Asymmetrical Competition between *Aedes aegypti* and *Culex quinquefasciatus* (Diptera: Culicidae) in breeding sites with mixed cultures

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Aedes aegypti and *Culex quinquefasciatus* are mosquito vectors for several tropical diseases that represent a current public health problem. The ecological requirements for each species are different and both species show high biological adaptability, however, they can be found in the same breeding sites. The purpose of this study was to assess the effect of asymmetrical competition between *Ae. aegypti* and *Cx. quinquefasciatus* on the larval development cycle. Our findings show that under field simulation conditions, temperature, space, sun protection and food were adequate for larval development and there was no asymmetrical competition between *Ae. aegypti* and *Cx. quinquefasciatus* mixed cultures in different *Cx. quinquefasciatus/ Ae. aegypti* ratios. Otherwise, under laboratory conditions in which different doses of food were evaluated, it was observed that competition between the two species takes place. Larval association where food was scarce (0.95 mg/larva) showed that *Ae. aegypti* had a higher adult emergence than *Cx. quinqueasciatus* and was capable of depriving *Cx. quinquefasciatus* of the food it needs to develop. In an intermediate dose of food (1.9 mg/larva), *Cx. quinquefasciatus* adult weight decreases and larval development time increases when *Cx. quinquefasciatus/ Ae. aegypti* ratio was low. Also, temperature effect was assessed demonstrating that *Cx. quinquefasciatus* was more vulnerable to changes in temperature. We propose that *Ae. aegypti* is more successfully in exploiting microhabitats when food is scarce, due to its scrape active feeding habitats and rapidly larval development. Therefore, in conditions of food paucity both species will compete, and *Ae. aegypti* will prevail.

Keywords: *Aedes aegypti*; *Culex quinquefasciatus*; Interespecific competition.

1. Introduction

Aedes aegypti and *Culex quinquefasciatus* are mosquito vectors for several tropical diseases that represent a current public health problem: Malaria, zika fever, yellow fever, chikungunya, West-Nile fever, filariasis, and encephalitis (Morrison *et al.*, 2008; Hill & Conelly, 2009). Both species tend to colonize mainly urban and suburban areas in tropical countries where the climate conditions suit the development of these vectors (Rueda *et al.*, 1990) and can be found sharing the same breeding sites, despite the differences in ecological requirements for each species: *Cx. quinquefasciatus* have a predilection for polluted waters rich in organic matter, whereas *A. aegypti* have a preference for clean and stagnant waters (Rozeendal, 1997); with few exceptions (Barrera *et al.*, 2008; Burke *et al.*, 2010).

Several studies have shown a high biological adaptability for both species. Chadee *et al*. (1998) reported multiple breeding sites for *Ae. aegypti* including rock holes, tree holes, leaf axils, ground pools and tires among others. Powell & Tabachnick (2013), reported at least twelve populations distributed over Africa, Asia and the Americas, demonstrating that the species is adaptively flexible and maintains significant genetic variation. Furthermore, *Ae. aegypti* is an opportunistic feeder, capable of rapidly responding to changes in environments and possess egg dormancy (Powell & Tabachnick 2013).

On the other hand, *Cx. quinquefasciatus* has been reported as an opportunistic feeder that takes blood from a wide range of mammals and birds (Molaei *et al.,* 2007), has olfactory memory, a mechanism that would provide the most accurate site indicator to a gravid female mosquito (McCall & Eaton, 2001), and proliferates in urban areas where blocked drains, pools collected from run-off domestic sewage and pit latrines provide ideal larval environments; therefore, it is a species considered tolerant to extreme

values of pH, high organic material, and high salinity (McCall & Eaton, 2001). Also, *Cx. quinquefasciatus* survives from eclosion to adult emergence in temperatures range from 15-34°C (Rueda *et al.,* 1990).

Due to this shared trait, *Ae. aegypti* and *Cx. quinquefasciatus* are species of epidemiological importance, which distributions overlap in much of tropics (Farajollah *et al*., 2005; Kraemer *et al*., 2015). The larvae of both species can occur in natural and artificial containers but differ in feeding modes. *Ae. aegypti* have a shredding feeding mode whereas *Cx. quinquefasciatus* have a collecting-filtering feeding mode (Merritt *et al.,* 1992). Hence, the importance of making studies where the interaction between the two species can be evaluated is indispensable. Moreover, biological control directed to mixed populations, also requires information about this interaction under different environmental variables that can be affecting the development and survival of mosquitoes larvae. With the aim of designing population and control strategy models, in this study we evaluated the differences in preimaginal development of *Ae. aegypti* and *Cx. quinquefasciatus* under different laboratory conditions of food and temperature and under field simulated conditions.

2. Materials and Methods

Larvae collection

Field *Ae. aegypti* and *Cx. quinquefasciatus* larvae were collected at San Joaquin, La Mesa, Cundinamarca, Colombia (4°38'24.1"N 74°31'17.9"W), from artificial containers filled with rain water located in suburban areas of the municipality. It was taken into account that the larvae collected came from containers where the two species were in coexistence.

Field Simulation Bioassays

In order to assess the differences in preimaginal development and competition for food between *Ae. aegypti* and *Cx. quinquefasciatus* in natural simulated conditions of development, we carry out bioassays at San Joaquin, La Mesa, Cundinamarca, Colombia in a place near the larvae collection site (4°38'25.1"N 74°31'16.7"W). Fifteen third-instar larvae of each species were placed into plastic containers with water filtration capacity according the following competition ratios *Cx. quinquefasciatus/Ae. aegypti* 0:1, 1:1 and 1:0. Three plastic containers were placed in a larger plastic container with 9 liters of rain water collected from the larvae collection site and placed in an open spot. Temperature was registered during the entire experiment, as well as adult production rate and body weight. Pupae were separated before imaginal ecdysis, and the adults obtained were weighed (KERN 770 v2.3).

Laboratory bioassays

Bioassays were carried out according to Carrieri *et al.* (2003). Briefly, *Ae. aegypti* and *Cx. quinquefasciatus* larvae collected were placed in a colony with conditions of 30°C at 70% of relative humidity with light periods of 16:8 L: D. Then, adults were fed with lamb's blood and the eggs obtained from F1 were used in our laboratory experiments. Trials were carried out in a climate-controlled chamber set at 75% RH; 16:8 L:D.

The eggs of both species were placed in separate containers with dechlorinated water and cat biscuits. Pools of first-instar larvae were separated and placed in 250 mL glass containers containing 200 ml dechlorinated water. Three food doses (cat biscuits) were tested at 30°C: 2.83, 1.9, and 0.95 mg/larva.

The influence of temperature was studied by comparing larval competition at 22°C and

30°C using the intermediate food dose of 1.9 mg/larva, which had been previously reported as being the most appropriate dosage for showing competition (Carrieri *et al.,* 2003).

The development time, body weight, and adult production rate corresponding to each temperature and food dose were quantified to establish the effect of competition between the two species at the following ratios of *Cx. quinquefasciatus/Ae. aegypti* larvae: 1:0; 2:1; 1:1; 1:2, and 0:1. Fifteen larvae were used as one unit in all the ratios (e.g. 2:1 were 30 *Cx. quinquefasciatus* larvae and 15 *Ae. aegypti* larvae).

To prevent the formation of a film on the water surface that could be dangerous to the larvae, during each of the first 6 days of larval development, the food was supplied in doses proportional to age, i.e., 10% on the first and second day, 15% on the third, 21% on the fourth, and 22% on the fifth and sixth day. Pupae were separated before imaginal ecdysis, and the adults obtained were placed at -20°C, then dried at 80°C for 24h and finally weighed (KERN 770 v2.3).

Statistical Analysis

The R v3.1.1 software was used for statistical analysis (R Core Team, 2016). Data was analyzed by Analysis of variance (ANOVA) followed by the Tukey-Kramer test to separate averages. No normal data was analyzed by Kruskal-Wallis test, followed by Mann–Whitney *U* test to separate averages in cases were significant differences were found.

Competition between the two species was measured using the relative crowding coefficient (RCC) as described by Harper (1977) and modified by Novak *et al*. (1993) and Oberg *et al*. (1996):

$$RCCs = \left\{ \frac{\frac{1}{2} * \left(\frac{Ae^{2:1}}{Cx^{2:1}}\right) + \left(\frac{Ae^{1:1}}{Cx^{1:1}}\right) + 2 * \left(\frac{Ae^{1:2}}{Cx^{1:2}}\right)}{3} \right\} / \left(\frac{Ae^{1:0}}{Cx^{1:0}}\right)$$

When RCC> 1, competition favors *Ae. aegypti,* and vice versa, if RCC< 1, *Cx. quinquefasciatus* prevails.

3. Results

Data obtained in field simulation experiments showed no significant differences in adult production rate of the two species between the three *Cx. quinquefasciatus/ Ae. aegypti* ratios evaluated (Table 1). In addition, means in adult wet weigh showed no significant differences between the ratios evaluated (Table 2). Together these results indicate that under field simulated conditions, no asymmetrical competition can be observed between both species, allowing their coexistence in the same breeding sites.

In regard to laboratory experiments, development time at food dose of 2.83 mg/larva showed no significant differences between *Ae. aegypti* development times on the different *Cx. quinquefasciatus/ Ae. aegypti* ratios evaluated. With food dose of 0.95 mg/larva at 2:1 and 1:1 ratios, *Ae. Aegypti* showed minor times of development, indicating a higher intraspecific vs. interspecific competition. However, with food dose of 1.9 mg/ larva at 2:1 and 1:2 ratios, *Ae. aegypti* showed minor times of development (Table 3). Presumably, *Ae. aegypti* larvae developed earlier in these treatments, because there was a greater availability of resources. Although the feed was supplied in mg / larva, these ratios had a higher number of larvae, showing that the larvae of *Ae. aegypti* can better exploit the resources that were initially intended for both species. Finally, at 1.9 mg/larva (22°C) we found significant differences between ratios tested, but it seems unclear what

effect does competition and temperature have on development time at this dose, according to the proportions evaluated.

In the case of *Cx. quinquefasciatus*, no significant differences were found in the dose of 2.83 mg/larva (Table 3). At the dose of 0.95 mg/larva, no significant differences were found either, but in two of the proportions evaluated (1: 1 and 1:2) where the number of individuals of *Cx. quinquefasciatus* with respect to the number of individuals of *Ae. aegypti* was greater, there was no development of individuals (Table 3). In addition, development times at 1.9 mg/larva showed significant differences, with significant minor development times for *Cx. quinquefasciatus* individuals where *Ae. egypti* individuals were at minor or equal ratios (1:0, 1:1, 2:1) (Table 3). At food dose of 1.9 mg/larva (22°C) we found that at 1:0 ratio, development time was significantly the largest of all. We assume that these larvae isolated from *Ae. Aegypti* do not have a selection pressure and therefore there will not be a significant advantage in developing faster. Thus, longer development times and a higher percentage of survivors could be found (Tables 3,4) Moreover, we found that development times of *Ae. aegypti* were always shorter than *Cx. quinquefasciatus* development times, although both species were affected by the availability of resources. However, the temperature affect further the development time of *Ae. aegypti* (Table 6).

Regarding to adult production rate, *Ae. aegypti* showed no significant differences in food dose of 1.9 mg/larva, 2.83 mg/larva and 1.9 mg/larva (22°C) among the ratios evaluated (Table 4). At dose 0.95 mg/larva, we found significant differences between *Cx. quinquefasciatus/Ae. aegypti* ratios, showing that yet again higher intraspecific vs. interspecific competition could be observed, due to the highest rates of adult production found at ratios 2:1 and 1:1 (Table 4).On the other hand, *Cx. quinquefasciatus* at dose of 0.95 mg/larva, showed no adult production in ratios where there were more or equal

individuals of *Ae. aegypti* (1:2, 1:1); and at a dose 1.9 mg/larva we found significant differences between the ratios assessed, but it seems unclear what effect does competition have on adult production at this dose. Further, dose of 1.9 mg/larva (22°C), showed that in ratios where there were more or equal individuals of *Ae. aegypti* (1:2, 1:1), there was minor rates of adult production. However, dose of 2.83 mg/larva, did not show significant differences. Moreover, overall adult production rates showed that food scarcity affects more *Cx. quinquefasciatus* percentage adult emergence than *Ae. aegypti* percentage (Table 6).

Regarding adult weight, *Ae. aegypti* showed at dose 0.95 mg/larva, 1.9 mg/larva and 1.9 mg/larva (22°C) a statistically significant increase in weight, directly related to the increase in *Cx. quinquefasciatus/Ae. aegypti* ratio (Table 5, Fig. 1). Whereas *Cx. quinquefasciatus* showed at those same doses, an increase in weight, inversely related to the increase in the *Cx. quinquefasciatus/Ae. aegypti* ratio (Fig. 1). At dose 2.83 mg/larva no correlation or significant differences were found for both species (Table 5, Fig. 1), therefore implying that in cases of food shortage or under intermediate resource conditions *Ae. aegypti* biomass is negatively affected by the presence of individuals of the same species rather than *Cx. quinquefasciatus* individuals. For *Cx. quinquefasciatus* we found that the biomass was negatively affected by the presence of *Ae. aegypti* individuals rather than individuals of the same species. Furthermore, mean weight of adults at different doses, showed that although individuals of *Cx. quinquefasciatus* weigh more when there is an extensive availability of resources (2.83 mg/larva), under conditions of scarcity there were no differences in the weight of individuals between both species or *Ae. aegypti* tend to be heavier; which would indicate *Ae. aegypti* has a better efficiency in converting food into biomass (Table 6).

Measures using relative crowding coefficient (RCC) showed that in all doses tested, competition favors *Ae. aegypti*, even though in cases where there was a wide availability of resources. Apparently low competition was observed in 0.95 mg/larva, which is explained by the modifications to the formula that were made by the lack of data in ratios 1:2 and 1:1 (Table 7).

4. Discussion

The main findings in this project show that under simulated field conditions, food, temperature, space and sun exposition were adequate for larvae development and no asymmetrical competition was observed between both species, allowing their coexistence in the same breeding sites.

Rueda *et al.* (1990) reported that at 30°C, *Cx. Quinquefasciatus* individuals, showed low weight gain and *Ae. aegypti* individuals showed low rates of adult production. In particular, our field simulated conditions showed that temperature was 30.16 ±1.70 °C, but contrary to expectations none of these biological parameters were affected by this temperature or the co-occurrence of the two species in the same breeding site. Likewise, biological parameters previously reported as optima at this temperature (development time, weight of *Ae. aegypti* and survival rate of *Cx. Quinquefasciatus*) were also not affected, since no differences were found when the larvae were isolated from the other species.

Studies on larval development for both species (Tun-lin *et al*, 2000; Suleman, 1982; Southwood *et al*, 1972) showed that competition for scarce food resources appeared to be more important than temperature in influencing *Ae. aegypti* development in the field, also food shortage affects more development in *Cx. quinquefasciatus* rather than density. Therefore, based on the fact that the larvae were in an under shed container with

9 liters of rainwater, reported as a successful container in larvae development (Tun-lin *et al*, 2000), given its wide content in organic matter, no competition for food was observed as expected. Besides, the volume of water per larva (approximately 12 mL per larva) has been reported as more than enough to avoid the density-dependent effect on larval development, which explains why there is no competition for space in our experiments. Nevertheless, larval mortality may be more influenced in the early larval stages by temperature and food shortages (Southwood *et al*, 1972), so our experiments in field simulation may have this bias.

Our experiments in field simulation conditions allow us to state that apparently, under conditions of wide availability of resources, a temperature of ±30°C, space and sun protection, no asymmetric competition is observed. However, studies are needed to evaluate competition between the two species in locations with different temperatures, space and protection of the sun, as well as to quantify the available food in detail, to clarify how the dynamics in the field can be. In addition, we suggest to evaluate under field conditions the effect of different immature larval stages of both species on the asymmetrical competition.

Results under laboratory conditions, at 30°C and 22°C, showed that *Ae. aegypti* is more successful than *Cx. quinquefasciatus* in exploiting artificial microhabitats either when food is scarce (0.95 mg/larva) or at intermediate concentration (1.95 mg/larva). Thus, in conditions of food paucity, the two species are brought into competition, and *Ae. aegypti* is capable of depriving *Cx. quinquefasciatus* of the food it needs to develops. As a consequence, an increase in the time of development and a decrease in the overall weight of *Cx. quinquefasciatus* were found (Tables 3, 4, 5). Further, RCCs measured in our experiments support this assumption (Table 7).

Change in temperature caused that *Ae. aegypti* time of development increased more in relation to the increase in time measured for *Cx. quinquefasciatus*. However, *Ae. aegypti* time of development at 22°C was lower than the time for *Cx. quinquefasciatus*. Besides, *Cx. quinquefasciatus* percentage of emergent adults and weight of adults were affected to a greater extent by the change in temperature (Table 6), suggesting that even though *Cx. quinquefasciatus* survives from eclosion to adult emergence in a wide range of temperatures, only certain temperatures are most likely to develop and be successful (Rueda *et al.,* 1990). Moreover, despite *Ae. aegypti* has a narrower surviving range of temperatures, it seems that in ranges that survives it is more efficient at converting food into biomass and having higher survival rates.

We suggest these results mean asymmetrical competition between both species in conditions of food paucity and apparently in a range of temperature of 22-30°C. Intrinsic factors of each species may explain the asymmetry of this competition. First, *Ae. aegypti* rather than *Cx. quinquefasciatus* uses an active feeding mode: Shredding. The shredding feeding mode in larval mosquitoes consists of chewing, biting, or gnawing off small fragments from coarse particulate organic matter such as leaves, filaments of macroalgae, or other plant parts. Yet these larvae may also feed off of dead invertebrates, often of their own kind (Merrit *et al,* 1992). Instead, *Cx. quinquefasciatus* is considered a passive collector-filter, a feeding mode that be defined as the removal of particulate organic material from suspension (Merrit *et al,* 1992). Second, differences found in time of the larval development for both species, being *Ae. aegypti* capable of faster developing times (our results and Rueda *et al.,* 1990; Tun-lin *et al,.* 2000). Third better efficiency converting food into biomass in *Ae. Aegypti* presumably due to its exponential lipogenesis (Timmerman & Briegel, 1999).

Additionally, we found an interesting pattern in the weight of adults for both species according to the amount of individuals of the same species when food is limited. In the case of *Ae. aegypti*, we found an inverse relationship between biomass and *Ae. aegypti/Cx. quinquefasciatus* ratio, which would indicate a higher intraspecific vs. interspecific competition. In the other hand, for *Cx. quinquefasciatus* we found a direct relationship between biomass and the ratio *Cx. quinquefasciatus / Ae. Aegypti*, indicating a higher interspecific vs. intraspecific competition (Fig 1 A, B, D, E, F, H). Besides, the slopes are showing how strong the effect of intraspecific or interspecific competition is, and also demonstrating the effect of food shortage.

Previous studies, have shown how *Ae. aegypti* is an opportunistic species which can easily colonize empty niches (Powell & Tabachnick, 2013). Therefore, according to our results there seem to be no obstacles to the spread of *Ae. aegypti* deriving from competition with the species *Cx. quinquefasciatus*, at least in conditions of food paucity and a temperature range of 22-30°C. Nevertheless, additional field studies are needed in this regard, to elucidate how dynamics can vary if the environmental conditions change.

5. Acknowledgements

6. References

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Table 1. Effect of competition on the adult percent adult emergence on simulated field conditions

Cx. quinquefasciatus/ Ae. aegypti Ratio	N	*Ae. aegypti* (Percent adult emergence ±SD)	*Cx. quinquefasciatus* (Percent adult emergence ±SD)
1:0	6		0.90±0.06a
1:1	6	0.92±0.08a	0.92±0.08a
0:1	6	0.94±0.08a	

Means within a column followed by the same letter are not significantly different according to Student t-test.

Table 2. Effect of competition on the adult wet weight means (mg) on simulated field conditions

Cx. quinquefasciatus/ Ae. aegypti Ratio	N	*Ae. aegypti* (Adult weight ± SD)			*Cx. quinquefasciatus* (Adult weight ± SD)		
		Female	Male	Total	Female	Male	Total
1:0	6				1,00±0,01a	0,81±0,05a	0,94±0,03a
1:1	6	0,94±0,05a	0,83±0,03a	0,91±0,03a	1,02±0,05a	0,79±0,07a	0,94±0,05a
0:1	6	0,93±0,04a	0,82±0,07a	0,90±0,05a			

Means within a column followed by the same letter are not significantly different according to Student t-test.

Table 3. Effect of competition on the days from egg eclosion to adult emergence

Cx. quinquefasciatus/ Ae. aegypti Ratio	N	Ae. aegypti development time (days from egg immersion to adult emergence ± S.D.)			Cx. quinquefasciatus development time (days from egg immersion to adult emergence ± S.D.		
		Female	Male	Total	Female	Male	Total
0.95 mg/larva							
1:0	3				12±0.00a	13±0.00a	12.33±0.57a
2:1	3	9.81±0.06 a	9.33 ±0.25a	9.63±0.59a	14±0.00a	13.67±0.57a	13.66±0.57a
1:1	3	11.25±1.24ab	10.26±0.50ab	10.68±0.73ab			
1:2	3	11.93±0.14ab	11.72±0.29ab	12.08±0.02b			
0:1	3	12.67±1.15b	12.33±1.89ab	12.27±1.00b			
1.9 mg/larva							
1:0	3				9.25±0.25a	8.24±0.21a	8.76±0.35a
2:1	3	6.02±0.22a	5.58±0.14a	5.71±0.64a	9.94±0.46a	8.58±0.20ab	9.23±0.15a
1:1	3	8.01±0.27b	8.14±0.43b	8.26±0.54b	9.44±0.50a	8.28±0.28a	8.68±0.29a
1:2	3	6.40±0.14a	6.14±0.01c	6.12±0.23a	10.44±0.59a	9.41±0.52b	9.97±0.25b
0:1	3	8.41±0.13b	8.05±0.28b	8.25±0.14b			
2.83 mg/larva							
1:0	3				9.52±0.14a	8.89±0.11a	9.19±0.20a
2:1	3	8.06±0.10a	7.85±0.43a	7.91±0.22a	9.43±0.24a	8.48±0.24a	8.93±0.15a
1:1	3	7.65±0.36a	7.32±0.15a	7.28±0.29a	9.87±0.64a	8.53±0.54a	9.29±0.61a
1:2	3	8.00±0.61a	7.48±0.38a	7.67±0.47a	9.74±0.12a	8.16±0.17a	9.00±0.18a
0:1	3	8.40±0.10a	7.74±0.13a	8.05±0.16a			
1.9 mg/larva (22°C)							
1:0	3				17.80±1.14a	16.51±0.81a	17.07±0.40a
2:1	3	16.73±0.38a	15.32±0.39a	16.01±0.55a	16.35±1.03ab	14.36±0.41b	15.43±0.60b
1:1	3	13.87±0.74b	13.68±0.59b	13.81±0.52b		15.45±0.51ab	15.45±0.51b
1:2	3	16.73±0.46a	14.90±0.70ab	15.61±0.45a	14.50±0.71b	14.67±0.87b	14.74±0.79b
0:1	3	14.89±0.99b	15.15±0.32a	15.35±0.17a			

Means within a column followed by the same letter are not significantly different according to Tukey-Kramer test or Mann–Whitney U test in case of no normality. Student t-test was used in case of 2 treatments

Table 4. Effect of competition on the percent adult emergence

Cx. quinquefasciatus/ *Ae. aegypti* ratio	N	*Ae. aegypti* (Percent adult emergence ±SD)	*Cx. quinquefasciatus* (Percent adult emergence ±SD)
0.95 mg/larva			
1:0	3		0.18±0.04a
2:1	3	0.80 ±0.02a	0.18±0.02a
1:1	3	0.78±0.10a	
1:2	3	0.54±0.07b	
0:1	3	0.24±0.04c	
1.9 mg/larva			
1:0	3		0.62±0.03 a
2:1	3	0.97±0.04a	0.93±0.03b
1:1	3	0.93±0.07a	0.6±0.11a
1:2	3	0.95±0.08a	1b
0:1	3	0.84±0.10a	
2.83 mg/larva			
1:0	3		0.96±0.04a
2:1	3	0.87±0.07a	0.97±0.03a
1:1	3	0.93±0.11a	0.91±0.04a
1:2	3	0.93±0.06a	0.96±0.04a
0:1	3	0.93±0.07a	
1.9 mg/larva (22°C)			
1:0	3		0.84±0.04a
2:1	3	0.87±0.00a	0.74±0.02a
1:1	3	0.89±0.04a	0.42±0.08b
1:2	3	0.88±0.05a	0.64±0.20ab
0:1	3	1.00±0.00a	

Means within a column followed by the same letter are not significantly different according to Tukey-Kramer test or Mann–Whitney *U* test in case of no normality. Student t-test was used in case of 2 treatments

Table 5. Effect of competition on the adult weight means (mg)

Cx. quinquefasciatus/ Ae. aegypti ratio	N	Ae. aegypti (Adult weight ± SD)			Cx. quinquefasciatus (Adult weight ± SD)		
		Female	Male	Total	Female	Male	Total
0.95 mg/larva							
1:0	3				0.42±0.03a	0.37±0.01a	0.40±0.01a
2:1	3	0.58±0.07a	0.37±0.03a	0.47±0.03a	0.37±0.03a	0.33±0.01b	0.35±0.01b
1:1	3	0.41±0.06b	0.28±0.05b	0.35±0.03b			
1:2	3	0.25±0.02c	0.19±0.01c	0.22±0.02c			
0:1	3	0.35±0.05bc	0.16±0.01c	0.25±0.03c			
1.9 mg/larva							
1:0	3				0.61±0.03a	0.34±0.02a	0.48±0.02a
2:1	3	0.71±0.03a	0.40±0.02a	0.56±0.02a	0.42±0.06b	0.36±0.03a	0.39±0.04b
1:1	3	0.62±0.04b	0.39±0.04a	0.50±0.03b	0.56±0.05a	0.32±0.04a	0.44±0.04ab
1:2	3	0.55±0.01c	0.37±0.01a	0.46±0.01bc	0.54±0.04ab	0.38±0.06a	0.46±0.01ab
0:1	3	0.49±0.01c	0.37±0.01a	0.43±0.01c			
2.83 mg/larva							
1:0	3				0.64±0.04a	0.39±0.02a	0.52±0.03a
2:1	3	0.86±0.06a	0.52±0.05a	0.69±0.01a	0.63±0.06a	0.40±0.02a	0.51±0.03a
1:1	3	0.75±0.07a	0.45±0.04a	0.60±0.03a	0.63±0.06a	0.37±0.04ab	0.50±0.04a
1:2	3	0.75±0.05a	0.48±0.05a	0.61±0.05a	0.67±0.04a	0.31±0.02b	0.49±0.02a
0:1	3	0.74±0.05a	0.42±0.02a	0.58±0.01a			
1.9 mg/larva (22°C)							
1:0	3				0.29±0.01a	0.23±0.02a	0.26±0.02ab
2:1	3	0.71±0.08a	0.48±0.07ab	0.60±0.07a	0.35±0.06a	0.30±0.05a	0.32±0.06b
1:1	3	0.89±0.10b	0.54±0.07b	0.52±0.03b		0.23±0.02a	0.23±0.02a
1:2	3	0.6±0.04a	0.43±0.01ab	0.51±0.03b	0.30±0.00a	0.30±0.04a	0.30±0.03ab
0:1	3	0.56±0.02a	0.34±0.05a	0.45±0.02c			

Means within a column followed by the same letter are not significantly different according to Tukey-Kramer test or Mann–Whitney *U* test in case of no normality. Student t-test was used in case of 2 treatments

Table 6. Comparison between some biological parameters (±SD) of *Ae. aegypti* and *Cx. quinquefasciatus* at different food doses

	0.95 mg/larva	1.9 mg/larva	2.83 mg/larva	1.9 mg/larva (22°C)
Percent adult emergence				
Ae. aegypti	0.59±0.24a	0.92±0.08a	0.92±0.07a	0.91±0.06a
Cx. quinquefasciatus	0.18±0.03b	0.79±0.19b	0.94±0.04a	0.66±0.19b
Days from egg immersion to adult emergence				
Ae. aegypti	11.17±1.27a	7.10±1.29a	7.73±0.40a	15.20±0.95a
Cx. quinquefasciatus	13±0.89b	9.16±0.59b	9.10±0.33b	15.67±1.03a
Mean adult weight (mg)				
Ae. aegypti	0.32±0.11a	0.49±0.05a	0.51±0.03a	0.56±0.11a
Cx. quinquefasciatus	0.38±0.03a	0.44±0.04b	0.62±0.05b	0.28±0.05b

Means within a column followed by the same letter are not significantly different according to Student t-test.

Table 7. *Cx. quinquefasciatus* and *Ae. aegypti* Relative Crowding Coefficient (RCC). When RCC> 1, competition favors *Ae. aegypti,* and vice versa, if RCC<1, *Cx. quinquefasciatus* prevails.

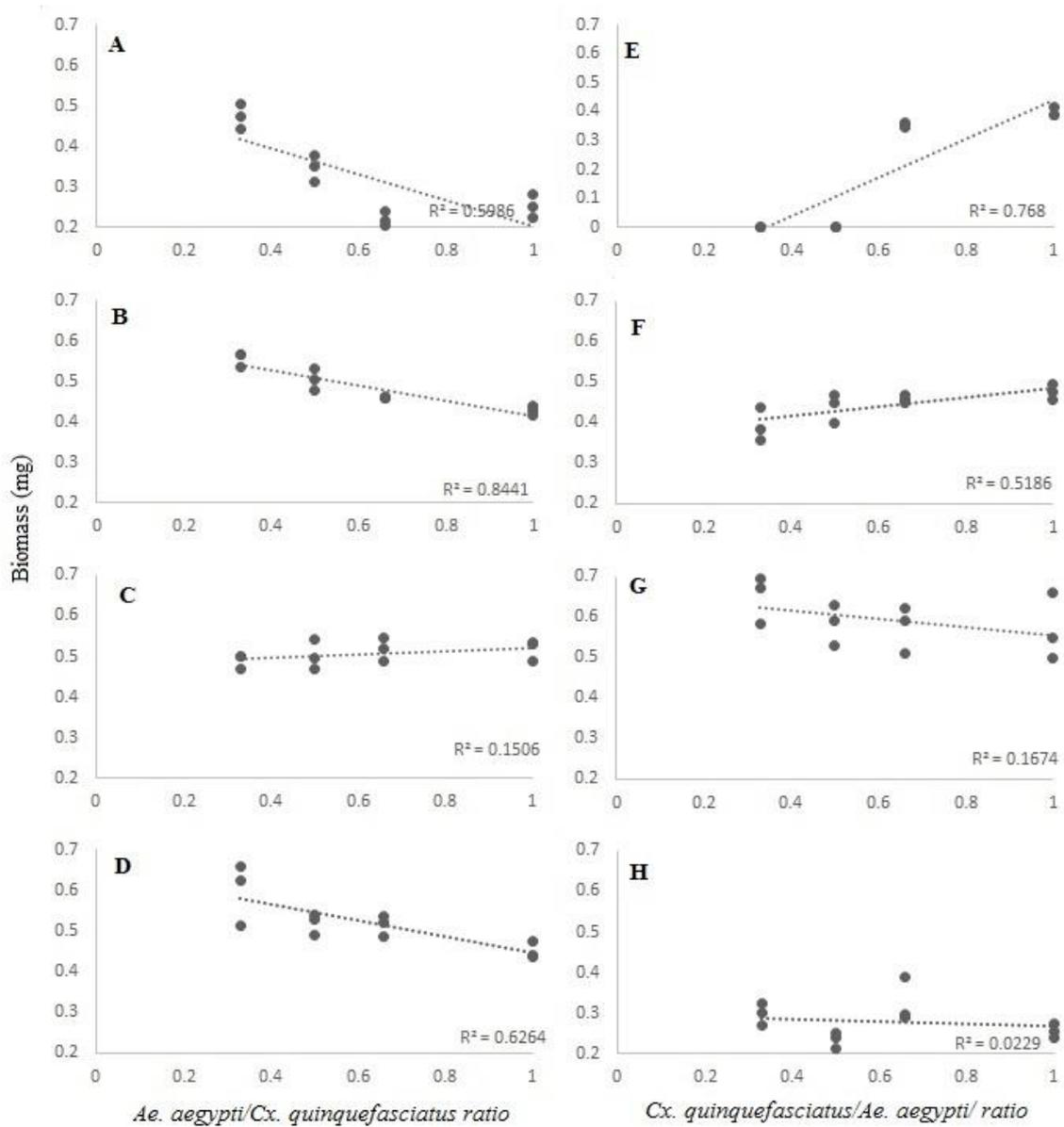| Food Dose	Relative Crowding Coefficient
0.95mg/larva	1.085547776
1.9 mg/larva	1.601418242
2.83 mg/larva	1.305095523
1.9 mg/larva (22°C)	1.40680578

Figure 1. Relationship between adult biomass produced and different competition ratios: (A) *Ae. aegypti* adults with food dose 0.95 mg/larva. (B) *Ae. aegypti* adults with food dose 1.9 mg/larva. (C) *Ae. aegypti* adults with food dose 2.83 mg/larva. (D) *Ae. aegypti* adults with food dose 1.9 mg/larva at 22°C. (E) *Cx. quinquefasciatus* adults with food dose 0.95 mg/larva (F) *Cx. quinquefasciatus* adults with food dose 1.9 mg/larva (G) *Cx. quinquefasciatus* adults with food dose 2.83 mg/larva (H) *Cx. quinquefasciatus* adults with food dose 1.9 mg/larva at 22°C.