Roelofs, 1983; Linn et al., 1984). Males were scored for three key behaviors in the flight sequence: taking flight (TF), initiation of upwind flight (UP), and source contact (SC). During each 2-h test period, three treatments were tested, with ten males tested with each treatment. Treatments were always tested in the order of increasing blend complexity. Analysis was made of the number of males exhibiting each behavior in the

sequence, based on the total tested to each treatment ($N = 70$ for CL, 50 for OFM, and 50 for RBLR). Statistical comparison of treatments was based on χ^2 2×2 test of independence with Yates' correction (Sokal and Rohlf, 1969), or the method of adjusted significance levels for proportions (Ryan, 1960, $P < 0.05$).

RESULTS

For all three species, at all dosages, males took flight (TF, Figure 1) in significantly higher percentages to the complete blend of components when compared to single components or the *Z/E* mix (with the exception of the highest dosage tested for RBLR and CL, Figure 1). However, the effect of the blend on male behavior was even more dramatic at the initiation of upwind flight (UP, Figure 1) phase of the response, with significantly higher percentages of males responding to the complete blends at all dosages tested. Male sensitivity, evidenced by the lowest dosage at which complete flights to the source occurred (SC, Figure 1), was also greater, by at least an order of magnitude, to the complete blends. Thus, addition of minor components at all dosages, and particularly at the lower dosages tested, significantly enhanced male sensitivity and recognition of the pheromone signal.

The enhancement of male response brought about by the addition of minor components, while in evidence for each species, varied considerably among species. For RBLR, males exhibited very little behavior with the Z11–14 : OAc isomer alone. The lower levels of activity to 3, 10, 30, and 100 μg were not significantly different from spontaneous levels of activation, and so it is questionable whether or not the observed activations were pheromone induced. Response levels to the *Z/E* mix were significantly enhanced over that with Z11–14 : OAc alone, with some males completing the flight at two dosages (30 and 100 μg) at which males did not initiate upwind flight to Z11–14 : OAc alone. With the full seven-component blend, male response was not only increased significantly with the 30-, 100-, and 300-μg dosages, but males also initiated upwind flight and reached the source with the 3- and 10-μg dosages.

With OFM, the taking flight response to Z8–12 : OAc alone was greater at the higher dosages when compared with RBLR, but addition of the *E* isomer did not significantly enhance this initial behavior. A low percentage of males initiated upwind flight to the *Z/E* mix, but the three-component blend was essential for complete flights at all dosages tested. Similar to the RBLR results, the full blend at the 0.001- and 0.01-μg dosages resulted in complete flights to the source, whereas these dosages did not elicit any upwind flight behavior with the *Z/E* mix.

For the CL, males took flight in higher percentages to all dosages tested, compared to OFM and RBLR. For male CL, blend enhancement of activity

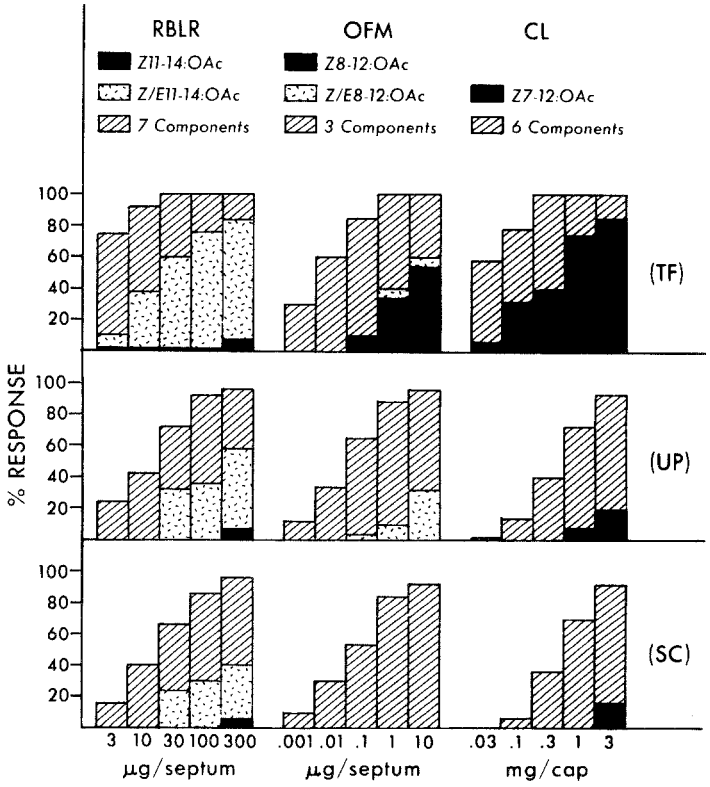

FIG. 1. Percentage of male RBLR, OFM, and CL exhibiting three key behaviors in the sustained flight tunnel (taking flight, TF; upwind flight over a 1.5-m distance, UP; and source contact, SC). Responses for each species are to the major component alone (solid area), the *Z/E* mixture (stippled area), and the complete blends (hatched area). Dosages represent the amount of the major component applied to the source, with the other components added to achieve the correct proportions (see Methods and Materials). $N = 70$ for CL, 50 for RBLR, and 50 for OFM to each blend–dosage combination.

was most evident in the upwind flight phase of the sequence. The lowest dosage of the main component to elicit upwind flight activity was 1 mg, whereas the lowest full blend dosage for this activity was 0.03 mg.

DISCUSSION

The results of the present study support the hypothesis that male moths are more sensitive to the female-released blend of components over a dynamic range

of release rates compared to the major component alone. In each case, the addition of the full complement of minor components not only enhanced male response over that observed to the single major component, but also resulted in completed flights to dosages of the major component that, when presented alone, did not elicit upwind flight activity. We conclude from this that the observations made with OFM by Baker and Cardé (1979) concerning the role of the blend do not constitute a specialized case, but rather that blend enhancement of male response represents an important general principle.

Pheromone Components and Behavioral Functions. The success of the present study and the previous reinvestigations was critically dependent on the fact that observations were made using the female-released blend of components (Baker et al., 1980; Bjostad et al., 1984b), rather than a partial blend (for CL, Linn and Gaston 1981a,b), or exaggerated ratios of components derived from field-screening trials (for RBLR, Baker et al., 1976; for OFM, Cardé et al., 1975a,b). It is clear from earlier results obtained with RBLR, OFM, and CL that certain minor components, when presented in exaggerated ratios, or when added singly to the major component, can significantly enhance trap catch and also give the appearance of controlling the expression of a specific aspect of the flight response. With RBLR, for example, field trials showed that addition of 12:OAc to the previously identified mixture of *Z*- and *E*11–14:OAc (8% *E*) (Roelofs and Arn, 1968; Klun et al., 1973) in a ratio of 1–1.5 times the level of *Z* isomer significantly increased trap catch (Roelofs and Comeau, 1968). Chemical analysis confirmed that 12:OAc was present in female glands, but at a ratio of only 3–5% of the *Z* isomer (Roelofs et al., 1975). At this ratio, however, 12:OAc did not enhance male activity in the field, and it was concluded on the basis of field trapping studies and crude airborne collections that females must be rapidly synthesizing and releasing larger amounts of 12:OAc.

Further observations of male RBLR flight behavior in the lab and the field (using only three of the recently identified seven components), showed that the *Z/E* isomer mix was responsible for upwind anemotactic flight and that 12:OAc significantly enhanced close-range approach, landing, and attempted copulations (Baker et al., 1976). Our most recent evidence indicates, however, that this is incorrect and that when 12:OAc is presented at the appropriate female-released ratio (3–5% of the *Z*11–14:OAc isomer), it does not enhance the response to the *Z/E* mix (Bjostad et al., 1984b). Rather, 12:OAc is one of seven pheromone components (12:OAc, *Z*9–12:OAc, *E*9–12:OAc, 11–12:OAc, 14:OAc, *Z*11–14:OAc, and *E*11–14:OAc) that act as a unit to enhance male sensitivity and flight response.

Similarly with OFM, 12:OH significantly enhanced trap catch when in a 3:1 ratio with the major component *Z*8–12:OAc (6% *E*) (Roelofs et al., 1969, 1973). As with RBLR, field and laboratory studies suggested that the *Z/E* mix was responsible for upwind anemotactic flight and that the relatively high ratio of 12:OH enhanced close-range behavior, including the dramatic hairpencil

courtship display (Cardé et al., 1975a,b). At this time the important blend constituent, Z8–12:OH, was not included in the tests. Subsequent studies showed, however, that Z8–12:OH is an important pheromone component, and that 12:OH, when presented at the appropriate ratio (<10% of the Z isomer and an equal or lower amount compared to Z8–12:OH), exhibited no discernable effect on male behavior. Further studies were then undertaken to show how variations in the ratio of components might affect male behavior (Linn and Roelofs, 1983). The results showed that off ratios did not enhance male response over that observed to the natural blend of components and that, over a wide range of *Z/E* mixtures, males were not able to compensate for the lack of the Z8–12:OH component and exhibit a complete flight to the source.

The CL provides a third, and somewhat different, example from that for RBLR and OFM. Characterization of the functional role of the minor component in this species was not the result of observations using an exaggerated ratio of components, rather they were the result of using a partially identified blend (Bjostad et al., 1980). The major component, Z7–12:OAc, was identified by Berger (1966). Subsequently Bjostad et al., (1980) identified 12:OAc from female gland and airborne collections, and it was shown that females released a 97:3 ratio of Z7–12:OAc to 12:OAc. The initial hypothesis was that 12:OAc was a short-range component, a hypothesis proposed directly from the results reported with RBLR (Baker et al., 1976), and OFM (Cardé et al., 1975a,b). The results of flight tunnel tests confirmed that 12:OAc did not appear to affect upwind flight, rather it enhanced close-range approach and contact with the source, when combined with Z7–12:OAc. It was concluded that the two-component system fit the existing paradigm concerning pheromone components and their functions (Linn and Gaston, 1981a,b).

As with RBLR and OFM, however, our most recent studies indicate that the previously observed effect with 12:OAc represents a response that can be exhibited by a low percentage of the male population to a partial pheromone blend (Linn et al., 1984). The male response to the six-component blend (Z7–12:OAc, 12:OAc, Z5–12:OAc, 11–12:OAc, Z7–14:OAc, and Z9–14:OAc) was in all respects superior to that observed with the two-component mix. These studies also involved subtraction tests with five-, four-, three-, and two-component blends containing Z7–12:OAc. The major component was necessary for any behavior to occur, but it was the only compound that fit this criterion. The subtraction tests showed that all five-component and several four-component blends elicited peak levels of response similar to those with the six-component mix. This suggested that the individual minor components were not acting to initiate specific behaviors, as several substitutions were possible. Rather, the minor components acted as an ensemble to enhance male response to the Z7–12:OAc component and thus effect optimal flight behavior.

Our present view is that individual minor components do not function to trigger specific behaviors in the response sequence, but rather that the full blend

acts as a unit in the male flight response and courtship sequence. It is evident from the above examples that these types of observations may be indicative of a partial, incorrectly characterized, or inappropriate blend of the pheromone and should not be proposed as evidence for the functional importance of individual compounds (Bradshaw et al., 1983), without comparative data for male response to female or gland effluvium/extracts (Linn et al., 1984).

Blend Perception and Active Space of the Pheromone. Data presented in this paper support the hypothesis that blends of components, rather than one predominating component, are acting at long distances from the source and are responsible for initiating and maintaining anemotactic flight (Baker and Roelofs, 1981). This conclusion is based on one of the major results of the present study that the blend of components markedly enhanced male sensitivity (or lowered the threshold for response) to the pheromone. We would conclude, with Baker and Roelofs (1981), that the active space of the pheromone is determined by the appropriate blend of components, not just the major component(s) (Nakamura and Kawasaki, 1977; Nakamura, 1979), and that the dimensions of the active space will be determined by the upper and lower concentration thresholds for the blend (Roelofs, 1978, Elkinton and Cardé, 1984).

The importance of sex pheromones as specific mate recognition signals is one of the formative concepts in research on chemical communication (Cardé and Baker, 1984; Roelofs and Brown, 1982). With the widespread awareness that moth species utilize multicomponent blends (Roelofs, 1980), it has become clear that signal specificity is a function of the composition and ratio of components, and the release rate of the blend (Roelofs and Cardé, 1977; Roelofs, 1978; Baker et al., 1981; Linn and Roelofs, 1983). The importance of a unique blend and ratio of components for the male is that it reduces the time spent in locating a mate. If it is the case, as is often stated, that females are a limiting resource for males, then rapid detection of the chemical signal is of critical importance for the male. A single component or isomer mix could certainly serve this purpose, if it were not for the fact that closely related, cohabitating, species often utilize the same compounds (Roelofs and Brown, 1982; Cardé and Baker, 1984). With a unique blend and ratio of compounds, however, specificity is added to the signal, enhancing the ability of the males to recognize the pheromone of conspecific females, and thus locate a female before conspecific males do, and also to aid the male in discriminating among closely related signals. Rapid detection and recognition of the signal aid the male in locating a female, and also in not wasting time and energy in false trail following (Cardé and Baker, 1984). We would argue that this strongly supports the concept that the blend is the active element in controlling male behavior at all distances from the female.

We recognize that our conclusions concerning the active space of the pheromone are based in large part on flight tunnel observations, but we would propose that they can be tested in the field. Baker and Roelofs (1981) determined

the upper and lower thresholds for the active space of OFM males to the three component blend over a dosage series. We propose to test males in the field, using the procedure developed by Baker and Roelofs, to the single Z8–12 : OAc isomer, the *Z/E* mix, and the three-component blend, over the same dosage series. Our hypothesis would be that the active space for the blend, at any dosage, would be significantly greater than to the single component or partial blend.

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REFERENCES

BAKER, T.C., and CARDÉ, R.T. 1979. Analysis of pheromone-mediated behaviors in male *Grapholitha molesta*, the Oriental fruit moth (Lepidoptera: Tortricidae). *Environ. Entomol.* 8:956–968.

BAKER, T.C., and ROELOFS, W.L. 1981. Initiation and termination of Oriental fruit moth male response to pheromone concentrations in the field. *Environ. Entomol.* 10:211–218.

BAKER, T., CARDÉ, R.T., and ROELOFS, W. 1976. Behavioral responses of male *Argyrotaenia velutinana* to components of its sex pheromone. *J. Chem. Ecol.* 2:333–352.

BAKER, T.C., CARDÉ, R.T., and MILLER, J.R. 1980. Oriental fruit moth pheromone component emission rates measured after collection by glass-surface adsorption. *J. Chem. Ecol.* 6:749–758.

BAKER, T.C., MEYER, W., and ROELOFS, W.L. 1981. Sex pheromone dosage and blend specificity of response by male Oriental fruit moths. *Entomol. Exp. Appl.* 30:269–279.

BERGER, R.S. 1966. Isolation, identification, and synthesis of the sex attractant of the cabbage looper, *Trichoplusia ni. Ann. Entomol. Soc. Am.* 59:767–771.

BJOSTAD, L.B., and ROELOFS, W.L. 1983. Sex pheromone biosynthesis in *Trichoplusia ni:* Key steps involve delta-11 desaturation and chain shortening. *Science* 220:1387–1389.

BJOSTAD, L.B., GASTON, L.K., NOBLE, L.L., MOYER, J.H., and SHOREY, H.H. 1980. Dodecyl acetate, a second pheromone component of the cabbage looper, *Trichoplusia ni. J. Chem. Ecol.* 6:727–734.

BJOSTAD, L.B., LINN, C.E., DU, J.W., and ROELOFS, W.L. 1984a. Identification of new sex pheromone components in *Trichopulsia ni* predicted from biosynthetic precursors. *J. Chem. Ecol.* 10:1309–1323.

BJOSTAD, L., LINN, C., and ROELOFS, W. 1984b. Identification of new sex pheromone components in *Trichoplusia ni* and *Argyrotania velutinana* predicted from biosynthetic precursors, pp. 223–238, *in* T.E. Acree and D.M. Soderlund (eds.). Semiochemistry: Flavors and Pheromones. Walter de Gruyter, Berlin.

BRADSHAW, J.W.A., BAKER, R., and LISK, J.C. 1983. Separate orientation and releaser components in a sex pheromone. *Nature* 304:265–267.

CARDÉ, R.T., and BAKER, T.C. 1984. Sexual communication with pheromones, pp. 355–383, *in* W.J. Bell and R.T. Cardé (eds.). Chemical Ecology of Insects. Chapman and Hall, London.

CARDÉ, R.T., BAKER, T., and ROELOFS, W. 1975a. Ethological function of components of a sex attractant system for Oriental fruit moth males, *Grapholitha molesta. J. Chem. Ecol.* 1:475–491.

CARDÉ, R.T., BAKER, T., and ROELOFS, W. 1975b. Behavioral role of individual components of a multichemical attractant system in the Oriental fruit moth. *Nature* 253:348–349.

CARDÉ, A.M., BAKER, T.C., and CARDÉ, R.T. 1979. Identification of a four-component sex pheromone of the female Oriental fruit moth, *Grapholitha molesta* (Lepidoptera: Tortricidae). *J. Chem. Ecol.* 5:423–427.

ELKINTON, J.S., and CARDÉ, R.T. 1984. Odor dispersion, pp. 73–92, *in* W.J. Bell and R.T. Cardé (eds.). Chemical Ecology in Insects. Chapman and Hall, London.

KLUN, J., CHAPMAN, O., MATTES, R., WOJTKOWSKI, P., BEROZA, M., and SONNET, P. 1973. Insect sex pheromones: Minor amount of opposite geometrical isomer critical to attraction. *Science* 181:661–663.

LINN, C.E., and GASTON, L.K. 1981a. Behavioral responses of male *Trichoplusia ni* in a sustained-flight tunnel to the two sex pheromone components. *Environ. Entomol.* 10:379–385.

LINN, C.E., and GASTON, L.K. 1981b. Behavioral function of the components and the blend of the sex pheromone of the cabbage looper, *Trichoplusia ni*. *Environ. Entomol.* 10:751–755.

LINN, C.E., JR., and ROELOFS, W.L. 1983. Effect of varying proportions of the alcohol component on sex pheromone blend discrimination in male Oriental fruit moths. *Physiol Entomol.* 8:291–306.

LINN, C.E., JR., BJOSTAD, L.B., DU, J.W., and ROELOFS, W.L. 1984. Redundancy in a chemical signal: Behavioral responses of male *Trichoplusia ni* to a 6-component sex pheromone blend. *J. Chem. Ecol.* 10:1635–1658.

MILLER, J.R., and ROELOFS, W.L. 1978. Sustained flight tunnel for measuring insect responses to windborne sex pheromones. *J. Chem. Ecol.* 4:142–149.

NAKAMURA, K. 1979. Effect of the minor component of the sex pheromone on the male orientation to pheromone source in *Spodoptera litura* (F.). *Chem. Rev. Insect* (Russia) 4:153–156.

NAKAMURA, K., and KAWASAKI, F. 1977. The active space of *Spodoptera litura* (F.) sex pheromone and the pheromone component determining this space. *Appl. Entomol. Zool.* 12:162–177.

ROELOFS, W.L. 1978. Threshold hypothesis for pheromone perception. *J. Chem. Ecol.* 4:685–699.

ROELOFS, W.L. 1980. Pheromones and their chemistry, pp. 583–602, *in* M. Locke and D.S. Smith (eds.). Insect Biology in the Future. Academic Press, New York.

ROELOFS, W., and ARN, H. 1968. Sex attractant of the redbanded leafroller moth. *Nature* 219:513.

ROELOFS, W.L, and BJOSTAD, L.B. 1984. Biosynthesis of lepidopteran pheromones. *Bioorg. Chem.* 12:279–298.

ROELOFS, W.L., and BROWN, R.L. 1982. Pheromones and evolutionary relationships of Tortricidae. *Annu. Rev. Ecol. Syst.* 13:395–422.

ROELOFS, W.L., and CARDÉ, R.T. 1977. Responses of Lepidoptera to synthetic sex pheromone chemicals and their analogues. *Annu. Rev. Entomol.* 22:377–405.

ROELOFS, W.L., and COMEAU, A. 1968. Sex pheromone perception. *Nature* 220:600–601.

ROELOFS, W., COMEAU, A., and SELLE, R. 1969. Sex pheromone of the Oriental fruit moth. *Nature* 224:723.

ROELOFS, W., CARDÉ, R.T., and TETTE, J. 1973. Oriental fruit moth attractant synergists. *Environ. Entomol.* 2:252–254.

ROELOFS, W., HILL, A., and CARDÉ, R.T. 1975. Sex pheromone components of the redbanded leafroller, *Argyrotaenia velutinana*. *J. Chem. Ecol.* 1:83–89.

RYAN, J.A. 1960. Significance tests for multiple comparison of proportions, variances, and other statistics. *Psychol. Bull.* 57:318–328.

SHOREY, H.H. and HALE, R.H. 1965. Mass-rearing of the larvae of nine noctuid species on a simple artificial medium. *J. Econ. Entomol.* 58:591.

SOKAL, R.R., and ROHLF, F.T. 1969. Biometry. W.H. Freeman, San Francisco.