Thresholds, Monitoring and Sampling

Development of a Pheromone-Based Monitoring System for *Neurocolpus longirostrus* (Knight) (Hemiptera: Miridae)

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Bioassay trials in 1996 with candidate *Neurocolpus longirostris* pheromones were in general inconclusive but had given some leads for continuing procedures and trials in 1997. As in previous trials, all field bioassays were placed in California buckeye, *Aesculus californica,* which was in bloom. As much as possible, traps were placed fully exposed on the periphery of buckeye trees in proximity to flowers but usually not in shade as had been done in previous trials. Following the lead provided by the 1997 field bioassays with *Calocoris norvegicus,* pheromone traps used for *Neurocolpus* in 1997 were standard Jackson traps using removable sticky insert liners. Five replications per treatment were used in all *Neurocolpus* field bioassays. Candidate pheromones were applied to rubber septa at reduced rates compared to the 1996 trials. In trials with sufficient bug collections, data were analyzed using Fisher's Protected LSD test at $P = 0.05$ significance level.

Following a review of volatile chemicals produced by male and female *N. longirostris* adults, the first field bioassays with synthetic *Neurocolpus* pheromones in 1997 were emplaced on May 12 near Dunlap, Fresno County, California. This test resulted in low numbers of *Neurocolpus* males trapped only in the treatment comprised of the complete (six-isomer) blend of female-produced volatile chemicals. These collections were made on the first day of exposure of this series of chemicals.

Following the initial test with the complete blend, a second series of pheromones was tested using a fivefold increase of pheromone on each rubber septa. In this trial, no bugs were collected until the third through sixth day of exposure, perhaps due to an arresting or repellent effect of the higher load rate of pheromones in the traps. As in the first trial, *Neurocolpus* males were trapped only in treatments containing the complete blend of female-produced volatiles.

A third bioassay, again using reduced rates of pheromone loads, continued to show improvement in collection of male *Neurocolpus* (Table 1, no. 3). For the first time, several treatments showed recurrent collections of male bugs, and one five-component blend (NL-97-37), was notably better than any of the other treatments in the trial. Also, the shift to Jackson traps rather than wing traps seemed to greatly reduce the number of female bugs collected, similar to results with the last 1997 *Calocoris* test.

The fourth series of Neurocolpus bioassays was designed to again compare pheromone load rates and blend composition on septa. Collection of bugs continued to improve for the duration of this test with the higher rates of the five-component blend (NL-97-51, -52)

significantly better than a three-component blend (NL-97-56, -57) at the same load rates (Table 1, no. 4).

The next series of *Neurocolpus* bioassays compared the best treatment (five-component blend) from Test 4 to various load rates of only a four-component blend. The results of this trial (Table 1, no. 5) showed that the highest dose rate of the four-component pheromone (NL-97-75) collected significantly more bugs than other treatments in the trial, with daily collections in most of the treatment replicates. It was noted that most of the bugs were collected after three to five days of lure exposure, indicating that the initial release rate of pheromone from the lures was again too high, thus supporting earlier observations that release rates that are too high will in fact keep bugs from moving into the traps. It was, however, observed during this bioassay series that male bugs that were eventually trapped were in close proximity to the pheromone source (on or inside rubber septa) indicating a strong attraction and movement directly to the septa. This is similar to collections of males responding to pheromones produced by virgin females placed in small cages inside sticky traps. Male *Neurocolpus* were also observed on buckeye flowers and foliage adjacent to the traps. This was also an indication that blend composition and release of pheromone was becoming more acceptable to responding male bugs. After one week of field exposure of the lures in this trial, the pheromones were no longer attracting males, suggesting that the release of attractive chemicals from septa in a proper blend and rate occurs over a very short period of time (about one week or less).

The sixth *Neurocolpus* bioassay was emplaced at a higher elevation (ca. 4000 ft) with better host conditions than in the (lower) Dunlap locations. In this trial the pheromone load rate on rubber septa was reduced from the previous trial and compared a four-component blend to a series of three-component blends. The results of this trial (Table 1, no. 6) showed that the four-component blend (NL-97-80) was superior to any of the three-component blends. It was also noted that male bugs were again attracted on the first day of trapping, probably due to improving pheromone blend composition and load rate on the lures.

It had been observed occasionally in previous 1997 trials and was strongly confirmed in this trial that *Neurocolpus* males are active on buckeye flowers early in the morning and usually in full sunlight. In addition to bugs being trapped on sticky trap surfaces, male bugs were observed on foliage near to traps and on outer trap surfaces between 6:00 and 9:00 a.m. PST. They were moving over the trap surface and foliage in an excited and agitated manner while temperatures were increasing from approximately 65° to 75°F on most days. It appeared from this behavior that the bugs were definitely responding to attractive chemicals coming from the pheromone traps. They had never been observed, over a period of many years, behaving in this manner or even exposed outside of protective buckeye flowers. Although male bugs were attracted to traps on the first day of pheromone presentation, maximum male response occurred on the third and fourth day of trapping, again indicating that initial release of pheromones from the rubber septa in this trial was perhaps still too high for optimum bug response.

The 1997 *Neurocolpus* pheromone bioassays produced significant advances in identification of attractive volatiles produced by female bugs and in pheromone blend composition and presentation. In addition, several interesting observations were made regarding bug behavior and activity. In all locations where bioassays were carried out in 1997, male

Neurocolpus responded to pheromone traps primarily in the early morning hours between sunup and 9:00 a.m. standard time. During these bioassays early morning temperatures ranged from approximately 65° to 75° or 80°F. On one occasion a freshly caught male bug was in a trap in sunlight when the temperature was 61°F. This possibly represents the lower limit of bug activity and response to pheromones. Both male and female bugs are active on flowers in the full sunlight in preference to flowers in shade or cooler locations. Males also seem to respond better to traps that were in open sunlight rather than in shady locations. However, this was not always the case with traps that had very attractive blends. As buckeye flowers began to senesce and decline in host suitability, the bug populations seemed to enter a migratory or searching mode of behavior. This could lead to active migration from drying buckeye to other potential hosts, which could be the reason *Neurocolpus* infests pistachio orchards some distance from the native buckeye host.

It was also established during these trials that the smaller Jackson trap was a very efficient trap for collection of *Neurocolpus* males responding to pheromones. This trap is easier to handle and less expensive than the larger wing traps used in previous trials. The successful 1997 *Neurocolpus* pheromone bioassays will provide a solid starting point for final identification and development of a commercial *Neurocolpus* trapping system in 1998.

Table 1. Collections of *Neurocolpus longirostris* in Jackson traps baited with selected pheromone isomer blends, Fresno County, California.

Bioassay No. 3

	No. *Neurocolpus* collected	
Pheromone blend	Males	Females
NL-97-31	4	0
NL-97-32	0	1
NL-97-33	4	0
NL-97-34	0	0
NL-97-35	0	0
NL-97-36	4	0
NL-97-37	17	1
NL-97-38 (blank)	1	0

Bioassay No. 4

	No. *Neurocolpus* collected	
Pheromone blend	Males	Females
NL-97-51	29a	0
NL-97-52	14ab	3
NL-97-53	2b	0
NL-97-54	3b	0
NL-97-55	2b	0
NL-97-56	2b	0
NL-97-57	0b	0
NL-97-58	3b	0
NL-97-59	0b	0
NL-97-60	1b	0
NL-97-61 (blank)	0b	0

Bioassay No. 5

	No. *Neurocolpus* collected	
Pheromone blend	Males	Females
NL-97-71	3a	2
NL-97-72	2a	0
NL-97-73	1a	0
NL-97-74	1a	0
NL-97-75	18b	1
NL-97-76	1a	0
NL-97-77	2a	0
NL-97-78	1a	1
NL-97-79 (blank)	1a	0

Bioassay No. 6

	No. *Neurocolpus* collected	
Pheromone blend	Males	Females
NL-97-80	35a	0
NL-97-81	6b	0
NL-97-82	0b	0
NL-97-83	0b	0
NL-97-84	2b	0
NL-97-85 (blank)	0b	0