30 Barclay, A.N., Letarte-Muirhead, M., Williams, A.F., and Faulkes, R.A., Nature *263* (1976) 563.
31 Snyder, S.H., and Goodman, R.R., J. Neurochem. *35* (1980) 5.
32 Wang, Y.S., and Mahler, H.R., J. Cell Biol. *71* (1976) 639.
33 Chiu, T.C., and Babitch, J.A., Biochim. biophys. Acta *510* (1978) 112.
34 Rees, R.P., Fedn Proc. *37* (1978) 2000.
35 Olden, K., Parent, J.B., and White, S.C., Biochim. biophys. Acta *65* (1982) 209.
36 Anderson, R.G.W., Vasite, E., Mello, R.J., Brown, M.S., and Goldstein, J.C., Cell *6* (1978) 919.
37 Lonberg-Holm, K., and Phillipson, L., eds. Virus Receptors. Chapman Hall, London 1981.
38 Carnegie, P.R., and Mackay, I.R., Lancet *2* (1975) 684.
39 Johnson, R.T., Brain *103* (1980) 447.
40 Lentz, T.L., Burrage, T.G., Smith, A., Crick, J., and Tignor, G.H., Science *215* (1982) 182.

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Monoaminergic neurons in the brain of goldfish as observed by immunohistochemical techniques

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Summary. With an immunofluorescent technique, catecholaminergic neurons were identified for the first time in the dorsal and medial thalamus and in the ventralis telencephali (the rostro-medial part of the lobus olfactorius) of the goldfish brain. Serotonin-containing neurons were found in the pretectal area.

The localization and distribution of monoaminergic neurons in the brains of lower vertebrates is important for the study of the phylogenetic development of such neuronal systems. Until now, most information about the central monoaminergic neurons of the vertebrates has been gathered by means of aldehyde-induced histofluorescent techniques[2-11]. These findings have been confirmed, mainly in higher vertebrates, by immunohistochemistry using antibodies against amine-synthesizing enzymes[12-14] and monoamines themselves[15]. Immunohistochemistry has revealed more extensive distribution of these monoaminergic neuron systems than was shown by histofluorescence. However, immunohistochemical information on monoaminergic neurons of the brains of lower vertebrates is almost lacking. Also, there are some discrepancies between the findings by the 2 methods in regard to monoamines contained in the neurons of the circumventricular organs of the frog[16,17].

In this paper, we report on the immunohistochemical localization of the monoaminergic neurons of the brain of the goldfish, especially of those neuron somata not hitherto detected in regions of the teleost brain, in comparison with the previously reported histofluorescent findings.

Materials and methods. Antiserum to bovine tyrosine hydroxylase (TH) was produced in rabbits and tested for its specificity as described previously[13]. Two kinds of rabbit antiserum to serotonin (5-HT) were used. One was purchased from Immuno Nuclear Corporation, Stillwater, Minn., USA, and the other was produced in our laboratory in rabbits using 5-HT coupled to bovine serum albumin (BSA) as antigen, according to the method of Steinbusch et al.[15,18]. The pre-immune serum and antiserum against BSA were used as controls. Control sera did not produce any specific staining in the brain sections of various animals. The 2 antisera to 5-HT produced specific 5-HT staining in brain sections of all the animals; similar results were obtained with the 2 antisera to 5-HT in dilutions of about 1:3000.

Brains of goldfish *(Carassius auratus)* were removed and fixed with Zamboni's solution (2% paraformaldehyde-0.2% picric acid in 0.1 M phosphate buffer. Subsequently they were processed for the indirect immunofluorescent staining. Details of the procedure were described earlier[12,16,17].

Results. At the level of the medulla oblongata, 2 distinct groups of TH-positive catecholaminergic neurons were observed in the reticular formation and in the post-obecular region. One cluster of catecholaminergic neurons extended bilaterally from the level of the rostral spinal region up to the middle portion of the expanded vagal lobe. The other group of catecholaminergic neurons was found to be located within the dorsal surface of the brain parenchyma of the post-obecular nucleus[9]. Processes of these neurons extended into the pia mater.

In the isthmic region of the upper rhombencephalon a small number of catecholaminergic neurons was found, bilaterally, at the ventral border of central gray. These neurons, situated dorso-laterally to the fasciculus longitudinalis medialis (FLM), were large in size but very few in numbers. At the same level of the isthmic tegmentum, 5-HT neurons were also seen in the midline portion between the FLM of both sides. This group of neurons, corresponding to the 5-HT neurons in brainstem raphé nuclei, could be followed up to the level of the caudal midbrain tegmentum. In contrast, no catecholaminergic neurons were found among the midbrain tegmentum.

In the diencephalon, a large number of monoaminergic neurons was found in close proximity to the ventricle. Hypothalamic 5-HT neurons, with processes reaching into the cerebro-spinal fluid (CSF) space, were clustered in the nucleus recessus posterioris (NRP) and the nucleus recessus lateralis (NRL) (fig. 1, b). TH-positive neurons were mostly situated within the brain parenchyma, and apparently had no contact with the CSF except for the rostro-medial portion of the NRL (the so-called paraventricular organ: PVO) (fig. 1, a). The most caudal catecholaminergic neurons of the hypothalamus were found in the nucleus posterior tuberis (NPT) lying rostromedially to the mammillary body. Some neurons were seen between the NRP and NRL in sagittal or horizontal sections (fig. 1, a). Their processes were not found to extend into the recessus posterioris and recessus lateralis. In contrast, the catecholaminergic neurons of the rostro-medial part of the NRL protrude with thick processes into the 3rd ventricle, together with the CSF-contacting 5-HT neurons of this nucleus (fig. 2, a and b). The NRP and the remaining part (ventro-

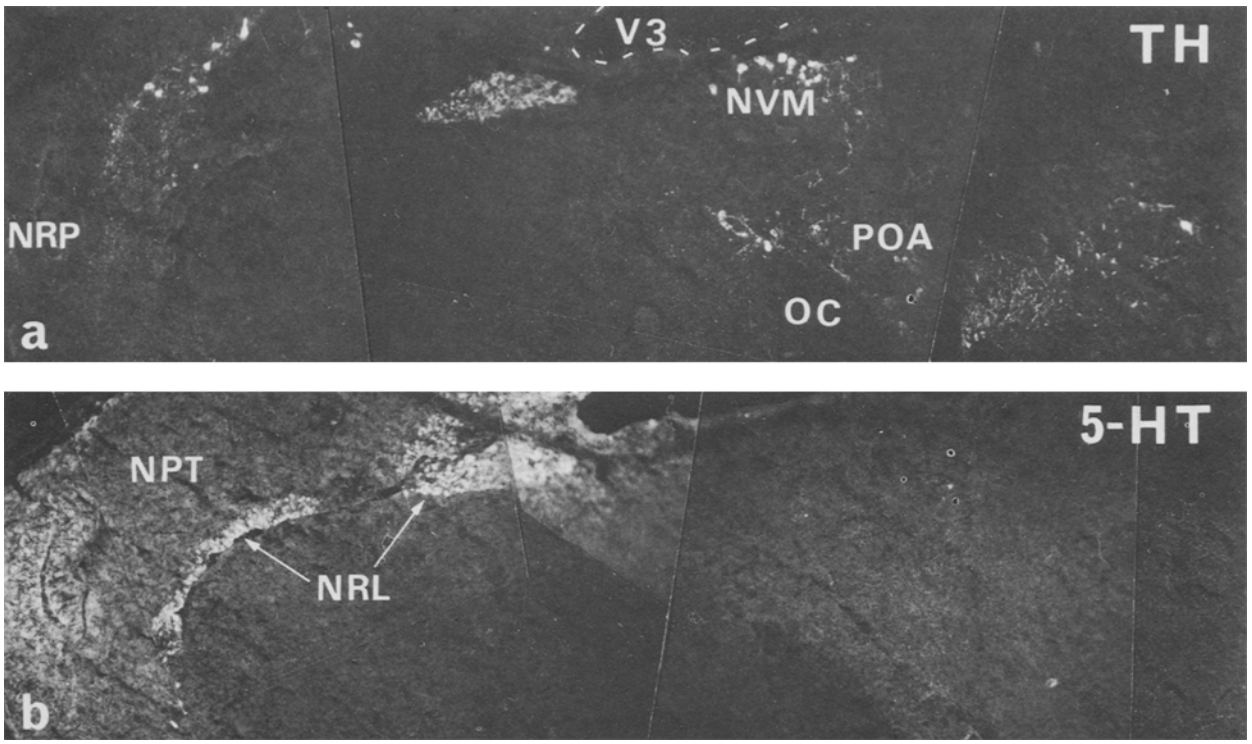

Figure 1. Immunofluorescent micrographs of TH- and 5-HT-positive neurons in serial sagittal sections of the diencephalon (right side is rostral, × 75). *a* TH-positive neurons were observed in the nucleus posterior tuberis (NPT), rostral portion of the nucleus recessus lateralis (NRL), the nucleus ventro-medialis thalami (NVM) and the preoptic area (POA). No specific TH-positive reaction was seen in the nucleus recessus posterioris (NRP) and caudal part of the NRL; *b* 5-HT-positive neurons were found in the NRP and almost all part of the NRL. Optic chiasma (OC), the 3rd ventricle (V3).

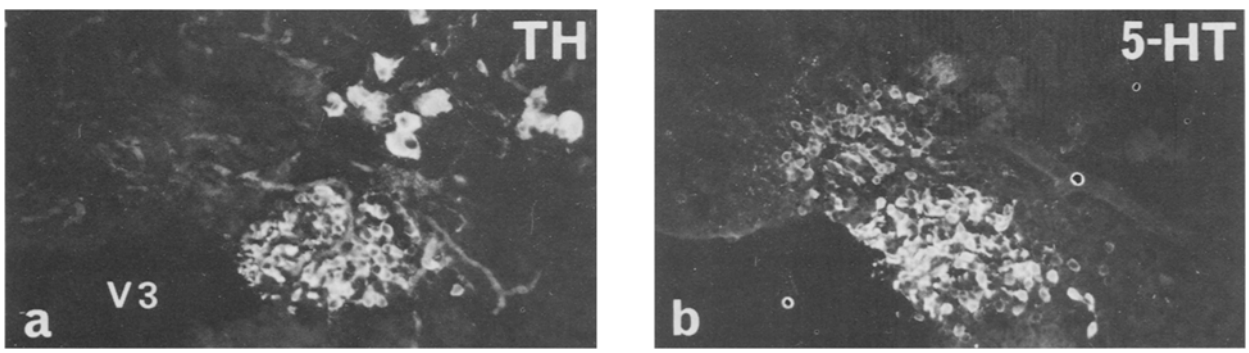

Figure 2. Immunofluorescent micrographs of the rostro-medial part of the NRL (so-called the paraventricular organ: PVO) in serial sagittal sections (right side is rostral, × 180). *a* TH-positive liquor contacting neurons and large-multipolar neurons (so-called the PVO-accompanying cells) were observed; *b* 5-HT-positive liquor contacting neurons were also found in this area but the PVO-accompanying cells were 5-HT-negative.

lateral part) of the NRL contained only 5-HT-containing neurons. Catecholaminergic neurons were also noticed around the PVO, namely the large multipolar PVO-accompanying cells[7] (fig. 2, a).

The thalamic region contained 2 distinct TH-positive cell groups, and 1 5-HT-containing cell group (fig. 1, a, fig. 3, a and b). One catecholaminergic neuron group was located lateral to the subcommissural organ (SCO) (which corresponded to the nucleus dorsolateralis thalami: NDL). These cells were found to lie just below the modified ependyma of the SCO, but not in contact with CSF (fig. 3, a). The other catecholaminergic neurons were found within the nucleus ventromedialis thalami (NVM)[19] (fig. 1, a); fibers from these neurons could be traced in the lateral and rostral directions. Thalamic 5-HT neurons were seen laterally to the NDL in the pretectal area[19] (fig. 3, b).

In the telencephalon, 2 groups of catecholaminergic neurons were observed; one in the preoptic area (POA) and another one in the nucleus olfactorius medialis (fig. 1, a, fig. 4, a and b). The catecholaminergic neurons of the preoptic nucleus were situated around the preoptic recess of the 3rd ventricle but did not extend into the ventricle. The other catecholaminergic neurons were found to be dispersed in the medial part of the lobus olfactorius, corresponding to the area ventralis telencephali pars dorsalis[19] (fig. 4, a and b). These neurons were medium-sized, bipolar

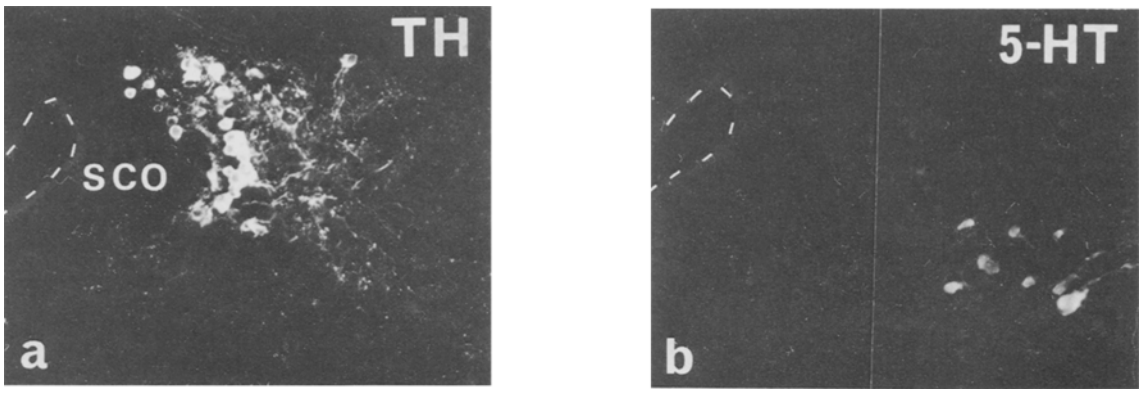

Figure 3. Immunofluorescent micrographs through the subcommissural organ (SCO) in serial transverse sections (× 180). *a* TH-positive neurons situated beneath the modified ependymal cells did not reach the ventricular lumen; *b* 5-HT-positive neurons were observed in the pretectal area ventro-lateral to the TH-positive neurons.

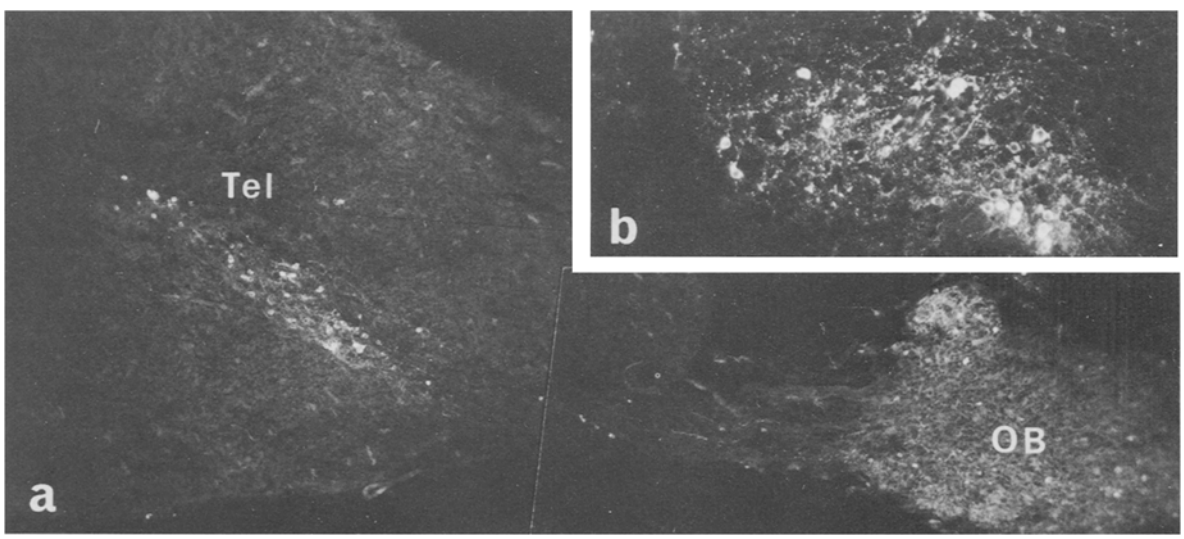

Figure 4. Immunofluorescent micrographs of TH-positive neurons of the telencephalon (Tel). *a* Sagittal section through the medial portion of the telencephalon and olfactory bulb. The fibers of TH-positive neurons in the medial olfactory area could be followed along the olfactory tract into the olfactory bulb (OB). A small number of neurons were also found among fibers between telencephalon and olfactory bulb (× 75); *b* High power magnification of these telencephalic TH-positive neurons in the transverse plane. These catecholaminergic neurons were oval in shape with fine varicose fibers (× 190).

or multipolar with oval perikarya; they formed bilateral bands reaching from the level of the anterior commissure to the rostral portion of the telencephalon. The fibers from these neurons appeared to project to the olfactory bulb (fig. 4, a).

In the olfactory bulb, a large number of catecholaminergic neurons were observed in the glomerular and mitral layers but only a few in the granular layer (fig. 4, a).

Discussion. In general, the distribution of central monoaminergic neuronal perikarya of the goldfish was found to be more extensive than that described for other teleosts and lamprey investigated by histofluorescent methods[2–11]. 'New sites' of catecholaminergic neurons found in the present immunohistochemical study are as follows; the nucleus dorsolateralis thalami, the nucleus ventromedialis thalami, and the area ventralis telencephali pars dorsalis according to the stereotaxic atlas of Peter and Gill[19]. For the first time, 5-HT neurons were found in the pretectal area.

The presence of green fluorescent cells in the posterior part of the telencephalon has been reported by Lefranc et al.[4,5] but this finding has not been confirmed so far[7–11]. In contrast to negative histofluorescent findings, our immunohistochemical study, using antibody to TH, also revealed the presence of catecholaminergic neurons in the rostro-medial part of the lobus olfactorius (fig. 4, a and b). The source of the varicose fibers from these catecholaminergic neurons could be traced into the olfactory tract up to the olfactory bulb (fig. 4, a). This finding suggests that some of the catecholaminergic fibers of telencephalon originate in somata located in the telencephalon itself.

When using the histofluorescence method, green- or yellow-fluorescent cells were found in the NRP and NRL; these cells were thought to be catecholamine- or 5-HT-containing ones, respectively[2,6,7,10,11]. In contrast, our immunohistochemical study indicates that TH-positive, i.e. catecholaminergic, neurons are located only within the rostro-medial portion of the NRL, not in the ventro-caudal part of the NRL nor in the NRP (fig. 1, a and b). This may suggest that monoamines are taken up from the CSF into the neurons of these area.

In addition, a small number of catecholaminergic neurons were observed in the NPT situated in the mesencephalo-

diencephalic junction (fig. 1, a). This region may correspond to the tuberculum posterius, which is incorporated into the bulk of the midbrain in higher vertebrates[6]. Therefore, it is of great interest, from a phylogenetic point of view, that a number of catecholaminergic neurons were found in the telencephalon (basal nuclei or paleostriatum) but a few catecholaminergic ones were noticed in the mesencephalodiencephalic junction.

1 Reprint requests should be addressed to I.N., Department of Anatomy, School of Medicine, Fujita-Gakuen University, Toyoake, Aichi 470-11 (Japan).
2 Baumgarten, H.G., and Braak, H., Z. Zellforsch. *80* (1967) 246.
3 Braak, H., and Baumgarten, H.G., Z. Zellforsch. *81* (1967) 416.
4 Lefranc, G., L'Hermite, A.L., and Tusque, J., C. r. Soc. Biol. Paris *163* (1969) 1193.
5 Lefranc, G., L'Hermite, A.L., and Tusque, J., C. r. Soc. Biol. Paris *164* (1970) 1629.
6 Baumgarten, H.G., Prog. Histochem. Cytochem. *4* (1972) 1.
7 Fremberg, M., van Veen, T., and Hartwig, H.G., Cell Tissue Res. *176* (1977) 1.
8 Parent, A., Dube, L., Braford, M.R., and Northcutt, R.G., J. comp. Neurol. *182* (1978) 495.
9 Watson, A.H.D., Cell Tissue Res. *208* (1980) 229.
10 Parent, A., and Northcutt, R.G., Brain Res. Bull. *9* (1982) 189.
11 Ochi, J., and Hosoya, Y., Histochemistry *40* (1974) 263.
12 Nagatsu, I., Acta histochem. cytochem. *7* (1974) 147.
13 Nagatsu, I., Kondo, Y., Inagaki, S., Karasawa, N., Kato, T., and Nagatsu, T., Acta histochem. cytochem. *10* (1977) 494.
14 Hökfelt, T., Johansson, O., Fuxe, K., Goldstein, M., and Park, D., Med. Biol. *54* (1976) 427.
15 Steinbusch, H.W.M., Neuroscience *6* (1981) 557.
16 Yoshida, M., Nagatsu, I., Kondo, Y., Karasawa, N., Ohno. T., Spatz, M., and Nagatsu, T., Experientia *38* (1982) 1097.
17 Nagatsu, I., Karasawa, N., Yoshida, M., Kondo, Y., Sato, T., Niimi, H., and Nagatsu, T., Biomed. Res. *3* (1982) 623.
18 Steinbusch, H.W.M., Verhofstad, A.A.J., and Joosten, H.W.J., Neuroscience *3* (1978) 811.
19 Peter, R.E., and Gill, V.E., J. comp. Neurol. *159* (1975) 69.

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Fatal interspecific mating of two *Heliothis* species induced by synthetic sex pheromone[1]

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Summary. Field tests showed that 3 components of the *Heliothis virescens* sex pheromone when deployed individually, significantly disrupt pheromone communication of males of this species and of *H. zea*. Field and wind tunnel tests indicated that males of *H. zea* are attracted to and mate with the females of *H. virescens* when the atmosphere is permeated with *(Z)*-9-tetradecenal or (*Z*)-11-hexadecen-1-ol. This mating is fatal to both individuals because of morphological incompatbility of their genitalia. Habituation of *H. zea* males to these compounds is the most likely reason for this attraction between 2 species that show reproductive isolation in nature.

The bollworm *(Heliothis zea)* and the tobacco budworm *(H. virescens)* are major pests of a large number of crops in the United States. The average annual loss due to crop damage plus cost of control for these 2 pests in the U.S. was estimated in 1976 to exceed 1 billion dollars[2]. Of comparable inportance are the environmental hazards associated with broad spectrum insecticides used to control these pests. Recent developments in the identification and synthesis of female sex pheromones of many pest insects have created great interest in their use in pest management as trap lures and as mating disruptants[3,4]. The 4 components of the *H. zea* female sex pheromone [(Z)-11-hexadecenal (Z-11-HDAL), *(Z)*-9-hexadecenal (Z-9-HDAL), *(Z)*-7-hexadecenal (Z-7-HDAL) and hexadecanal (HDAL)] are also

Table 1. Field evaluation of individual components of *Heliothis* spp. pheromones as mating disruptants and /or confusants for *H. zea* and *H. virescens* males by air permeation in cotton fields over a 9-day post-treatment period[a,b]

Treatment	$\bar{X}$ Number feral ♂♂ captured/trap/night in traps baited with:				Mating between tethered *Heliothis* spp. ♀♀ and feral *Heliothis* spp. ♂♂			
	H. zea ♀♀		*H. virescens* ♀♀		% *H. zea* ♀♀ mated to		% *H. virescens* ♀♀ mated to	
	H. zea ♂♂	*H. virescens* ♂♂	*H. zea* ♂♂	*H. virescens* ♂♂	*H. zea* ♂♂	*H. virescens* ♂♂	*H. zea* ♂♂	*H. virescens* ♂♂
Z-11-HDAL	0.03^{c}	0.00^{b}	0.00^{e}	0.58^{d}	1.56^{d}	0.00^{a}	0.00^{e}	7.19^{c}
Z-9-TDAL	$8.53^{b,**}$	0.64^{a}	$18.17^{a,*,**}$	3.03^{c}	38.75^{b}	0.00^{a}	18.75^{a}	15.10^{b}
TDAL	$9.64^{b,*,**}$	0.03^{b}	1.08^{d}	$8.83^{a,*,**}$	28.44^{c}	0.00^{a}	6.25^{c}	29.69^{a}
Z-11-HDOL	$10.72^{b,**}$	0.03^{b}	6.17^{b}	$5.53^{b,*}$	27.19^{c}	0.00^{a}	8.33^{b}	26.67^{a}
Untreated check	$13.44^{a,*,**}$	0.03^{b}	1.56^{c}	$10.61^{a,*,**}$	48.75^{a}	0.00^{a}	1.56^{d}	35.52^{a}

[a] Hypothesis on trap and mating table data were tested using ranks of counts or percentages rather than actual counts or percentages to address potential non-normality of data. [b] Ranks of means followed by different letters in the same column are significantly different at the 5% level of probability by Duncan's multiple range test. * Indicates significant difference between mean ranks between the numbers in columns 1 vs 3 or between the numbers in columns 2 vs 4 for each treatment. Pairwise t-test ($p < 0.05$). ** Indicates significant difference between mean ranks between the numbers in columns 1 vs 2 or between the numbers in columns 3 vs 4 for each treatment. Pairwise t-test ($p < 0.05$).