A comparison of the development, reproductive biology, and pheromone-based communication of two Indonesian soybean pod borers (Lepidoptera: Pyralidae)

by

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B.Sc., University of Guelph, 1990

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A COMPARISON OF THE DEVELOPMENT, REPRODUCTIVE BIOLOGY, AND

PHEROMONE-BASED COMMUNICATION OF TWO INDONESIAN SOYBEAN POD BORERS

(LEPIDOPTERA: PYRALIDAE)

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Abstract

Experiments conducted in West Java, Indonesia investigated the developmental biology and reproductive behavior of two sympatric soybean pod borers, *Etiella zinckenella* Trietschke and *E. hobsoni* Butler (Lepidoptera: Pyralidae). It was determined that 1) significant interspecies differences occurred between the egg, larval, pupal, and total egg-to-adult developmental periods of laboratory raised *E. zinckenella* and *E. hobsoni*; 2) the pre-pupal and total egg-to-adult developmental periods of female *E. zinckenella* were significantly shorter than for males; 3) the longevity of virgin-female *E. zinckenella* was significantly longer than that of virgin-male *E. zinckenella* or virgin-male and female *E. hobsoni*; 4) interspecies differences occurred in the female: male sex-ratios of laboratory raised adults; 5) peak mating for both species occurred on the second night after eclosion; 6) interspecies differences occurred in the temporal distribution of calling and mating behaviors; 7) repeated mating was observed for both species at a very low frequency; 8) interspecific mating did not occur; 9) female *E. zinckenella* were significantly more fecund (160.8 ± 49.0 eggs) than *E. hobsoni* (32.5 ± 16.9 eggs); 10) the duration of copulation of *E. zinckenella* (123.6 ± 12.5 min) was significantly longer than that of *E. hobsoni* (73.7 ± 16.1 min); 11) virgin-female baited traps caught only conspecific males, and reduced numbers of males were captured in traps simultaneously baited with virgin-females of each species; 12) wing-traps baited with two conspecific virgin-females caught (\( \bar{x} \pm SE \)) 2.65 ± 0.41 male *E. zinckenella* and 1.61 ± 0.40 male *E. hobsoni* per night; 13) the greatest number of males of both species were caught in traps placed at, or above the canopy level; and 14) no males were attracted to traps baited with synthetic candidate pheromone blends previously demonstrated to be attractive to *E. zinckenella* in Egypt and Hungary. This report demonstrates distinct biological differences and reproductive isolation between the two *Etiella* spp. and suggests that *E. zinckenella* in Indonesia is genetically different from *E. zinckenella* in Europe and North Africa.
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Introduction

Soybeans in Indonesia

Soybeans, *Glycine max* Merril, contribute an important source of protein and vegetable oil to the Indonesian diet and provide a wide array of popular food products such as *tempeh*, *tofu*, *edamame*, sprouts, soya sauce, and cooking oil. Indonesia is currently unable to meet its domestic demand for soybeans and imports a large proportion of its consumption (Pandey et al. 1987). Production of food crops such as soybean is a government priority and programs are in place to expand and intensify soybean production (Wardojo 1991). Insect pests are a significant limitation to soybean yields (Kalshoven 1981).

Soybeans are grown primarily in the lowland areas as a *Palawija* crop, i.e. a secondary crop grown during the dry season when water is too limited for *Sawah*, or paddy rice production. Rarely, a farmer with abundant irrigation may cultivate three rice crops per year. Typically, one or two rice crops are grown, with *Palawija* crops, fresh market produce, or dry fallow periods filling in the remainder of the cropping cycle. Some soybean is also grown in the highland areas during the rainy season, but this amounts to only a small percentage of total production. Seasonal peaks in soybean plantings occur during the months of April, July, and November (Biro Pusat Statistik 1991); comparatively few soybeans are planted in November because the rainy season typically lasts from December to April. Because of their position in the cropping cycle, most soybeans grown in the lowland areas are short season (ca. 90 day) varieties to facilitate maximum paddy rice production. Over 80% of Indonesian soybean production occurs on the island of Java, although significant production areas also occur on the islands of Sumatra, Sulawesi, Bali, Lombok and the Nusa Tenggara Barat (Sumarno et al. 1989). Eastern Java is the largest single production area, accounting for > 50% of total production in 1980.

Soybeans yields are limited by numerous noxious insects, diseases and weed species (Soetarjo et al. 1987; JICA 1990). During the seedling stage, attacks by the beanfly, *Opiomyia phaseoli* Tryon, and the shoot borers, *Melanagromyza sojae* Zehntner and *Melanagromyza*
dolichostigma De Meijere, frequently require insecticidal seed treatments to prevent major losses in germination and subsequent yields. Sporadic attacks by the soybean leaf beetle, Phaedonia inclusa Stal, and white fly Bemisia tabaci Gennadius, also occur during this stage. During the vegetative stage soybeans are attacked by a wide range of defoliators: the armyworm, Spodoptera litura F., leaf-folder, Omiodes indicata F., tortricid, Adoxophyes privatana Walker, green semi-looper, Chrysodeixis chalcites Esper, corn earworm, Helicoverpa armigera Huebner, and soybean leaf beetle Phaedonia inclusa Stal. Insecticide applications to control these pests are more common than necessary since they are often kept in check by natural enemies in unsprayed fields (Van den Berg 1992). As pods develop and seeds mature the main pests are the pod borers Etiella zinckenella Treitschke and Etiella hobsoni Butler, and the pod suckers Nezara viridula L., Riptortus linearis F., and Piezodorus rubrofasciatus F. In some areas pod feeding by late instar corn earworm also occurs during this stage. Damage to the harvestable pods and seeds often causes major losses.

*Etiella* spp. in Indonesia

**General biology**

The genus *Etiella* Zeller comprises seven species (Whalley 1973), probably Australasian in origin. Only *E. zinckenella* is worldwide in distribution; the other six species are restricted to various parts of Australasia and the Orient. Human activity is presumed to have contributed to the rapid and widespread dissemination of *E. zinckenella*, since little morphological variation is observed between geographically dispersed populations (Whalley 1973). *E. zinckenella* and *E. hobsoni* are the only *Etiella* spp. reported as pests of soybean in Indonesia (Naito and Hamotto, 1987).

Specific differences were reported between the egg, larval, and pupal stages of *E. zinckenella* and *E. hobsoni* (Naito and Hamoto, 1987), yet from personal observation these differences are unreliable for discrimination between the two species. The adult stages, however, have notable differences in size, color, wing patterns, and morphology of the genitalia (Whalley 1973, Naito and Hamoto 1987). Because the larval stages are indistinguishable, most Indonesian
reports simply categorize damage due to *Etiella* spp., or erroneously assume it is due to *E. zinckenella* alone.

*E. zinckenella* can feed on 30 species in 21 genera of legumes, many of which are cultivated (Naito 1961), and may also feed on some non-legumes (Whalley 1973). Geographic biotypes of *E. zinckenella* apparently exist; it is a common pest of soybean but not the red kidney bean *Phaseolus vulgaris* L. in Asia while the reverse occurs in North America (Talekar 1987). Information on the biology of *E. hobsoni* is very limited (Whalley 1973), and little is known in Indonesia about the host-range of either *Etiella* spp. in nature (Naito and Harnoto 1987).

Laboratory feeding studies indicate that both Indonesian species were able to develop normally on sunhemp, *Crotalaria juncea* L., hyacinth bean, *Dolichos lablab* L., mungbean, *Phaseolus aureus* L., red kidney bean, *Phaseolus vulgaris* L., common pea, *Pisum sativum* L., bush bean, *Vigna hybrida* L., cowpea, *Vigna unguiculata* L., and string bean, *Vigna sesquipedalis* Frew. The highest percentage reached maturity when fed on soybean (Naito and Harnoto 1984). However, in nature *E. zinckenella* larvae were found only on *Crotalaria juncea* L. and both species were found on *Tephrosia purpurea* Pers. Oviposition preferences may play a key role in determining the host range of *Etiella* spp. (Hattori 1988), a hypothesis which is supported by preferential oviposition by both *Etiella* spp. on field grown soybeans over red kidney beans (Naito and Harnoto 1987).

Population trends of *Etiella* spp. correspond closely with the seasonality of soybean production in lowland Java. Seasonal extinctions of *Etiella* spp. populations occur during the rainy season from December to April when soybeans are seldom grown. On continuously grown soybean fields, populations were also extremely low during this period (Naito and Harnoto 1987). During the dry season when most soybean plantings occur, their numbers increased rapidly until August, remained high until December, and then declined dramatically with the onset of heavy rains. On the alternate host, *C. juncea*, *E. zinckenella* populations were reportedly substantially higher during the rainy season than during the dry season (Mangundojo 1959).
The durations (days, $\bar{x} \pm SE$) of egg development were nearly identical for *E. zinckenella* (4.16 ± 0.01, n=173, range: 4-5) and *E. hobsoni* (4.24 ± 0.02, n=100, range: 4-5) (Naito and Hamoto, 1984). The duration of the larval stage (days) of *E. hobsoni* (16.2 ± 2.1, n=66, range: 13-19) was slightly longer than for *E. zinckenella* (14.8 ± 1.5, n=67, range: 12-19), while pupal stages were identical in duration (11.2 ± 1.1, n=37, range: 8-13 for *E. zinckenella* and 11.2 ± 1.1, n=66, range: 8-13 for *E. hobsoni*). The duration of the larval stage of female *E. zinckenella* was shorter than for males, whereas the opposite relationship was found for *E. hobsoni*.

The entire life-cycle of *E. zinckenella* fed on pigeon pea, *Cajanus cajan* L., was completed in about 22-24 days at 25°C (Bindra and Singh 1969) and the durations of the egg, larval, and pupal stages were reported as 5, 7-9, and 10 days, respectively. At 22 and 13.5°C the durations of the egg stage were increased from 5 to 33 days. At 29°C the pupal stage was completed in 9 days, but it increased to 62 days at 15°C. The larval stage was prolonged when food was limited or poor in quality. The life-span of unmated adults fed on sugar solution was 8 to 20 days at 26°C, but mated moths lived a maximum of 11 days. The longevity of adults was extended by low temperatures, and reduced by high temperatures. In temperate climates the larvae hibernate in small cocoons and pupate the following spring, 3-6 months later. The longevity of *E. zinckenella* females was longer than that of males in two consecutive years of experimentation (Hattori and Sato 1983).

**Damage by *Etiella* Spp.**

*Etiella* spp. are considered to be major pests of soybeans in Indonesia. Larval *Etiella* spp. feed on soybean seeds, causing direct losses in yield and additional losses in the quality and sale price of damaged seeds. *E. zinckenella* is considered a more serious pest of soybean than *E. hobsoni* because it is the predominant species on Java where most Indonesian soybean is grown (Naito et al. 1986). The degree of damage caused by *E. hobsoni* infestations has been reported to be greater (Naito et al. 1986) or lesser (Djuwarso et al. 1992) than by *E. zinckenella*, perhaps reflecting fluctuations in the relative population levels. From January to June *E.
zinckenella comprised <10% of all larval Etiella spp. sampled, but its relative population increased to over 60% after July (Naito and Harnoto 1987).

In some areas populations of Etiella spp. seldom reach severe levels (Van den Berg 1992), yet severe infestations can cause losses of up to 80% (Talekar 1987). Yield losses of 26% have been reported (Supriyatin 1990), but overall losses to Etiella spp. have been estimated at 9% (Soejitno 1987). Damage tends to be greatest in late-planted fields, and outbreaks are more common following drought stress (Van den Berg 1992). Higher damage levels are reported in Central and Western Java than in Eastern Java (Naito et al. 1983). According to one farmer, damaged seeds are separated manually and are sold for about half the price of undamaged seed.

Control of Etiella spp. populations:

Control of Etiella spp. using contact insecticides is difficult since the larva feed within the pods under a closed canopy structure (Talekar 1987). Supriyatin(1990) reported 7% seed damage despite weekly applications of Cypermethrin from the flowering stage until harvest. Systemic agents might provide additional protection, but could also leave unacceptably high residues within the harvested seeds. The young larvae are vulnerable to contact chemicals, because they crawl around the pod surface for 15 to 20 min after hatching, prior to selecting an entry point, spinning a small web and boring into the pod for another 10 to 70 min (Bindra and Singh 1969). To ensure that larvae receive a lethal dose prior to entering the pods, insecticide applications must be precisely timed (Harnoto et al. 1984). However, prolonged oviposition by E. zinckenella females and overlapping generational cycles (Hattori and Sato 1983) may demand several insecticide applications for effective control (Soejitno 1987).

At least 67 species of parasites and hyperparasites have been reported to attack E. zinckenella world-wide (Bindra and Singh 1969), but no reports were found on the natural enemies of E. hobotsoni. Very few reports are available on the impact of biological controls on Indonesian Etiella spp. populations. Phaneratoma spp. and Agathus spp. are common larval parasites (Van der Berg 1992). Temelucha etiellae and Pristimerus naitoi (Kusigemati, 1985) have also been reported as larval parasites, and some parasitism of Etiella spp. eggs by
Trichogrammateoidea bactrai bactrai has been observed under field conditions (A. Naito, personal com., Bogor Research Institute for Food Crops, Bogor, West Java, Indonesia).

Reproductive Biology of Etiella spp.

No information is currently available on the calling and mating behavior of E. hobsoni, although several reports are available for E. zinckenella. Female E. zinckenella reportedly became sexually mature on the second day following eclosion (Hirai 1982) when they assumed a typical pyralid calling posture, with the wings held apart and the abdomen bent upwards at a ca. 90° exposing the pheromone gland (Hirai 1982). Some females (5%) mated on the second day after eclosion, peak mating (51%) occurred on the third day, and the remainder mated on the fourth (20%), fifth (22%), and seventh (2%) days. The duration of copulation was 71.4 ± 6.9 min (n=7) (Hirai 1982).

In a growth cabinet with a 16L:8D photoperiod, mating was also first observed during the second day after eclosion (15%), peak mating occurred on the 3rd day (41%), and the remainder of matings occurred on the 4th (22%), 5th (7%), 6th (7%), 7th (4%), and 8th (4%) days (Hattori and Sato 1983). Copulation was first observed during the 3rd hour of darkness (4%), peak mating occurred during the 4th hour (37%), and then mating declined during the 5th (33%), 6th (11%), 7th (7%) and 8th hours (7%). Copulation endured for 72 ± 4 min. Calling behavior and copulation occurred earlier on the third than the second day after eclosion (Hirai 1982). Under a natural photoperiod (Fukuyama, Japan, Sept.-Oct.) peak mating occurred during the 5th hour of darkness, but occurred during the 7th hour under a 12L:12D photoperiod in a growth chamber.

During courtship and mating E. zinckenella males approached a calling female from the rear with vigorous wing fluttering and touched the posterior region of her abdomen with his antennae or maxillary palpi (Hirai 1982). The male then walked around the female to take a head to head position. A receptive female would remain stationary and permit the male to place his head under hers, whence he extruded his brush organ (Hirai 1981) which contacted her maxillary palpi or antennae. The male and females then extended their abdominal tips towards each other.
The male seized the female abdominal terminalia with his claspers and the pair then assumed a tail to tail position. The male wings rested above the females in the final position.

*E. zinckenella* females raised on pigeon peas can lay 50-200 eggs (Hill 1975). On soybean the mean number of eggs laid per female has been variously reported as 117 (Tsutsui 1950), 70.8 and 112.9 (Hattoni and Sato 1983). One report indicates the number of eggs laid per female over five days on potted soybean plants as 137.3 for *E. zinckenella* and 102.0 for *E. hobsoni* (Naito and Harnoto 1987).

For *E. zinckenella* the mean daily oviposition was uniformly spread without a prominent peak (Hattori and Sato, 1983), yet a delay of mating by 6 days did not significantly reduce the number of eggs per female. The combined pre-mating and pre-oviposition period of *E. zinckenella* averaged 5.6 days. The preoviposition period among females that mated at different ages was not significantly different, but varied widely among individuals. This variation implies that oviposition in the field by female *E. zinckenella* occurs over a relatively long duration, which may explain the prevalence of overlapping life-stages in soybean fields (Hattori and Sato 1983).

**Pheromone identification**

Four components of the female sex pheromone of Hungarian and Egyptian *E. zinckenella* were identified using coupled gas chromatographic-electroantennographic detection (GC-EAD) analysis, followed by field trapping experiments (Toth et al. 1989). Flame ionization detector (FID) and male antennae responses (EAD) of *E. zinckenella* occurred at retention times identical to synthetic standards of (Z)-11-tetradecenyl acetate (Z-11-14:OAc), (E)-11-tetradecenyl acetate (E-11-14:OAc), (Z)-9-tetradecenyl acetate (Z-9-14:OAc), and saturated tetradecenyl acetate (C-14:OAc). A prominent FID peak corresponding to Z11-16:OAc did not elicit a response by male antennae. Similar EAD responses were observed for a range of C14 acetates and alcohols, yet the acetates, particularly Z-11-14:OAc, E-11-14:OAc, and Z-9-14:OAc, evoked the strongest responses.

Rubber septa baited with synthetic candidate pheromone components in ratios similar to those observed in glandular extracts attracted significant numbers of male moths to traps in both
Hungarian and in Egyptian field trials. Blends of Z-11-14:OAc, E-11-14:OAc, and Z-9-14:OAc, and C-14:OAc in a 10:1:1:5 ratio elicited the highest responses at doses of 1000 and 100 μg, respectively, but significant numbers of males were also trapped using a 10 μg dose. In component subtraction experiments, omission of the C-14:OAc component resulted in a higher number of males captured. When the E11-14:OAc component was omitted the number of males trapped was dramatically reduced, and no males were trapped by blends lacking the Z9-14:OAc component. When Z-11-16:OAc was added to the four component mixture in different amounts no significant increase in trap catches was observed. No previous reports concerning the pheromone-based communication of *E. nobsoni* were found.
Objectives

Because a synthetic pheromone blend had been successfully tested for *E. zinckenella* in Hungary and Egypt, my goal was to conduct experiments which could contribute towards the development of a pheromone-based pest management strategy for the Indonesian *Etiella* spp.. However, the literature available regarding the biology and control of *Etiella* spp. pod-borers in Indonesia is very limited, particularly for *E. hobsoni*. Therefore, the following experimental objectives were chosen to provide information on the development, reproductive biology, and pheromone-based communication of *E. zinckenella* and *E. hobsoni*:

1) compare the durations of the developmental stages by sex for both species,
2) compare the temporal distribution of calling and mating behaviors of both species,
3) compare the fecundity and duration of mating of both species,
4) field test pheromone blends previously tested in Hungary and Egypt on Indonesian *Etiella* spp. populations,
5) perform GC-EAD analysis of pheromone gland extracts of both species,
6) field test candidate pheromone components for Indonesian populations,
7) determine the effect of trap height on male trap catches,
8) test for cross attraction between the natural pheromone plumes of both species, and
9) test for heterospecific-interference between the natural pheromone plumes of both species.
Materials and Methods

Rearing

Larvae were collected in infested soybean pods from farmers' fields in various locations throughout West Java, Indonesia, from July through November 93 and reared for several generations under ambient laboratory conditions. The photo regime in the laboratory was roughly 12L:12D, the temperature ranged from 25-30° with an average of about 27°C, and the relative humidity ranged from 60-95%.

Infested pods were placed on paper in plastic containers (20 x 10 x 30 cm) fitted with fine mesh screen-cloth covers that allowed aeration and prevented condensation. In containers containing late instar larvae, sawdust was placed under the paper layer to provide pupation sites. Every few days the containers were inspected for pupae which were held in separate containers (10 x 10 x 20 cm) until the adults eclosed.

Newly eclosed adults were sorted by species and transferred into separate fine mesh mating cages (25x 25 x 25 cm) containing fresh soybean pods as an oviposition substrate. Every few days the moths were provided with a 20% honey-water solution on cotton wicks. Each day the egg-laden pods were transferred to a separate container.

Approximately one day prior to hatching, the egg-laden pods were transferred into tightly sealed plastic containers containing fresh mature pods. This latter step was necessary to prevent the dispersal of the larvae, which were very small and escaped through all but a completely sealed container. Small silica gel bags were placed in the sealed containers to prevent condensation. Each day pods containing new larval entry holes were transferred into aerated containers as above.

Development

To determine the duration of the egg-stage for each species the egg-laden pods from rearing cages were placed in small tightly sealed plastic containers (5 x 10 x 20 cm) with fresh pods, and small silica gel bags to prevent excess humidity. Each day the containers were inspected and the newly-hatched larvae were counted and removed until all of the eggs had
hatched. Each pod in the container was inspected at 40X magnification and the number of entry holes was counted.

Newly-hatched larvae were transferred singly onto large fresh pods placed inside 50 mL test tubes with cotton plugs. The pods were inspected the following day to ensure that the larvae had entered the pods, and only tubes containing larvae that entered within 24 h were kept. The tubes were then inspected each day until the mature larvae (recognized by their size and red color) had emerged from the pods. The duration of the larval period was determined as the number of days between egg hatching and emergence from the pods.

The prepupal stage was measured as the number of days between emergence from the pods until pupation, and the pupal stage as the number of days between pupation and the eclosion of the adult. The newly-eclosed adults were sexed by comparing their antennae and for each test tube the total number of days from oviposition until adult eclosion was summed to determine the development time for each sex and species. Sex ratios of each species were calculated from the above experiment, and from field samples.

To determine adult longevity, individual adults were placed in 250 mL Ehrlenmeyer flasks held under ambient laboratory conditions. The tops were sealed with fine mesh screen cloth penetrated by a cotton wick intermittently soaked in 10% sucrose. The flasks were inspected each morning and the numbers of live and dead moths recorded.

Reproductive biology

Larvae were reared under ambient laboratory conditions with a normal photoregime (ca. 12L:12D) until pupation, and were then transferred into a reverse-phase photoregime 12D:12L for the remainder of their development. To examine the calling behavior of unmated females, newly-eclosed laboratory-reared female moths of each species were transferred singly into fine mesh cages (25 x 25 x 25 cm). One side of these cages was made of transparent acetate to facilitate observation. A 20% honey water solution was supplied on cotton wicks which were replenished every few days. Several fresh soybean pods were also placed in each cage. The adult females
were inspected every 10-15 min under a red light during the dark-phase, and the initiation and termination of calling by each female was recorded.

To test the hypothesis that *E. zinckenella* and *E. hobsoni* are reproductively isolated, newly eclosed pairs of moths were maintained in observation cages as above in all four possible species and sex combinations: 1) female *E. zinckenella* with male *E. zinckenella* (n=17 normal phase, n=6 reverse phase); 2) female *E. zinckenella* with male *E. hobsoni* (n=12 normal phase, n=8 reverse phase); 3) female *E. hobsoni* with male *E. hobsoni* (n=8 normal phase, n=14 reverse phase); and 4) female *E. hobsoni* with male *E. zinckenella* (n=10 normal phase, n=10 reverse phase). The replicates in this experiment were subdivided by photophase to test whether the observations obtained using the normal and reverse-phase photoregimes were consistent. In addition to 20% honey water, each cage was also supplied with a petri-dish containing five large fresh soybean pods as oviposition substrates. The cages were inspected every 10-15 min under a red light during the dark phase and the initiation and termination of calling and mating was recorded. Each day the soybean pods were replaced with fresh pods, and the numbers of red (fertile) and white (infertile) eggs were counted on the third day following oviposition to determine the fecundity of each mated female.

Frequency of mating was assessed with newly-eclosed pairs of moths maintained as above in observation cages with a reverse-phase photoregime and a daily supply of fresh soybean pods. For each species the cages were divided into three treatments: 1) male removed following one copulation (*E. zinckenella*: n=11, *E. hobsoni*: n=10); 2) female replaced by a newly-eclosed female following each copulation (*E. zinckenella*: n=7, *E. hobsoni*: n=12); 3) male was replaced by a newly-eclosed male following each copulation (*E. zinckenella*: n=12, n=12 *E. hobsoni*: n=12). The moths were observed every 10-15 min. throughout the dark phase and the initiation and termination of calling and mating was recorded. The numbers of red (fertile) and white (infertile) eggs were counted as above. In each of the above mating experiments, mated females were placed in 70% ethanol following death, and were subsequently dissected to count the number of spermatophore present in the bursa copulatrix.
Pheromone-based communication

For pheromone analyses, newly-eclosed females from field collected larvae were held under ambient conditions and a natural photo regime in 300 mL transparent plastic cups (five females per cup) covered with fine mesh screen. A 20% honey water solution on cotton wicks was replenished every few days. When three or more females per cup were calling, the females' abdomens were compressed and pheromone glands forcibly extruded. 10-15 glands were removed with microscissors and extracted in 30 μL of HPLC grade hexane for approximately 10 min at room temperature. The extract was then transferred using a micro-syringe into a small goose-necked glass vial which was stored on dry ice until flame sealed. Aliquots containing one female equivalent of pheromone extract were subsequently subjected to gas chromatographic-electroantennographic analysis (GC-EAD) (Arm et al. 1975) on fused silica columns coated with J&W Scientific (Folsom, CA) DB-5, DB-210 (30 m x 0.25 or 0.32 mm ID) or DB-23 (30 m x 0.32 mm ID), with helium as the carrier gas.

The Toth et al. (1988) pheromone blend was tested in a soybean field in Garut, West Java, Indonesia during August 1993. Wing traps, rubber septa, and test compounds were provided by Phero Tech Inc., Delta, B.C., Canada. Four treatments in traps 15 m apart were arranged in a 4 x 4 Latin square: 1) a pheromone blend consisting of Z11-14:OAc (1000 μg): E11-14:OAc (100 μg): Z9-14:OAc (100 μg): C-14:OAc (500 μg); 2) three virgin female E. zinckenella (three days old); 3) three virgin female E. hobsoni (three days old); and 4) an empty control. Virgin females were placed in groups of three inside modified plastic 35 mm film cases with the open top and bottoms covered with a fine mesh screen secured with an elastic band, and nutrient provided by a small cotton wick soaked in 20% honey water solution. All baits were suspended inside a wing trap. Each morning for eight days all captured males were counted and removed and the 20% honey water solution replenished. The females were replaced every three days (or

1 Electrophysiological and chemical analyses, as well as preparation of lures for field tests of candidate blends (Tables 10,11) were done by R. Gries and G. Gries, Center for Pest Management, Department of Biological Sciences, Simon Fraser University.
earlier if dead). Additional field tests were performed using a variety of synthetic candidate pheromone components at different locations in West Java. In Tables 10 and 11 the component blends used, experimental design, number of replicates, trap spacing, duration, and location for each experiment are specified.

Two experiments were conducted to determine the effect of trap height on captures of males for both species. Traps baited with two virgin females provided with 20% honey water were placed 10 m apart at three different heights above the ground level: 1) 30 cm (about half canopy height); 2) at canopy level; and 3) at 30 cm above the canopy. The experiments were set up as separate 3x3 Latin squares for each species. Each trap was inspected every two days, the number of captured males of each species counted and removed, and the 20% honey water solution replenished.

To test for the effect of heterospecific pheromone plumes on the captures of male _E. zinckenella_ and _E. hobsoni_, virgin females were suspended as above within wing traps in a 4x4 Latin square with the following treatments: 1) two _E. zinckenella_ females; 2) two _E. hobsoni_ females; 3) two _E. zinckenella_ females and two _E. hobsoni_ females (in separate cages); and 4) unbaited control traps.

**Statistical Analysis**

Means, standard errors, t-tests, ANOVA, and linear regressions were calculated using Minitab Version 8.2 (Minitab Inc., State College, PA, 16801). Moth trap catches were transformed by \( \log_{10}(x+1) \) to ensure equal variances (Zar 1984) prior to ANOVA. Following ANOVA means were separated using Tukey's multiple range test (Zar 1984). In all cases \( \alpha=0.05 \).
Results

Development

Significant differences were observed between the durations of the life stages of *E. zinckenella* and *E. hobsoni* (Table 1). The durations of the egg, larval, and the total egg-to-adult periods of *E. hobsoni* were significantly longer than those of *E. zinckenella*. However, the pupal stages of both sexes of *E. zinckenella* were significantly longer than those of *E. hobsoni*. No significant species differences were observed between the durations of the pre-pupal stages. The pre-pupal period of female *E. zinckenella* was shorter than for males and female *E. zinckenella* eclosed about one day earlier than males. No other significant differences between the sexes were observed for *E. zinckenella* or *E. hobsoni*.

Virgin female *E. zinckenella* lived longer than virgin female *E. hobsoni* or virgin male *E. zinckenella* (Table 2). No difference was found between male and female *E. hobsoni*, or between male *E. zinckenella* and *E. hobsoni*. The sex ratios of laboratory-raised adults were female-biased at 1.18:1 and 1.56:1 for *E. zinckenella* and *E. hobsoni*, respectively. However, a field sample of *E. zinckenella* adults in November 1993 revealed a ratio of 1:1.24.

Reproductive Biology

*E. zinckenella* and *E. hobsoni* females both initiated calling behavior by curving their abdomens up between their wings, evertg the ovipositor and exposing the pheromone gland. For both species calling behavior was observed only during the scoto-phase, and typically ended within 10-20 min after onset of the photophase. The ovipositor was often observed to move back and forth during calling periods. Mating in both species proceeded as described for *E. zinckenella* (Hirai 1982). A male approaches a calling female from the rear, probing her ovipositor and pheromone gland with his antennae. The male then became excited, first wing-fanning, circling, and twitching his antennae, and then approaching the female head-on, placing his head under her's, with his antenna along his back. Copulation was initiated when both the male and female extended their abdomens towards each other while still face to face. The male claspers then
Table 1. Comparison of development durations by life stage for *Etiella zinckenella* (E.z.) and *Etiella hobsoni* (E.h.).

<table>
<thead>
<tr>
<th>Sex</th>
<th>Life stage</th>
<th>Number sampled</th>
<th>Duration in days (x ± SE)</th>
<th>t-test <em>P</em> between species</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>E.z. E.h.</td>
<td>E.z. E.h.</td>
<td></td>
</tr>
<tr>
<td>Both</td>
<td>Eggs</td>
<td>1150 797</td>
<td>4.72 ± 0.02</td>
<td>5.68 ± 0.04</td>
</tr>
<tr>
<td>Females</td>
<td>Larva</td>
<td>41 21</td>
<td>10.59 ± 0.17</td>
<td>12.33 ± 0.28</td>
</tr>
<tr>
<td></td>
<td>Pre-pupa</td>
<td>41 21</td>
<td>4.29 ± 0.19</td>
<td>4.57 ± 0.21</td>
</tr>
<tr>
<td></td>
<td>Pupa</td>
<td>45 21</td>
<td>13.07 ± 0.16</td>
<td>12.38 ± 0.27</td>
</tr>
<tr>
<td></td>
<td>Total (incl. eggs)</td>
<td>45 21</td>
<td>33.44 ± 0.19</td>
<td>35.38 ± 0.36</td>
</tr>
<tr>
<td>Males</td>
<td>Larva</td>
<td>52 11</td>
<td>10.58 ± 0.18</td>
<td>12.46 ± 0.37</td>
</tr>
<tr>
<td></td>
<td>Pre-pupa</td>
<td>52 10</td>
<td>5.02 ± 0.24</td>
<td>4.80 ± 0.36</td>
</tr>
<tr>
<td></td>
<td>Pupa</td>
<td>53 10</td>
<td>13.30 ± 0.13</td>
<td>12.00 ± 0.26</td>
</tr>
<tr>
<td></td>
<td>Total (incl. eggs)</td>
<td>53 11</td>
<td>34.49 ± 0.23</td>
<td>35.27 ± 0.49</td>
</tr>
</tbody>
</table>

*All differences between sexes were only found between the durations of male and female pre-pupal periods (t-test, *P*=0.0190) and total egg-to-adult periods (t-test, *P*=0.0007) for *E. zinckenella.*
Table 2. Comparison of the mean ages of mortality of *Etiella zinckenella* (E.z.) and *E. hobsoni* (E.h.) adults.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Number sampled</th>
<th>Age of mortality, days post-eclosion ($\bar{x} \pm SE$)</th>
<th>t-test $P$ between species</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E.z.</td>
<td>E.h.</td>
<td>E.z.</td>
</tr>
<tr>
<td>Female</td>
<td>11</td>
<td>15</td>
<td>11.27 $\pm$ 1.47</td>
</tr>
<tr>
<td>Male</td>
<td>14</td>
<td>16</td>
<td>7.64 $\pm$ 0.66</td>
</tr>
<tr>
<td>t-test $P$ between sexes</td>
<td>-</td>
<td>-</td>
<td>0.040</td>
</tr>
</tbody>
</table>
seized the female genitalia, and the individuals rotated to a rear to rear position. Males not following the above sequence were rejected by females. Occasionally, males in crowded rearing cages appeared to encite female calling behavior by repeatedly probing her abdominal terminalia with his antennae. No differences in the above sequence of events were observed between *E. zinckenella* and *E. hobsoni*.

The percentage of virgin-females calling by age after eclosion differed markedly between species (Fig. 1). Only a single female *E. zinckenella* among 20 began calling on the first night after eclosion and this female exhibited only several short calling bouts. On the second night after emergence 30% of the *E. zinckenella* females began calling, and thereafter the percentage of calling females increased steadily with age. 75% of 16 *E. hobsoni* females called on the first night after eclosion (Fig. 1). The percentage peaked at 94% on the second night after eclosion, and declined to about 50% by day six. These trends were generally repeated in subsequent mating experiments (data not shown). On average, *E. zinckenella* females began calling about four hours earlier, and called for twice as long as *E. hobsoni* females (Table 3). The mean durations of calling bouts of individual females were not significantly different, but over the entire experimental period, roughly twice the number of calling bouts were observed for *E. zinckenella* compared to *E. hobsoni*.

The differences in the onset and duration of calling throughout scotophase are shown in Fig. 2. Calling by *E. zinckenella* females was distributed more evenly than that by *E. hobsoni* females. The data in Fig. 2 on the second night after eclosion also reflect the high numbers of *E. hobsoni* and the low numbers of *E. zinckenella* calling at that time (Fig. 1). The mean onset of calling by *E. zinckenella* females occurred consistently earlier over a 7-day period than for *E. hobsoni*, and over time, females of both species called earlier in the scotophase (Fig. 3). Taking the averages for days 1-3 and 5-7, the mean onset of calling time advanced by 1.97 h for *E. zinckenella* and by 1.12 h for *E. hobsoni*. The duration of individual calling bouts and the total duration of calling per night increased with age for both species (Figs. 4,5). Although *E. hobsoni* initially called for longer durations than *E. zinckenella* (Fig. 5) and achieved the longest calling
Fig. 1. Trends in percentage of virgin female *Etiella zinckenella* and *E. hobsoni* calling by post-eclosion age.
Table 3. Comparison of the calling parameters of virgin female *Etiella zinckenella* and *E. hobsoni*.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th><em>E. zinckenella</em></th>
<th><em>E. hobsoni</em></th>
<th>t-test P between species</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>number of hours N Range</td>
<td>number of hours N Range</td>
<td></td>
</tr>
<tr>
<td>Onset of calling in scotophase</td>
<td>78 0.4-10.8 2.86 ± 0.34</td>
<td>77 3.5-11.9 6.86 ± 0.21</td>
<td>0.0000</td>
</tr>
<tr>
<td>Net calling per female per night</td>
<td>80 0.02-11.9 7.81 ± 0.37</td>
<td>74 0.5-8.48 3.87 ± 0.26</td>
<td>0.0000</td>
</tr>
<tr>
<td>Duration of individual calling bouts</td>
<td>203 0.01-11.9 2.63 ± 0.18</td>
<td>109 0.08-8.5 2.63 ± 0.22</td>
<td>1.0000</td>
</tr>
</tbody>
</table>
Fig. 2. Temporal distribution of calling behavior throughout the scotophase on the second day after eclosion by virgin female *Etiella zinckenella* \((n=20)\) and *E. hobsoni* \((n=16)\).
Fig. 3. Mean onset of calling times in relation to days post-eclosion for virgin female *Etiella zinckenella* and *E. hobsoni*.

Number of Hours into Scotophase

<table>
<thead>
<tr>
<th>Days after Eclosion</th>
<th>E.Z. (20)</th>
<th>(20)</th>
<th>(20)</th>
<th>(20)</th>
<th>(20)</th>
<th>(15)</th>
<th>(14)</th>
</tr>
</thead>
</table>
Fig. 4. Average duration of individual calling bouts by virgin female *Etiella zinckeneilla* and *E. hobsoni*

Days Post-eclosion, with number of Surviving Females in Parentheses

- E.z.: (20) (20) (20) (20) (20) (15) (14) (14) (11) (n.a.)
Fig. 5. Net duration of calling per night in relation to days post-eclosion by virgin female *Etiella zinckenella* and *E. hobsoni*.

*Etiella zinckenella*

*Etiella hobsoni*

Days Post-eclosion, with number of Surviving Females in Parentheses

E.Z.: (20) (20) (20) (20) (15) (14) (14) (11) (n.a.)

bout duration eight days after eclosion (Fig. 4), the total duration of calling per night by female *E. zinckenella* rose over four days to be consistently double that for *E. hobsoni* (Fig. 5). The close similarity in the duration of individual calling bouts between species (Table 3) is also evident in Fig. 4.

The calling and mating behaviors of both species were highly similar under the normal and reverse-phase photoregimes (Table 4), except that the mean post-eclosion age of mating occurred earlier for *E. zinckenella* under the normal than the reverse-phase photoregime. No significant difference was detected between the mean onset of calling times of *E. zinckenella*, but calling times may have occurred slightly earlier for *E. hobsoni* under the normal than the reverse-phase photoregime. A greater percentage of virgin female *E. zinckenella* called on the first night following eclosion under the normal-phase photoregime (41%, n=29) than under the reverse-phase photoregime (14%, n=14), but this difference was not significant (Chi-square test: *P*=0.10). No significant difference in the mean onset of copulation time was detected for either species, and the mean age of mating was the same for *E. hobsoni* under both light regimes. For both species the trends in calling activity with increasing age were also the same. Therefore, apart from the difference in the age of mating of *E. zinckenella*, the effect of the photoregimes used in these experiments was minimal.

For *E. zinckenella* peak mating occurred during the second night, the mean post-eclosion age of copulation was 4.8 ± 2.5 days, and mating continued until day 10 (Fig 6). Under normal photophase conditions *E. zinckenella* peak mating also occurred during the second night, but the mean post-eclosion age of copulation was 2.50 ± 0.56 days, and mating continued until day 5 (Table 4). For *E. hobsoni* peak mating also occurred on the second night, the mean age of copulation was 1.9 ± 0.9 days post-eclosion, and no mating occurred after day 4. Under normal photophase conditions *E. hobsoni* peak mating also occurred during the second night, the mean post-eclosion age of copulation was 1.33 ± 0.33 days, and mating continued until day 2 (Table 4). No significant difference was detected between the mean post-eclosion ages of mating for *E. zinckenella* and *E. hobsoni* (t-test, *P*=0.12) under the normal-phase photoregime (Table 4), but
Table 4. Comparison of calling and mating trends observed under normal and reverse photo-regime data for interspecific mating experiment.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>E. zinckenella</th>
<th>E. hobsoni</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal photo-phase</td>
<td>Reverse photo-phase</td>
</tr>
<tr>
<td>mean onset of calling $^1$ (X±SE)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>N=36</td>
<td>N=48</td>
</tr>
<tr>
<td>mean age of mating $^2$ (days 1-5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>N=6</td>
<td>N=5</td>
</tr>
<tr>
<td>mean age of mating $^2$ (days 1-2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>N=6</td>
<td>N=5</td>
</tr>
<tr>
<td>Call age increase</td>
<td>17 increased</td>
<td>6 increased</td>
</tr>
</tbody>
</table>

$^1$ number of hours into scotophase

$^2$ number of days following eclosion
Fig. 6. Distribution of calling and mating behavior in relation to days post-eclosion by female/male pairs of *Etiella zinckenella* and *E. hobsoni*.
the mean post eclosion age of mating for *E. zinckenella* was significantly higher than for *E. hobsoni* under the reverse phase photoregime. Calling activity by unmated females of both species continued well beyond the last day on which mating occurred for both photoregimes. Although most mating by *E. zinckenella* pairs occurred during the later half of the scotophase, 31% occurred during the first half (Fig. 7). By contrast, *E. hobsoni* copulations were observed only during the second half of scotophase. These trends in mating activity correspond with the observed trends in calling behavior (Fig. 2).

Calling activity was not inhibited in females paired with heterospecific males (Chi-square test: $P<0.90$), but no courtship behavior was observed in such pairings (Table 5). Copulation and fertile eggs were only obtained between conspecific male and female pairings. In no case did mating occur if females failed to call. A substantially higher percentage of female *E. hobsoni* called than female *E. zinckenella* (Table 6), but among calling females no difference in the percentage of matings was observed between species. Repeated calling after mating and repeated mating was observed in both species, but at a very low frequency.

The fecundity of female *E. zinckenella* ($160.8 \pm 49.0$ eggs per female, $N=8$, range: 0-356) was much higher than for female *E. hobsoni* ($32.5 \pm 16.9$, $N=13$, range: 0-214) (t-test, $P=0.0380$). A possible confounding factor in measuring the fecundity was that females were sometimes observed ovipositing on cage walls and first instar larvae, possibly from eggs contaminating the cages, were sometimes found on pods present in the cages for only one day. A separate fecundity measurement conducted within glass Erlenmeyer flasks for *E. zinckenella* females yielded $208.7 \pm 52.4$ eggs per female ($N=9$, range: 0-580), not significantly different from the previously recorded value (t-test, $P=0.5100$). Only a single measurement of 127 eggs laid per mated *E. hobsoni* female was obtained by this method. The pooled oviposition data from both methods of measurement is shown in Fig. 8 which indicates that *E. zinckenella* exceeds *E. hobsoni* in the number of eggs laid per female per day for the first five days, after which they are approximately equal. The duration of the oviposition period for *E. zinckenella* ($6.11 \pm 0.72$ d, $n=15$) females was not significantly longer than that of *E. hobsoni* females.
Fig. 7. Temporal distribution of mating throughout the scotophase by male/female pairs of *Etiella zinckenella* \((n=16)\) and *E. hobsoni* \((n=23)\)
Table 5. Occurrence of calling behavior and mating in con- and heterospecific male/female pairings of *Etilella zinckernellia* (E.z.) and *E. hobsoni* (E.h.).

<table>
<thead>
<tr>
<th>Pairing combination</th>
<th>E.h. Female</th>
<th>E.h. Male</th>
<th>E.z. Female</th>
<th>E.z. Male</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>23</td>
<td>18</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>number of females calling</td>
<td>18</td>
<td>10</td>
<td>22</td>
<td>18</td>
</tr>
<tr>
<td>mating</td>
<td>9</td>
<td>0</td>
<td>8</td>
<td>0</td>
</tr>
</tbody>
</table>
Table 6. Calling, mating and repetition of these behaviors by female *Etiella zinckenella* and *Etiella hobsoni* in conspecific pairings.

<table>
<thead>
<tr>
<th>Female Behavior</th>
<th><em>Etiella zinckenella</em></th>
<th><em>Etiella hobsoni</em></th>
<th>$X^2$ test $P$ between species</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. tested</td>
<td>% exhibiting behavior</td>
<td>No. tested</td>
</tr>
<tr>
<td>Calling</td>
<td>30</td>
<td>40.0</td>
<td>34</td>
</tr>
<tr>
<td>Mating by calling females</td>
<td>12</td>
<td>50.0</td>
<td>32</td>
</tr>
<tr>
<td>Calling by mated females</td>
<td>4</td>
<td>0.0$^a$</td>
<td>10</td>
</tr>
<tr>
<td>Repeated mating</td>
<td>0</td>
<td>0.0</td>
<td>1$^b$</td>
</tr>
</tbody>
</table>

$^a$Although calling following mating, and repeated mating for *E. zinckenella* was not observed in this experiment, both of these behaviors were observed in a single pair of *E. zinckenella* during another mating experiment.

$^b$A single pair of *E. hobsoni* was tested for repeated mating out of two *E. hobsoni* females which were observed to repeat calling behavior after mating. In this case the same pair were observed to mate three times.

$^c$Conditions of chi-square test violated (expected frequencies too low)
Figure 8. Mean number of eggs laid per day by number of days following mating for *Etiella zinckenella* (E.z.) (n=15) and *E. hobsoni* (E.h.) (n=8).

| No. days following mating | E.z.: 0 (12) (12) (13) (10) (9) (7) (6) (5) (4) (3) (2) (1) (0) | E.h.: 2 (3) (3) (5) (4) (3) (2) (0) (2) (1) (2) (0) (0) (0) |
(3.87±1.17 d, n=8) (t-test, P=0.13). Similarly, a larger proportion of mated female *E. zinckenella* (15/18) oviposited than *E. hobsoni* (8/15) but this difference was not significant (Chi-square test, P>0.05). Considering only the females which laid eggs, the mean number of eggs laid per female was 239.7 ± 36.9 (N=15, range: 59-423 eggs) for *E. zinckenella*, significantly higher than 65.4 ±2.65 (N=8, range: 2-214 eggs) for *E. hobsoni* (t-test, P=0.001). *E. zinckenella* pairs remained in copula for 123.6 ± 12.5 min (N=10, range: 35-182 min), significantly longer than the 73.7 ± 16.1 min (N=10, range: 19-273 min) for *E. hobsoni* (t-test, P=0.0210). No significant correlation was observed between the duration of copulation and the fecundity of either species (Fig. 9) (linear regression, E.z.: R²=0.022, P=0.728, E.h.: R²=0.059, P=0.449). A single spermatophore was transferred during copulation by each species.

**Pheromone-Based Communication**

Virgin female-baited traps set out during the rainy season failed to catch any males until observable adult *Etiella* spp. populations returned at the beginning of the dry season. In soybean fields where adult *Etiella* spp. were frequently observed, traps baited with two caged virgin females caught between 0 and 8 conspecific adult males per night. The mean number of moths caught per trap per night was higher for *E. zinckenella* (\(\bar{x}=2.65 \pm 0.41\)) than *E. hobsoni* (\(\bar{x}=1.61 \pm 0.40\)), but *E. zinckenella* field populations were generally higher. The number of moths per trap varied significantly with the trap height (Table 7). Catches in traps below the canopy level were reduced by 91% for *E. zinckenella* compared to those at or above the canopy level, and no *E. hobsoni* were trapped below the canopy level.

The number of *E. zinckenella* males captured in traps baited with both conspecific females was reduced by 67% compared to traps baited with only conspecific females (Table 8). In turn the number of captured male *E. hobsoni* was reduced by 48% in the presence of heterospecific females, but the reduced capture level was statistically intermediate between that to conspecific females and to unbaited control traps. Only conspecific males were caught in traps baited with two females of the same species.
Figure 9. Scatterplot of fecundity versus duration of copulation for *Etiella zinckenella* (n=8) and *E. hobsoni* (n=13).

* Non-significant linear regression (E.z.: $R^2=0.022$, $P=0.728$, E.h.: $R^2=0.059$, $P=0.449$)

<table>
<thead>
<tr>
<th>Species</th>
<th>Trap height (cm)</th>
<th>Trap position</th>
<th>Number of males captured ($\bar{x} \pm SE$)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Etiella zinckenella</em></td>
<td>30</td>
<td>below canopy</td>
<td>0.67 ± 0.33</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>canopy level</td>
<td>7.33 ± 2.33</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>above canopy</td>
<td>8.00 ± 3.79</td>
</tr>
<tr>
<td><em>Etiella hobsoni</em></td>
<td>30</td>
<td>below canopy</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>canopy level</td>
<td>0.67 ± 0.67</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>above canopy</td>
<td>3.33 ± 2.40</td>
</tr>
</tbody>
</table>

\(^a\)Significant treatment variation detected (ANOVA, $P=0.007$ (E.z.) and $P=0.034$ (E.h.)) but no significant separation of means (Tukey's multiple range test, $P>0.05$).
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of males captured ($\bar{x} \pm SE$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E. zinckenella</td>
</tr>
<tr>
<td>Unbaited control</td>
<td>$0.00 \pm 0.00$ b</td>
</tr>
<tr>
<td>Two $E. zinckenella$ females</td>
<td>$10.75 \pm 2.95$ a</td>
</tr>
<tr>
<td>Two $E. hobsoni$ females</td>
<td>$0.00 \pm 0.00$ b</td>
</tr>
<tr>
<td>Two females of each species</td>
<td>$3.50 \pm 2.02$ b</td>
</tr>
</tbody>
</table>

* Treatments within a column followed by different letters differ significantly (Tukey’s Multiple Range Test, $P<0.05$).
Quantities of pheromone components in glandular extracts of both species were low (Fig. 10). In *E. zinckenella*, EAD-active compounds were no, or only barely FID-detectable. In *E. hobsoni*, the most EAD-active compound occurred well above FID-detection threshold, but additional compounds were not FID-detectable. Comparative GC chromatography of synthetic standards tentatively identified EAD-active compounds as C12:OAc, Z9-12:OAc, E9-12:OAc, Z11-14:OAc, E11-14:OAc, and Z9-14:OAc for *E. zinckenella*, and Z11-14:OAc, E11-14:OAc, Z9-14:OAc and Z11-16:OAc for *E. hobsoni* (Fig. 10). A major EAD-active compound in extracts of *E. zinckenella* which coeluted with Z11-14:OAc also elicited the strongest antennae response. The most EAD-active compound in extracts of *E. hobsoni* coeluted with Z11-16:OAc.

Traps baited with a Toth et al. (1989) pheromone blend failed to attract males of either species (Table 9), while traps baited with virgin females attracted conspecific males. High numbers of *E. hobsoni* males were attracted to traps baited with conspecific females, but the number of *E. zinckenella* males attracted to virgin female-baited traps was very low. Additional three-component blends (Toth et al. 1989) were tested at two different dosages (Table 10, experiment 2: Z11-14:OAc (10 μg): E11-14:OAc (1 μg): Z9-14:OAc (1 μg) and experiment 3: Z11-14:OAc (100 μg): E11-14:OAc (10 μg): Z9-14:OAc (10 μg)) but neither treatment caught significant numbers of male moths despite substantial adult field populations. A single two component treatment (Table 10, experiment 4: of Z11-14:OAc (100 μg): E9-12:OAc (5 μg)) attracted significant numbers of male *E. zinckenella*. However, the number of moths trapped by this blend was very low compared to the numbers typically caught in virgin female-baited traps (note that Tables 10 and 11 show the total number of moths caught per treatment whereas Tables 7-9 give the mean numbers trapped). Thus, over a 24 day period, six replicates of the two component treatment caught about the same number male *E. zinckenella* as could be expected on one or two nights using a single trap baited with two virgin females. (The single female *E. zinckenella* caught by this treatment was removed prior to any males being caught in this trap, and the males caught by this treatment were distributed among four of the six replicates). No lures tested for *E. hobsoni* attracted significant numbers of males (Table 11).
Fig. 10. Flame ionization detector (FID) and electroantennographic detector (EAD: male *Etiella zinckenella* or *E. hobsoni* antenna) responses to one female equivalent of female pheromone gland extracts. Chromatography: DB-23 coated fused silica column (30 m x 0.32 mm ID); temperature program: 70°C (1 min), 20°C/min. to 140°C, 2°C/min. to 200°C. Structural assignments of EAD-active compounds are tentative and need to be confirmed by mass spectrometry.

<table>
<thead>
<tr>
<th>Treatment</th>
<th><em>E. zinckenella</em></th>
<th><em>E. hobsoni</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Unbaited control</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00  a</td>
</tr>
<tr>
<td>Three <em>E. zinckenella</em> females</td>
<td>1.00 ± 0.58</td>
<td>0.00 ± 0.00  a</td>
</tr>
<tr>
<td>Three <em>E. hobsoni</em> females</td>
<td>0.00 ± 0.00</td>
<td>20.75 ± 3.33 b</td>
</tr>
<tr>
<td>Toth et al. (1989) synthetic pheromone blend&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00  a</td>
</tr>
</tbody>
</table>

<sup>a</sup> ANOVA *P*=0.1170 for *E.*z. Means for *E.*h. followed by different letters are significantly different (Tukey’s Multiple Range Test, *P*<0.05).

<sup>b</sup>(Z-11-14:0Ac (1000 µg), E-11-14:0Ac (100 µg), Z-9-14:0Ac (100 µg), and C-14:0Ac (500 µg))
### Table 10. Summary of candidate pheromone blends field tested for *Etiella zinckenella*

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>Amounts (µg) of synthetic compounds loaded onto lures</th>
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<th>Female</th>
<th>Male</th>
<th>Female</th>
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<tbody>
<tr>
<td></td>
<td>Etiella zinckenella</td>
<td>Etiella hobsoni</td>
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</tr>
<tr>
<td></td>
<td>E11-16</td>
<td>Z11-14</td>
<td>E11-14</td>
<td>C-12</td>
<td>Z9-12</td>
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<tr>
<td>1a</td>
<td>1000</td>
<td>100</td>
<td>100</td>
<td>500</td>
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</table>

* Tested at Garut, 22 - 29 August, 1993, Latin square, n=4, spacing=15 m, traps placed at canopy level.

* Tested at Cianjur, 29 September - 21 October, 1993, Latin square, n=6, spacing=10 m, traps placed at canopy level.

* Tested at Cianjur, 21 October - 3 November, 1993, RCBD, n=5, spacing=10 m, traps placed at ca. 30 cm above canopy.

* Tested at Bogor, 2 - 26 August, 1994, Latin square, n=6, spacing=7.5 m, traps placed at ca. 30 cm above canopy. Significant treatment variation observed for number of male E.z. trapped (ANOVA, P=0.0000).
Table 11. Summary of candidate pheromone blends field tested for *Etiella hobsoni*

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>Z11-</th>
<th>E11-</th>
<th>Z9-</th>
<th>Z11-</th>
<th>Z11-</th>
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<tr>
<td></td>
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</table>

Total no. trapped

<table>
<thead>
<tr>
<th>Etiella hobsoni</th>
<th>Etiella zinckenella</th>
</tr>
</thead>
<tbody>
<tr>
<td>male</td>
<td>female</td>
</tr>
<tr>
<td>female</td>
<td>female</td>
</tr>
</tbody>
</table>

a tested at Cianjur, 30 Sept. - 21 Oct., 1993, Latin square, n=5, trap spacing=10 m, placed at canopy level.
b tested at Cianjur, 22 Oct. - 3 Nov., 1993, RCBD, n=3, trap spacing=10 m, placed at ca. 30 cm above canopy.
c tested at Bogor, 17 - 20 November, 1993, Latin square, n=5, spacing=7 m, traps placed at ca. 30 cm above canopy.
d tested at Bogor, 4 Dec. - 15 Dec., 1993, RCBD, n=5, trap spacing=7 m, placed at ca. 30 cm above canopy.
Discussion

Significant differences occur between the developmental periods of laboratory-raised *E. zinckenella* and *E. hobsoni* (Table 1). The egg, larval, and total egg-to-adult developmental periods of both sexes of *E. zinckenella* were shorter than those of *E. hobsoni*, whereas the pupal periods of both sexes of *E. zinckenella* were longer than those of *E. hobsoni*. Overall, *E. zinckenella* females eclosed about 2 days earlier than female *E. hobsoni*, and *E. zinckenella* males eclosed about one day earlier than male *E. hobsoni*. Differences from previously reported findings (Naito and Hamoto 1984) include: a longer duration of the egg stage of *E. hobsoni* than for *E. zinckenella*, previously reported to be the same; a longer duration for the pupal stage of *E. zinckenella* than for *E. hobsoni*, previously reported to be the same; no difference in the duration of the larval stages of male and female *E. hobsoni*, previously reported to be longer for *E. hobsoni* females, no difference in the duration of the larval stages of male and female *E. zinckenella*, previously reported to be longer for *E. zinckenella* males. A much longer total duration of development at 27°C for *E. zinckenella* occurred than previously reported by Bindra and Singh (1969), who found the life cycle to be completed within 22-24 days at 25°C, and within a shorter period at higher temperatures. The latter difference is possibly related to the soybean diet I used for *E. zinckenella*, whereas Bindra and Singh (1969) raised their larvae on pigeon pea. The overall durations of the egg-to-adult stages of both *E. zinckenella* and *E. hobsoni* were also longer in my experiment than reported by Naito and Hamoto (1984), perhaps reflecting different laboratory conditions. All of the other developmental durations I measured were greater than those of Naito and Hamoto (1984), except for the duration of the larval stages of each species which were about four days longer in their report. This latter difference is apparently due to differences in experimental method; I measured the duration of the larval and prepupal stages separately, whereas Naito and Hamoto (1984) combined the pre-pupal stage and larval stages. Newly hatched larvae were found to be highly phototactic in agreement with a previous report (Bindra and Singh 1969), and I also observed this behavior among late instar larvae. First instar larvae that had escaped in the laboratory would frequently form spinnerets and suspend
themselves near windows. This may be an innate mechanism promoting wind-born dispersal, a common trait in numerous lepidopteran species, particularly among the Tortricidae (Barbosa et al. 1989).

Female *E. zinckenella* eclosed earlier than males, perhaps due to a shorter pre-pupal period (Table 1), but no corresponding differences were observed for *E. hobsoni*. Despite their earlier eclosion, however, *E. zinckenella* females appear to reach sexual maturity relatively later after eclosion than *E. hobsoni* females. That is, most virgin female *E. hobsoni* began calling on the first night after eclosion (Figs. 1, 6), whereas few *E. zinckenella* females called on the first night after eclosion (Figs. 1, 6) (Hirai 1982; Hattori and Sato 1983). Under a reverse-phase photoregime, the mean post-eclosion mating age of *E. zinckenella* was significantly greater than that of *E. hobsoni* (Table 4). Under a normal-phase photoregime, the mean post-eclosion mating age of *E. zinckenella* was also greater than that of *E. hobsoni*, but this difference was not statistically significant. Unfortunately, the number of observations (n=3) for *E. hobsoni* in the later comparison was very low. Using data pooled from both photoregimes (Fig. 6), peak mating for both species occurred on the second night after eclosion, however, mating by *E. zinckenella* continued until the ninth day, whereas the mating by *E. hobsoni* continued only until the fifth day. Previous reports indicate that peak mating by *E. zinckenella* occurs on the third day following eclosion (Hirai 1982; Hattori and Sato 1983). An earlier mean onset of calling time with increasing age was previously indicated for *E. zinckenella* (Hirai 1982); this trend was observed for *E. zinckenella* and to a lesser extent for *E. hobsoni* (Fig 3). An advance in the onset of calling times may provide a means by which aging unmated females are most likely to attract responsive males.

A smaller percentage of *E. zinckenella* than *E. hobsoni* females called (Table 6), although those which called mated with approximately the same frequency. The lower percentage of calling by *E. zinckenella* females may reflect their need to call in a natural environment. In nature, *E. zinckenella* females may actually have a higher likelihood of mating than *E. hobsoni* since they called for twice as long per night (Table 3), lived significantly longer (Table 2), and had a greater
range in mating ages (Table 4, Fig. 6). Conversely, the lower percentage of calling by E. zinckenella females could be due to different mating strategies between the two species. Perhaps only the most reproductively fit E. zinckenella females initiate mating behavior, but compensate by having a higher fecundity. However, this hypothesis is contradicted a nonsignificant difference in the proportion of oviposition mated female E. hobsoni (8/15) and E. zinckenella (15/18) (Chi-squared test, P>0.05). Males beyond a certain age may have a reduced response to calling females, because females of both species continued calling beyond the maximum observed age of mating (Fig. 6). Rare incidences of repeated mating observed for both E. zinckenella and E. hobsoni (Table 6) refute a previous report that E. zinckenella are unable to mate more than once (Abul-Nasr and Awadalla 1957), yet its occurrence is so infrequent that its possible role in nature is questionable. Verification of repeated mating in the field would require the recovery of two or more spermatophores from captured females.

E. zinckenella and E. hobsoni appear to be completely reproductively isolated; no interspecific mating or courtship behavior was observed by me (Table 5), or by Naito and Hamoto (1984). The sympatric nature of these two species, their overlapping developmental and mating periods, and their close taxonomic relationship suggest that interspecific barriers should exist. The natural pheromone plumes of each species attract only conspecific males (Tables 8, 9), and plays an important role in preventing interspecific pairings. Interspecies differences in the temporal distribution of calling and mating behaviors (Figs. 2, 6, 7) may also provide partial barriers to interspecific mating, as may the earlier maturation of E. zinckenella relative to E. hobsoni may (Table 1). However, substantial overlap in the age and timing of mating nevertheless occurs.

The fecundity of E. zinckenella females was higher than in previous reports using soybeans as hosts (Tsutsui 1950; Hattori and Sato 1983), but was similar to that on other host plants (Bindra and Singh 1969; Hill 1975). The fecundity of E. zinckenella females also was nearly four times higher than that of E. hobsoni. The duration of copulation for E. zinckenella was nearly twice as long as reported for Japanese (Hirai 1982; Hattori and Sato 1983) and Egyptian
(Abul-Nasr and Awadalla 1957) populations. The duration of copulation of *E. zinckenella* was also nearly twice as long as for *E. hobsoni*, but no correlation was detected between the duration of copulation and the fecundity for either species (Fig. 9). These results suggest divergent reproductive strategies by each species. *E. zinckenella* remains vulnerable to predation for a longer time while *in copulo*, but compensates for this vulnerability by producing a larger number of eggs, while the strategy of *E. hobsoni* is the reverse.

The higher fecundity, larger size (Naito and Hamoto 1984), longer life-span (Table 2) and greater range in mating ages of *E. zinckenella* females (Table 4) suggests that *E. zinckenella* may have a higher outbreak potential than *E. hobsoni*. This hypothesis is supported by the observation that populations of the two species tend to be equal in relative abundance early in the dry season, whereas by the end of the season *E. zinckenella* is predominant (Naito and Hamoto 1984). The low fecundity of *E. hobsoni* may also explain why it was much more difficult to maintain in laboratory culture than *E. zinckenella*. Additionally, the more rapid larval development of *E. zinckenella* than *E. hobsoni* (Table 1) despite their larger size (Naito and Hamoto 1984) suggests that *E. zinckenella* may be more highly adapted to the soybean host than *E. hobsoni*. *E. hobsoni* was only recently discovered as a pest of soybean in Indonesia (Naito and Hamoto 1984). However, the two species are virtually indistinguishable during the larval stages, and the readily distinguishable adults are nocturnal and thus seldom observed closely. Therefore, *E. hobsoni* may have been incorrectly assumed to be *E. zinckenella* for a considerable period prior to its proper identification.

The life-cycles of both species closely match the pod and seed development periods of the short-season soybean varieties which are predominant in the lowland areas of Indonesia. Thus only one generation of *Etiella* spp. can reach maturity within a single planting. The common practice of staggering planting dates in Indonesia greatly favours *Etiella* spp. outbreaks as the season progresses, since fields are small and successive generations will emerge within range of abundant susceptible host material. In support of this hypothesis, I found heavy *Etiella* spp. infestations only in late planted areas. Synchronized plantings could therefore be expected to
cause a considerable reduction in *Etiella* spp. populations, but this practice might not be practical for Indonesian farmers, or may require coordination by local agricultural authorities. Kalshoven (1981) recommended that plantings within an area be restricted to within a 40 day period, the approximate intergenerational period for both *Etiella* spp. Even with synchronized plantings, however, overlapping generations of *E. zinckenella* may nevertheless still occur due to immigration from surrounding areas, and the high variability in the age of mating (Table 4, Fig. 6) and oviposition (Figure 8) by *E. zinckenella* females (Hattori and Sato 1983). Prolonged oviposition by *E. zinckenella* may therefore hinder efforts to optimize the timing of insecticide applications based on adult populations, or may require more than one application to adequately control this species. The variability in the age of mating (Table 4, Fig. 6) and oviposition (Figure 8) by *E. hobsoni* females was less than that of *E. zinckenella* females, and therefore, efforts to precisely time insecticide applications may be more effective at controlling this species.

My results indicate that there must be pronounced geographic variation in the pheromone composition of *E. zinckenella*. Despite the fact that caged females attracted significant numbers of males (Tables 7, 8, 9) no response whatsoever occurred at any dose to pheromone blends tested successfully in both Hungary and Egypt (Tables 9, 10). Variation in pheromone composition has been previously reported among geographically isolated populations of several lepidopteran species including the European corn borer, *Ostrinia nubilalis* Hübner (Cardé et al. 1978), the turnip moth, *Agrotis segetum* L. (Toth et al. 1992), the fruit-tree leafroller, *Archips argyrospilus* Walker (Deland et al. 1993), and the obliquebanded leafroller, *Choristoneura rosaceana* Harris (Thomson et al. 1991). The existence of such geographic variation indicates that regionally-specific identification of pheromone blends will be required before pheromones are used in the management of this species. It further suggests that strain-specific resistance to pheromone-based management strategies could occur. In *O. nubilalis* female sex-pheromone production, male response to different pheromone blends, and reproductive isolation between strain-types is governed by relatively few alleles (Liebherr and Roelofs 1975, Klun and Maini 1979, Roelofs et al. 1987).
The number of males attracted to traps baited with three virgin-female *E. zinckenella* (Table 9) was low compared to the number of males attracted to traps baited with three virgin-female *E. hobsoni*, or to the numbers caught by traps baited with two virgin females of either species (Tables 7,8). Perhaps overcrowding within the small bait cages caused reduced calling by *E. zinckenella* females. *E. hobsoni* females are significantly smaller sized than *E. zinckenella* females (Naito and Hamotto, 1987), and would therefore experience relatively less crowding. In contrast, competition by wild female *E. zinckenella* may have resulted in the low number of males caught during this experiment. Reduced male trap catches have been attributed to female competition in a number of lepidopterous species (McNeil 1991).

Lack of cross attraction between *E. zinckenella* and *E. hobsoni* (Table 8) indicates highly specific chemical communication systems. This specificity could prove useful in developing a selective monitoring system that could detect seasonal and regional variations in *E. zinckenella* and *E. hobsoni* populations. Conversely, because the response of males to traps baited with both con- and heterospecific females was partially inhibited (Table 8), it may also be possible to develop a pheromone blend capable of simultaneously disrupting mating by both species. The GC-EAD analyses of *E. zinckenella* and *E. hobsoni* pheromone gland extracts (Fig. 10) suggested several differences in their pheromone composition. Z11-14:OAc appeared to occur in extracts of both species, elicited the strongest EAD response by *E. zinckenella* males, and was FID detectable only in *E. zinckenella* extracts. Z11-16:OAc appeared to be present only in *E. hobsoni* extracts, elicited the strongest EAD response by *E. hobsoni* males, and was the only FID-detectable, EAD-active compound in *E. hobsoni* extracts. Therefore Z11-14:OAc and Z11-16:OAc may be major pheromone components of *E. zinckenella* and *E. hobsoni*, respectively. E11-14:OAc and Z9-14:OAc seemed to be present in extracts of both species, but Z9-12:OAc, E9-12:OAc, and C-12:OAc may be *E. zinckenella* specific. The compounds Z11-14:OAc, E11-14:OAc, Z9-14:OAc, and C-14:OAc were previously reported as pheromone components of *E. zinckenella* from Hungary and Egypt (Toth et al. 1989). Z11-16:OAc was also present in *E. zinckenella* pheromone gland extracts but appeared to have no effect on attracting males.
Structural assignments of the above compounds need to be confirmed by GC-mass spectrometry. Low quantities of pheromone components in the glandular extracts also complicated estimates of concentrations which may be critical if component ratios impart specificity of *E. zinckenella* and *E. hobsoni* pheromone blends. The above sex pheromone components are common to a variety of species, and altered pheromone component ratios are an important means for imparting the specificity of chemical communication channels (Cardé and Baker 1984). Synthetic Z11-14:OAc (100 µg) and E9-12:OAc (5 µg) attracted significant numbers of male *E. zinckenella*. However, attractiveness was extremely low in comparison to virgin-female baited traps, suggesting that additional components are essential or dosages or component ratios were incorrect. Testing the two component blend over a range of dosages, and in different ratios would be a good starting point for future pheromone experiments for Indonesian *E. zinckenella*. 


