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Effect of Diet On Symbiont Density Within An Invasive Agricultural Pest

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By

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B.S., Brown University, 2009

Advisor: Nicole Gerardo

An abstract of
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Abstract

Effect of Diet On Symbiont Density Within An Invasive Agricultural Pest

By Alexander Chang

Many insects are host to microbial symbionts that often play a pivotal role in shaping the insects' abilities to utilize resources. Symbioses can be context-dependent, as different biotic and abiotic factors can alter the relationship between symbiont and host. Because of these two factors, the symbionts of invasive insects are important for study as their host ranges can be dictated by their symbionts, and their relationships with their symbionts can be altered by the novel conditions invasive organisms must face. Here, we investigate the relationship between diet and within-host symbiont populations in the stinkbug *Megacopta cribraria*, which invaded North America in 2009. To examine the host-symbiont association in its new environment, we reared *M. cribraria* on two alternative host plants, kudzu vines (*Pueraria lobata*) and soybeans (*Glycine max*), and measured within-host symbiont densities at various stages of development. We found that juveniles and adult females, but not adult males, reared on soybean had significantly lower symbiont densities than those raised on kudzu. The lowered fitness of the symbiont in insects reared on soybean is surprising as the symbiont is genetically similar to the Japanese strain which confers host usage of soybean; it may be that the soybean in the United States differs from that of Japan. Additionally, the lowered symbiont densities found in adult females reared on soy may impact symbiont transmission, which in turn would limit the potential range for *Megacopta cribraria* in the United States. These results highlight the impact of environmental context on host-symbiont partnerships.

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Chapter 1: Introduction

Symbiosis in Context

Symbioses are common in nature; organisms from all five kingdoms contain members that form mutualisms with microbial symbionts (Janzen 1985). These relationships are of particular importance in insects, where approximately 10% of species have nutritional symbionts (Paracer & Ahmadjian 2000). Many of these insects are pests because they harbor symbionts that allow them to utilize otherwise inaccessible resources, including some agricultural crops. For example, termites are reliant on their microbiota to digest cellulose, and aphids rely on symbiotic bacteria to supplement their diet of plant sap with necessary amino acids (Batra & Batra 1979, Buchner 1965).

Symbioses are frequently context-dependent. Mutualisms require that both players receive benefits that exceed the cost of maintaing the mutualism. Changes to the costs and benefits can lead to altered relationships between host and symbionts, and these changes can be caused by extrinsic environmental parameters. For example, when nitrogen levels in soil are high, legumes that associate with nitrogen-fixing rhozibia will shut down nodules where they house symbiotic bacteria (Denison & Harter 1995). Changes can also be intrinsic; black flies have a symbiotic fungus that is commensal in well-fed larvae, mutualistic in starved larvae, and parasitic in adults (McCreadie et al. 2004).

Context dependence becomes especially important for invasive species as introduction to a new environment results in a radical change in conditions. Different temperatures, humidity, predators, and resource availabilities are all problems an invasive species must confront in order to become established. For invasive species that associate

with symbionts, these novel conditions have the potential to alter the relationship between host and symbiont. From an agricultural perspective, this is important because the host ranges of many pest species are dependent on bacterial mutualisms (Douglas 2007).

Study System

Megacopta cribraria is a sap sucking insect of the Plataspidae family, natively found in Asia and Australia (Hsiao *et al.* 1997; Ishihara 1973). *M. cribraria* is an agricultural pest in China (Guanguan *et al.* 2006) that causes significant damage to soybean crops (Zhixing *et al.* 1996). In Japan, *M. cribraria* is not considered a pest species, but its sister taxa *M. punctatissima*, is a pest (Hosokawa *et al.* 2007). Both species are host to a symbiont, a γ-proteobacteria named '*Candidatus* Ishikawaella capsulata', that is purported to provide essential nutrients not found in the bugs' diet (Hosokawa *et al.* 2008). To pass on the symbiont, females place symbiont capsules in between their eggs during oviposition (Fig 1-1). When hatched, the nymphs are symbiont-free but acquire their symbionts by sucking on these symbiont capsules (Fukatsu & Hosokawa 2002). Without the symbiont, insects experience much higher mortality, delayed development, and abnormal coloration (Fig 1-2)(Hosokawa *et al.* 2007). Prior work has shown that the pest status of *Megacopta* is dependent on the strain of symbiont carried. When provided with symbiont capsules from the pest species *M. puncatissima*, Japanese *M. cribraria*, which subsist primarily on kudzu and perform poorly on soybean, perform well on soybean (Hosokawa *et al.* 2007).

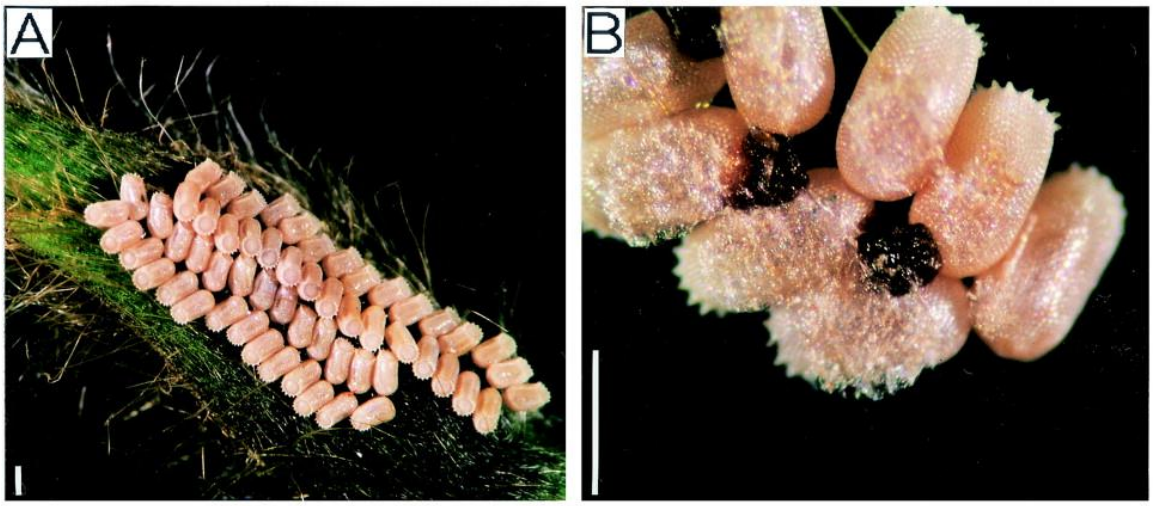

Fig. 1-1. *Megacopta punctatissima* egg clusters. A) Two sets of egg clusters on *Pueraria lobata*. B) The underside of an egg cluster; the black masses between the eggs are symbiont capsules. (adapted from Fukatsu & Hosokawa 2002)

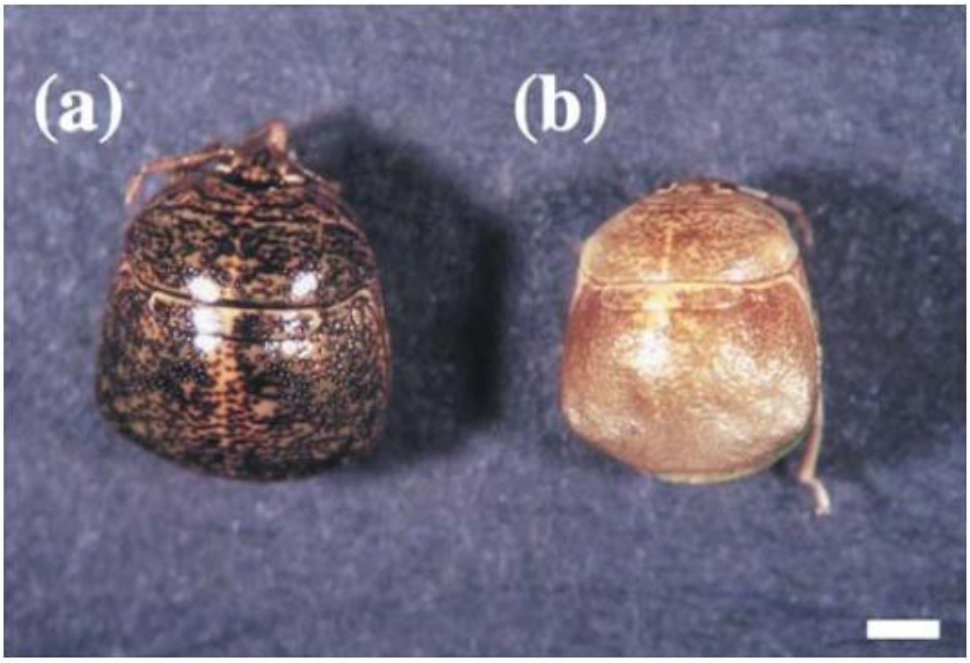

Fig 1-2. Two *Megacopta punctatissima* female adults. The female on the left is a normal female while the female on the right was raised without symbionts.

In October 2009, *M. cribraria* was identified in northeast Georgia after several thousand of the insects were found on two homes near a field of kudzu (Suiter *et al.* 2010). Since then it has spread to eight states in the southeastern United States (Fig 1-3) (Ruberson *et al.*, 2012), and while closely associated with kudzu, has been observed feeding on soybean (Musser *et al.*, 2012). Whole genome sequencing of the symbiont has revealed that the source of this invasion is most likely Japan, and that its genome is extremely similar to that of the *Ishikawaella* symbiont in the pest *M. punctatissima* (Brown *et al.* in review). Preliminary data indicate that, like *M. punctatissima*, invasive *M. cribraria* can develop on both soybean and kudzu, though with relatively delayed development times on soybean (Couret, unpublished data).

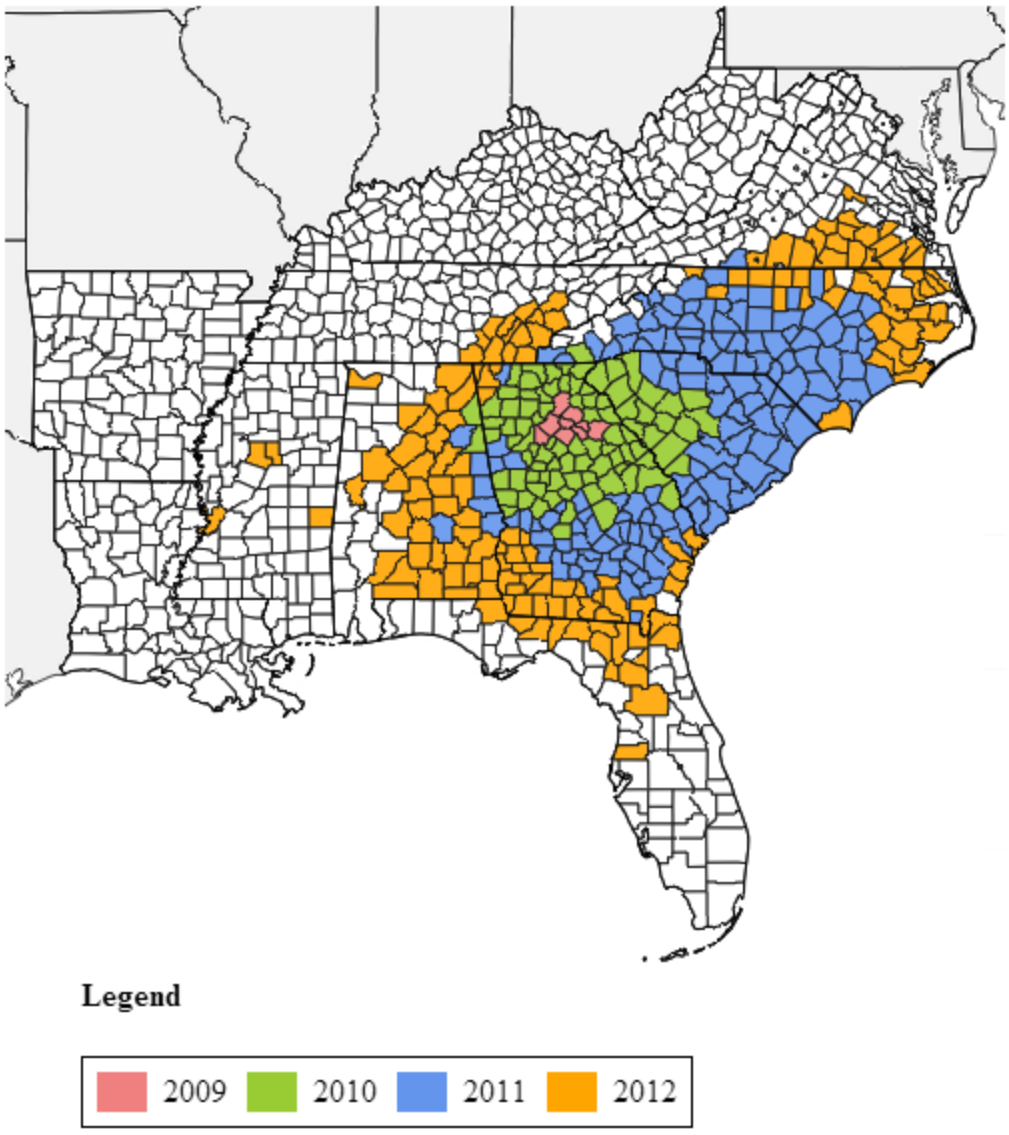

Fig. 1-3. Distribution map for *M. cribraria* in the Southeast United States. (adapted from http://www.kudzubug.org/distribution_map.cfm)

Chapter 2

Experiment

Introduction

Here, we explore the impact of host plant species on the maintenance of symbionts within invasive *M. cribraria.* We measure symbiont densities across developmental stages and between sexes of individuals reared on kudzu and soybean.

Experimental Procedures

Origin of Insects. *Megacopta* egg clusters were collected from wild kudzu (*Pueraria lobata*) vines and from the surface of structures within Atlanta, GA between March and June of 2012. These egg clusters were mixed and then divided into two groups. One group of eggs was placed at the base of a potted kudzu plant and the other at the bases of several soybean (*Glycine max*) plants. Plants were placed outdoors in large mesh enclosures where the insects were allowed to develop. Adult males (n = 24) and second (n = 20), third (n = 12), and fourth instar nymphs (n = 24) were collected with soft forceps and then immediately frozen at -20C to await DNA extraction. To examine any possible interaction between sex, diet, and symbiont density a second experiment was performed with just adults of both sexes as nymphs of both sexes are morphologically identical. For this experiment, insects were raised in an outdoor garden in large mesh enclosures. Ten adult females and ten adult males were collected from both kudzu and soybean within one week of molting to adults and then frozen in liquid nitrogen. All insects were collected between April 2012 and July 2012.

DNA Extraction, Standard Preparation, and qPCR. Insects were frozen in liquid nitrogen and then crushed with a pestle. The homogenized tissue was processed with the DNEasy Blood and Tissue Kit (Qiagen, Valencia, CA) according to manufacturer specifications. Second instar nymphs were pooled into groups of 5 due to their small size, while the rest of the insects were processed individually. DNA was eluted with 200 uL of buffer EB for fourth instars and adults, while second and third instars were eluted with 100 uL of EB to obtain necessary DNA concentrations. Eluted DNA samples were stored at -20C until use.

Symbiont load was assayed in two ways. The first method, which is referred to here as the relative method, was through comparison of the symbiont *groE* concentration against concentration of the host *ef1-α* gene (primer sequences for *groE* available in Hosokawa *et al.* 2007, *ef1- α* primers are ef-F1-124 5'-CAA GGG ATG GGC TAT TGA AA-3' and ef-R1-124 5'- AAC CTG AAG GGG AAG CCT TA-3'). This method takes into account the number of copies of host *ef1-α*, which can be a proxy for the size of the insect and may reduce variation amongst biological replicates. Relative quantity was calculated by subtracting the symbiont *groE* CT from the host *ef1-α* CT to obtain the ΔCT. The ΔCT values were then standardized to one sample to obtain the ΔΔCt value and relative quantity was calculated with the formula $2^{\Delta\Delta Ct}$. The second method, referred to here as the absolute method, was performed through quantification of symbiont *groE* concentration by comparison to standards of a known concentration. Standards were generated by cloning the *groE* amplicon into a plasmid with the TOPO TA Cloning Kit (Invitrogen, Foster City, CA). This plasmid insert was then sequenced to confirm identity with the symbiont *groE* gene. This method describes the total symbiont load of

an insect irrespective of body size. Total symbiont load was calculated for the absolute data as ((copies of groe per 0.02 ng DNA * total ng DNA extracted) / 0.02).

All runs were done with Power SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA) on an ABI StepOne Plus (Applied Biosystems, Foster City, CA) with the following program: 10:00 @ 95C, 40x 0:30 60C, 1:00 95C. Samples were run at a final concentration of 2 ng/uL for *ef1-α* and 0.002 ng/uL for *groE,* with primer concentrations of 250 pM. Efficiencies for both primers were evaluated by running serial dilutions; they were 100% efficient at the DNA and primer concentrations used. Lack of non-specific amplification was confirmed by examining melt curves. Three technical replicates were performed for all samples and standards, with outliers being removed.

Statistical Analyses. Both relative and absolute data were log transformed. Because the insects from the developmental experiment were reared under different conditions insects of the sex comparison experiment, data were analyzed separately. Three individuals were removed from both developmental and adult datasets due to extremely low symbiont quantifications most likely caused by inefficient DNA extraction. Data were analyzed using the GLM function in R version 2.11 and were fitted using the quasipoisson distribution.

Results and Discussion

Host Plant Effect on Symbiont Maintenance. From the developmental experiment, the relative method (Table 2-1A, Fig 2-1) indicated that host plant plays a significant impact on within-host *Ishikawaella* population size; insects reared on kudzu have a significantly higher number of symbionts relative to body size ($F_{1,63} = 8.31$, $p = 0.005$). The absolute method (Table 2-1, Fig 2-2), however, did not indicate a significant interaction between plant and symbiont population size ($F_{1,63} = 1.35$, $p = 0.249$). In both cases the effect of host plant appeared to be minimal in adult males so additional models were run with just the data from the nymphal stages. With adult males removed, both assays indicate that rearing on soybean has a significantly negative impact on symbiont population levels ($F_{1,39} = 6.75$, $p = 0.013$ relative, $F_{1,39} = 6.71$, $p = 0.013$ absolute).

A. Symbiont density in *M. cribraria* reared on different host plants

Developmental Stage	Host Plant	N	Log No. of Symbiont *groE* copies per Insect	Log No. of Symbiont *groE* copies per Host *ef-1a* Copy
Second Instar	Kudzu	4*	16.54 ± 0.78	9.851 ± 0.35
	Soy	4*	15.93 ± 0.56	8.524 ± 0.88
	Overall	8	16.27 ± 0.71	8.68 ± 1.02
Third Instar	Kudzu	6	19.78 ± 0.48	3.291 ± 0.56
	Soy	5	18.20 ± 0.85	3.952 ± 0.61
	Overall	11	19.06 ± 1.04	3.59 ± 0.65
Fourth Instar	Kudzu	10	20.94 ± 0.48	4.112 ± 1.67
	Soy	12	20.44 ± 0.99	1.954 ± 1.64
	Overall	22	20.67 ± 0.82	2.99 ± 1.99
Adult Male	Kudzu	12	19.85 ± 0.45	1.898 ± 0.87
	Soy	12	20.07 ± 0.46	1.531 ± 0.45
	Overall	24	19.96 ± 0.46	1.71 ± 0.70

B. Symbiont density in Adult *M. cribraria* reared on different host plants

Adult Male	Kudzu	10	21.53 ± 0.26	2.95 ± 0.35
	Soy	7	21.61 ± 1.86	1.66 ± 3.88
	Overall	17	21.57 ± 1.15	2.42 ± 2.48
Adult Female	Kudzu	10	22.23 ± 0.46	0.94 ± 0.40
	Soy	10	20.41 ± 0.83	-2.49 ± 1.42
	Overall	20	21.32 ± 1.14	-0.78 ± 2.04

Table 2-1. Symbiont numbers in *M. cribraria* reared on different host plants.

* indicates that each sample of second instar nymphs is a pool of DNA from 5 individuals.

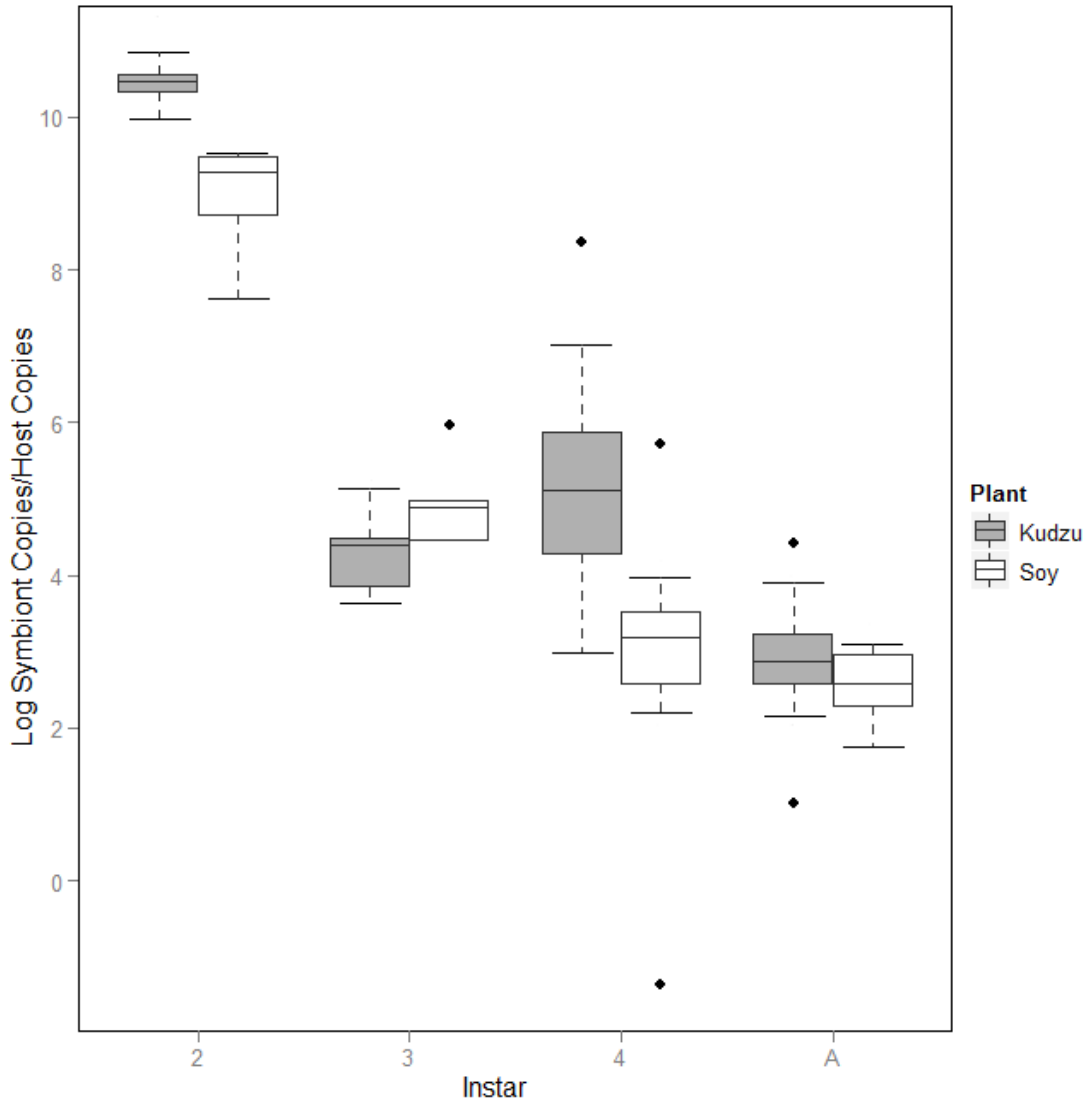

Fig 2-1. Relative symbiont numbers in *M. cribraria* across developmental stage and reared on alternative host plants. Symbiont load as symbiont gene amplification standardized relative to host gene amplification, providing an estimate of symbiont number relative to host body size. Error bars extend to the most extreme value within 1.5 times the interquartile range.

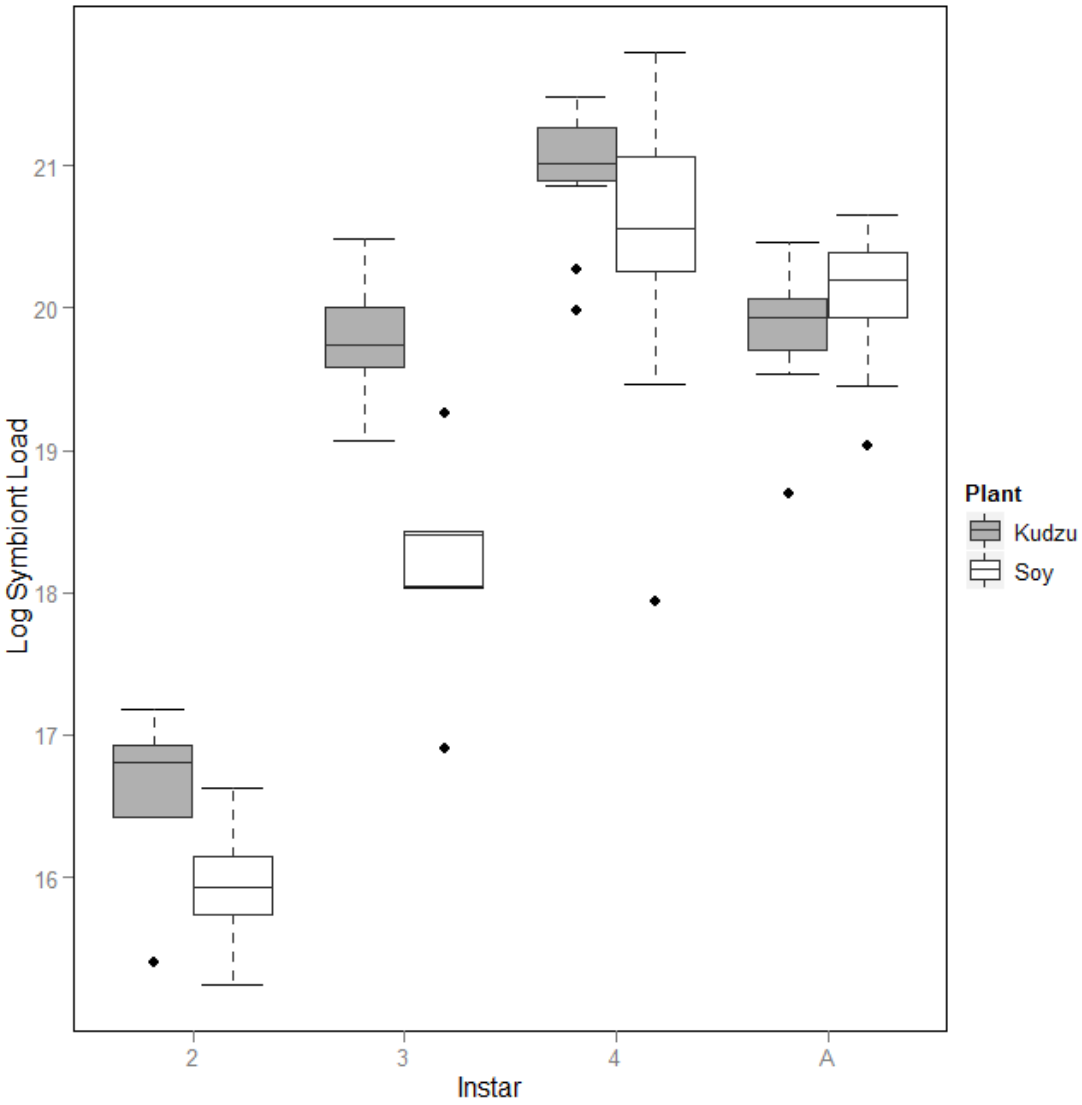

Fig 2-2. Absolute symbiont load in *M. cribraria* across developmental stage and reared on alternative host plants. Symbiont load as total number of symbionts per insect. Error bars extend to the most extreme value within 1.5 times the interquartile range.

For the experiments comparing adults of both sexes, both methods indicated that host plant significantly impacted symbiont population levels (Table 2-1 B, Fig 2-3, Fig 2-4) ($F_{1,35} = 20.58$, $p < 0.001$ relative, $F_{1,35} = 9.87$, $p = 0.004$ absolute) and also indicated a significant interaction between sex and plant ($F_{1,35} = 11.07$, $p = .025$ relative, $F_{1,35} = 9.19$, $p < 0.005$ absolute). Adult males reared on alternative host plants exhibited no significant difference in symbiont population ($t_{15} = 0.13$, $p = 0.89$ absolute, $t_{15} = 1.06$, $p = 0.30$ relative), which is consistent with the first experiment across development stages, but adult females raised on soybean exhibited significantly lower symbiont densities than those raised on kudzu ($t_{18} = 6.05$, $p < 0.001$ absolute, $t_{18} = 7.36$, $p < 0.001$ relative).

Preliminary data indicates that invasive *M. cribraria* raised on soybean have delayed development times compared to those reared on kudzu (Couret, unpublished data). This result, combined with the data presented here, suggests that soybean is a suboptimal host for both *M. cribraria* and its symbiont. It is probable that the insects perform poorly on soybean due to nutritional deficiencies but why soybean leads to lower symbiont populations is less clear. One hypothesis is that *M. cribraria* down regulates the symbiont population during times of nutritional stress, similar to how corals release their symbionts when exposed to stressful environmental conditions (Lesser 1997, Banaszak *et al*. 1995). An alternative explanation is that the symbionts simply have lower fitness given a diet of soybean sap. Metabolic profiling of insects reared on both diets could facilitate understanding the nutrients limiting growth and symbiont maintenance.

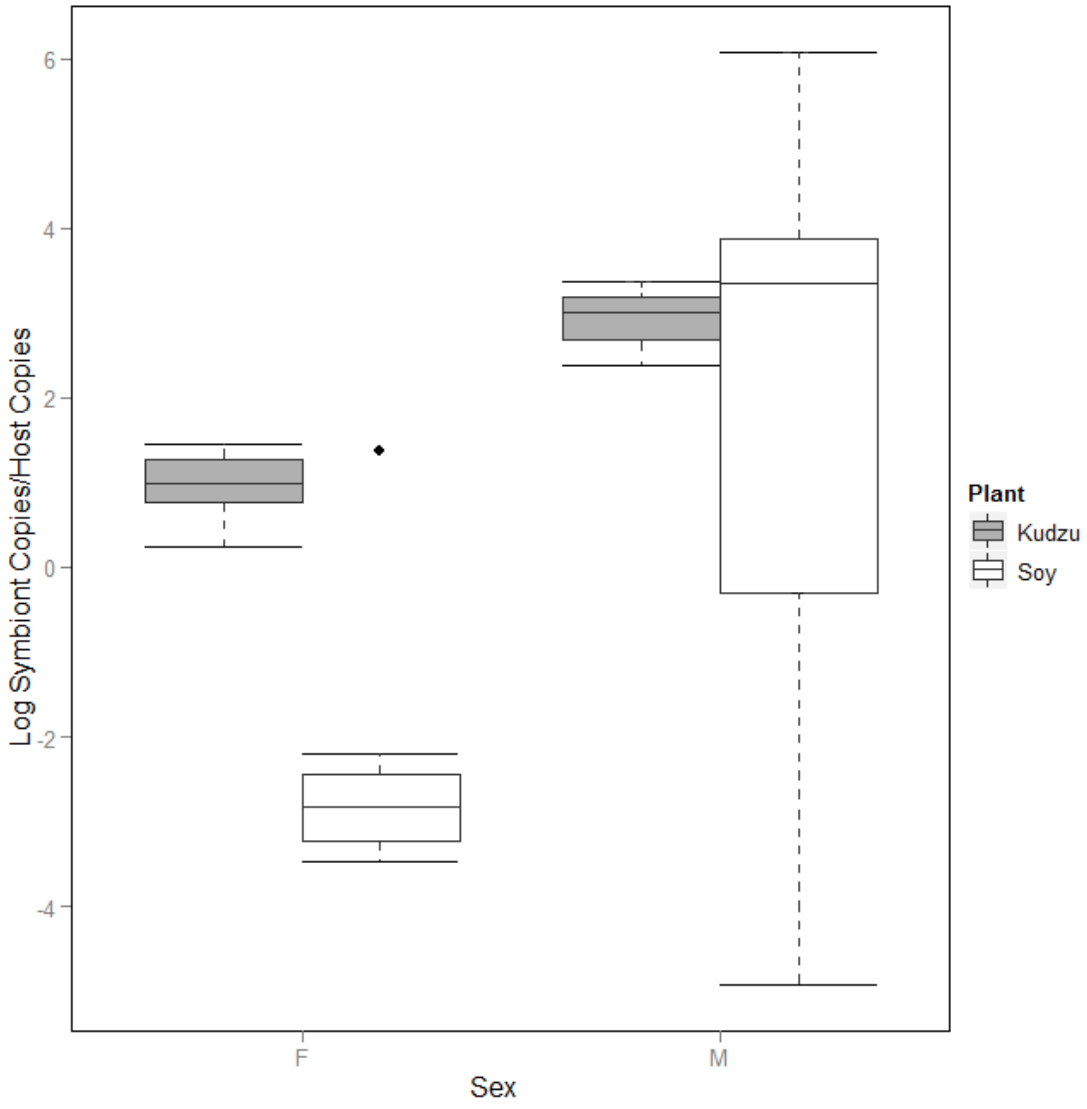

Fig 2-3. Relative symbiont numbers in young *M. cribraria* adults reared on alternative host plants. Symbiont load as symbiont gene amplification standardized relative to host gene amplification, providing an estimate of symbiont number relative to host body size. Error bars extend to the most extreme value within 1.5 times the interquartile range.

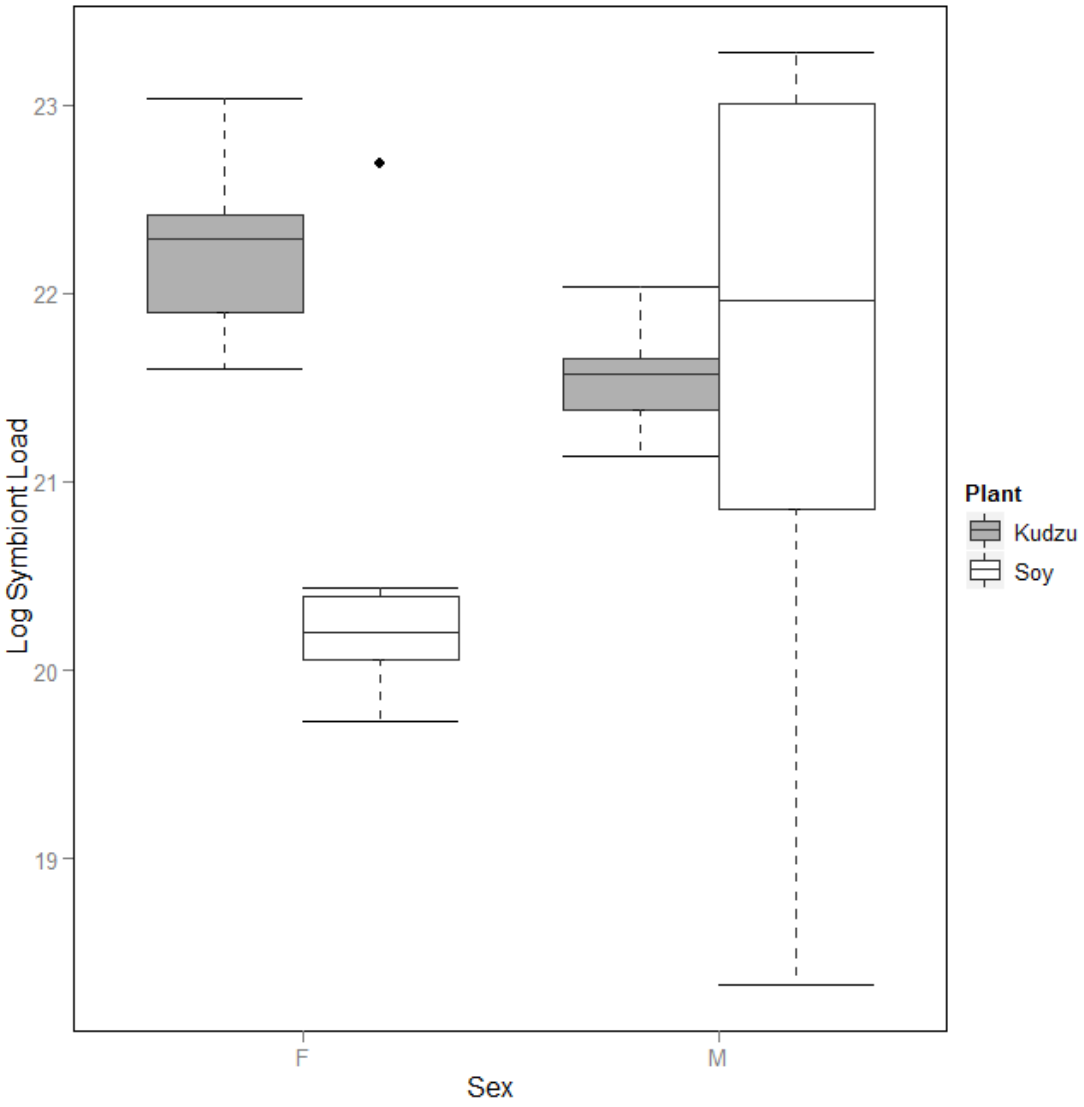

Fig 2-4. Absolute symbiont loads in young *M. cribraria* adults reared on alternative host plants. Symbiont load as total number of symbionts per insect. Error bars extend to the most extreme value within 1.5 times the interquartile range.

Curiously, while we found a significant effect of host plant on juveniles and adult females, we did not find an effect of host plant on symbiont load in adult males; this result was consistent across both datasets. This finding appears driven by a decrease in symbiont load in females reared on soybean compared to both females reared on kudzu and to males reared on both host plants. This may be because as these young females begin to develop both eggs and symbiont capsules, they are more energetically constrained than their female counterparts on more optimal host plants. This reproductive cost then manifests in less nutrients available for symbiont growth. Their male counterparts reared on soy, who are not allocating significant energy to reproduction can also allocate more towards symbiont growth.

The finding that symbiont populations in juveniles are affected by host plant while those in adult males are not (Fig. 2-1) is likely driven by the fact that the juvenile nymphs, which cannot be sexed, include both males and females. Like adults, juvenile females, but not males, may maintain decreased symbiont populations depending on host plant. Another possibility is that the rapid development of juveniles, regardless of sex, requires more energy allocation to be shuffled to development, and thus fewer nutrients are available for symbiont maintenance, leading to decreased symbiont densities when on sub-optimal host plants such as soybean.

Age and Symbiont Population. *Ishikawaella* population density increases significantly as the insects advance through their nymphal stages (Fig. 2-2) ($F_{1,63} = 49.63$, $p < 0.001$). This increase occurs during their developmental period between the second and fourth instars with an 85-fold increase in average symbiont load. While the total symbiont population increases during development (Fig. 2-2), the ratio of symbionts to

host cells decreases significantly (Fig. 2-1) ($F_{1,63} = 117.30$, $p < 0.001$) indicating that while both host cell and symbiont cell numbers are increasing, host cell numbers increase at a faster rate than symbiont cell numbers..

The absolute data also indicate that symbiont density peaks at the fourth instar but drops for males at adulthood. , Females and male adults have overall similar symbiont densities (Fig 2), indicating that females also experience a decrease in symbiont density at adulthood. Decreases in primary symbiont densities at adulthood have been reported in other insects (Wolschin *et al*. 2004, Nishikori *et al*. 2009), with the study by Nishikori showing an increase in lysozymal activity in aphids at adulthood, suggesting active destruction of symbionts by aphids at later life stages. Given that after maturity, the nutritional demands of growth and development are relaxed, it is likely that adults have decreased need for nutritional supplementation provided by their symbionts. This can be observed behaviorally as adults do not spend the majority of their time feeding like the juveniles do.

Conclusion. We have shown that effect of diet on symbiont density in *Megacopta cribraria* is context dependent; symbionts in juveniles and adult females have decreased population densities when their hosts are reared on soybean, but males are not affected. This is a surprising result as the symbiont genotype is extremely close to the genotype of the pest *M. punctatissima* which feeds on soybean. Metabolically, the only functional difference between the symbiont of the invasive insect and the Japanese pest *Megacopta* is a single amino acid change in a riboflavin synthase gene; it is possible that the fitness difference observed is due to differences in riboflavin availability between kudzu and soy (Brown, in review). A difference in the nutritional composition between soybean sap in

the United States and soybean sap in Japan could offer an explanation for this result as well.

The decreased symbiont titers we observed in adult females reared on soybean are of particular importance, as the symbiont capsules they provide to the next generation are necessary for transmission of the symbiont. It is known that *Megacopta* mothers provide an excess amount of symbionts to their offspring; however, the roughly four-fold decrease in symbiont titer we observed in females reared on soy may negatively impact transmission efficiency (Hosokawa 2007b). Studies of how the invasive *M. cribraria* performs on soybean over several generations should be performed to determine if the mutualistic relationship can persist on this important agricultural crop.

References

Banaszak, A. T., & Trench, R. K. (1995). Effects of ultraviolet (UV) radiation on marine microalgal-invertebrate symbioses. II. The synthesis of mycosporine-like amino acids in response to exposure to UV in *Anthopleura elegantissima* and *Cassiopeia xamachana*. *Journal of experimental marine biology and ecology*, *194*(2), 233-250.

Batra, L. R., Batra, S. W. T., & Batra, L. R. (1979). Termite-fungus mutualism. *Insect-Fungus Symbiosis: Mutualism and Commensalism*, 117-163.

Buchner, P. (1965). Endosymbiosis of Animals with Plant Microorganims.

Denison, R. F., & Harter, B. L. (1995). Nitrate Effects on Nodule Oxygen Permeability and Leghemoglobin (Nodule Oximetry and Computer Modeling).*Plant physiology*, *107*(4), 1355-1364.

Douglas, A. E. (2007). Symbiotic microorganisms: untapped resources for insect pest control. *TRENDS in Biotechnology*, *25*(8), 338-342.

Fukatsu, T., & Hosokawa, T. (2002). Capsule-transmitted gut symbiotic bacterium of the Japanese common plataspid stinkbug, Megacopta punctatissima. *Applied and Environmental Microbiology*, *68*(1), 389-396.

Hosokawa, T., Kikuchi, Y., Shimada, M., & Fukatsu, T. (2007). Obligate symbiont involved in pest status of host insect. *Proceedings of the Royal Society B: Biological Sciences*, *274*(1621), 1979-1984.

Hosokawa, T., Kikuchi, Y., & Fukatsu, T. (2007). How many symbionts are provided by mothers, acquired by offspring, and needed for successful vertical transmission in an obligate insect–bacterium mutualism?. *Molecular ecology*,*16*(24), 5316-5325.

Hosokawa, T., Kikuchi, Y., Shimada, M., & Fukatsu, T. (2008). Symbiont acquisition alters behaviour of stinkbug nymphs. *Biology letters*, *4*(1), 45-48.

Hsiao TY, Zheng LY, Ren SZ (1977) *A Handbook for the Determination of the Chinese Hemiptera-Heteroptera* Scientific Publishing Company, Beijing.

Ishihara T (1973) A list of Heteroptera from Hiroshima Prefecture. Part I. . *Entomology World* **5**.

Janzen, D. H. (1985). The natural history of mutualisms. *The biology of mutualism: Ecology and evolution*, 40-99.

Lesser, M. P. (1997). Oxidative stress causes coral bleaching during exposure to elevated temperatures. *Coral Reefs*, *16*(3), 187-192.
McCreadie, J. W., Beard, C. E., & Adler, P. H. (2005). Context-dependent symbiosis between black flies (Diptera: Simuliidae) and trichomycete fungi (Harpellales: Legeriomycetaceae). *Oikos*, *108*(2), 362-370.

Musser, F. R., Catchot Jr, A. L., Davis, J. A., Herbert Jr, D. A., Leonard, B. R., Lorenz, G. M., ... & Stewart, S. D. 2011 Soybean Insect Losses in the Southern US.

Nishikori, K., Morioka, K., Kubo, T., & Morioka, M. (2009). Age-and morph-dependent activation of the lysosomal system and Buchnera degradation in aphid endosymbiosis. *Journal of insect physiology*, *55*(4), 351-357.

Paracer, S., & Ahmadjian, V. (2000). *Symbiosis: an introduction to biological associations* (pp. 1-291). New York, NY: Oxford University Press.

Ruberson, J. R., Takasu, K., Buntin, G. D., Eger Jr, J. E., Gardner, W. A., Greene, J. K., ... & Toews, M. D. (2013). From Asian curiosity to eruptive American pest: Megacopta cribraria (Hemiptera: Plataspidae) and prospects for its biological control. *Applied Entomology and Zoology*, *48*(1), 3-13.

Suiter, D. R., Eger, J. E., Gardner, W. A., Kemerait, R. C., All, J. N., Roberts, P. M., ... & Douce, G. K. (2010). Discovery and distribution of Megacopta cribraria (Hemiptera: Heteroptera: Plataspidae) in northeast Georgia. *Journal of Integrated Pest Management*, *1*(1), F1-F4.

Wolschin, F., Hölldobler, B., Gross, R., & Zientz, E. (2004). Replication of the endosymbiotic bacterium Blochmannia floridanus is correlated with the developmental and reproductive stages of its ant host. *Applied and environmental microbiology*, *70*(7), 4096-4102.

Zhixing W, Huadi W, Guihua C, Zi Z, Caiwen T (1996) Occurence and control of Megacopta cribraria (Fabricius) on soybeans. *Plant Protection* **3**, 7-9.