



## MINUTE PIRATE BUG (*ORIUS INSIDIOSUS* SAY) POPULATIONS ON TRANSGENIC AND NON-TRANSGENIC MAIZE USING DIFFERENT SAMPLING TECHNIQUES

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### ABSTRACT

Field experiments were conducted to evaluate the populations of minute pirate bug [*Orius insidiosus* (Say)] using visual, sticky cards and destructive sampling techniques in transgenic and non-transgenic maize in three locations in Nebraska (Mead, Clay Center, and Concord) United States of America, during 2007 and 2008. All sampling methods revealed significant counts of *O. insidiosus* on CP4 EPSPS maize plus an insecticide application for control of first generation *O. nubilalis* at R2 (blister) sampling period. Similarly, visual observations of *O. insidiosus* on Cry1Ab x Cry3Bb1 x CP4 EPSPS maize yielded significantly higher mean adult counts at R2 (blister) sampling period for both years while, sticky cards and destructive sampling methods gave significant counts during 2007 and 2008, respectively. During both sampling periods (R1 and R2), mean adult counts of *O. insidiosus* differ significantly among the three sites at Mead, Clay Center, and Concord, Nebraska. Results from this research show that Cry1Ab maize, Cry1Ab x CP4 EPSPS maize, Cry1Ab x Cry3Bb1 x CP4 EPSPS maize, and CP4 EPSPS maize had no significant effects on *O. insidiosus* population abundance compared to CP4 EPSPS maize treated with insecticides.

**Keywords:** Minute pirate bug, maize, sampling techniques

### INTRODUCTION

Minute pirate bug, *Orius insidiosus* (Say) (Hemiptera: Anthocoridae) is a generalist predator which is frequently reported in ecological field studies as important non-target organism in transgenic maize (Al-deeb *et al.*, 2001; Musser *et al.*, 2004). In the Midwest, including Nebraska, *O. insidiosus* is a common predator in maize (Wright, 2004) and soybean fields (Brosius *et al.*, 2007).

*Orius* spp. are important natural enemies of pest insects and mites in many cropping systems such as in maize, soybeans,

vegetables, and fruit crops (Jarvis and Guthrie, 1987). Nearly all *Orius* spp. are predaceous as nymphs and adults (Lattin and Stanton, 1992). The primary food of *Orius* spp. consists of small insects and insect eggs, plant pollen, rarely plant sap, and others feed on both plant and animal (Armer *et al.*, 1998). Nymphs and adults of *O. insidiosus* are commonly found on maize silks, and serve as natural enemies of key maize pests such as larvae of *Ostrinia nubilalis* Hübner, *Helicoverpa armigera* Hübner (Wright, 2004), *Spodoptera frugiperda* Smith (Isenhour *et al.*, 1990), *Rhopalosiphum maidis* Fitch. (Rutledge *et al.*, 2004), *Frankliniella* spp. (van den Meiracker

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and Ramakers 1991; Riudavets, 1995) spider mites (*Oligonychus pratensis* Banks, *Tetranychus urticae* Koch), whiteflies (*Bemisia argentifolii* Bellows & Perring, and *B. tabaci* Gennadius), and eggs of other insects (van der Veire and Degheele, 1992; van Lenteren *et al.*, 1997). *Orius insidiosus* are commercially produced and sold as a biological control agent for glasshouse-grown vegetables and ornamental crops (Copping, 2004).

The potential impact of transgenic maize has evaluated *O. insidiosus* as a key non-target arthropod (Al-deeb *et al.*, 2001; Pilcher *et al.*, 2005). Effective and reliable sampling of *O. insidiosus* nymphs and adults is important in assessing the impact of transgenic maize on non-target organisms, particularly for environmental risk assessments. Several methods have been documented to assess the non-target organisms in transgenic maize by using visual observations, pitfall traps, sticky cards, sweep nets, and beat buckets (Al-deeb *et al.*, 2001; Head *et al.*, 2005; Pilcher *et al.*, 2005).

Visual observations, yellow sticky cards, and destructive sampling techniques have been used to monitor *O. insidiosus* nymphs and adults together with above ground arthropod pests for the non-target impact of transgenic plants (Udayagiri *et al.*, 1997; Musser *et al.*, 2004; Dively and Galen, 2005). Visual observations are commonly used to monitor insect pests and non-target arthropods in maize fields. This method is simple, easy to use, fast, and requires no supplies. However, it can be considered biased due to changes in insect behavior, time of sampling, entomological, and taxonomic expertise. Conversely, yellow sticky cards have the advantage of being unbiased for sampling time; results are less affected by visual acuity of field personnel, and are relatively rapid and inexpensive. Yellow sticky cards also attract several mobile species allowing comparison with other species. Destructive counts are done using bucket and beat sheets.

## MATERIALS AND METHODS

The experiments were conducted during 2007 and 2008 at three geographically different University of Nebraska-Lincoln experimental research stations. The field locations were, Agricultural Research and Development Center near Mead (N41°11.07' WO96°27.263' in 2007 and N41°11.09'WO96°27.411' in 2008), South Central Agricultural Laboratory near Clay Center (N40°34.216' WO98°07.958' in 2007 and N40°34.272' WO98°07.822' in 2008) and the Northeast Research and Extension Center - Haskell Agricultural Laboratory near Concord (N42°23.037' WO96°57.193' in 2007 and N42°23.149' WO96°57.331' in 2008) (Fig. 1). Soil types were Sharpsburg silty clay loam, Kennebec silty clay loam and Butler/Crete silt loam, respectively. All locations were previously planted with soybean in a no tillage system.

### Agronomic practices

Plantings were done in a no-till corn system on 5/10, 5/11 and 5/15 in 2007 and 5/19, 5/20 and 5/21 in 2008 at Mead, Clay Center, and Concord, respectively. Nutrient management, irrigation, and herbicide application were conducted for each treatment based on the normal agronomic requirements of each specific site.

## Experimental design and treatments

A randomized complete block design, replicated four times was used. The treatments in 2007 were: a) a Cry1Ab x CP4 EPSPS maize, b) a CP4 EPSPS maize, c) a CP4 EPSPS maize plus an insecticide application to control the first generation of *O. nubilalis*, d) a Cry1Ab x Cry3Bb1 x CP4 EPSPS maize, and e) a conventional maize. In 2008, the treatments were: a) a Cry1Ab maize, b) a CP4 EPSPS maize, c) a CP4 EPSPS maize plus an insecticide application to control the first generation of *O. nubilalis*, d) a CP4 EPSPS maize plus an insecticide application to control the second generation of *O. nubilalis*, e) a Cry1Ab x Cry3Bb1 x CP4 EPSPS maize and f) a conventional maize.

In the CP4 EPSPS maize plus an insecticide application to control the first generation of *O. nubilalis* both in 2007 and 2008, permethrin (Pounce® 1.5G) insecticide was applied at the recommended rate of 12 oz./1000 row ft band at Mead (7/3/07 and 7/14/08), Clay Center (7/4/07 and 7/15/08), and Concord (7/9/07 and 7/16/08) using an improvised jar shaker applicator uniformly applied at whorl maize stage (V9-V12 growth stages) (Ritchie *et al.*, 1993). In 2008, bifenthrin (Capture® 2 EC) insecticide was sprayed in a formulation of 6.66 ml for every 2 gallons (7,571 ml) of water using a carbon-gated sprayer for the control of second generation *O. nubilalis* at Mead (8/12/08), Clay Center (8/13/08) and Concord (8/11/08).

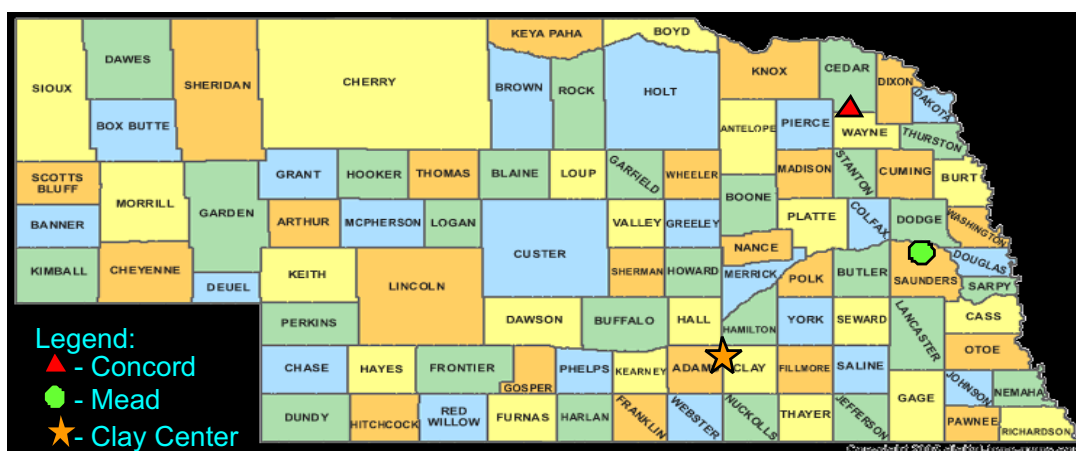
Each plot measured 60 square meters. There were 8 rows in each plot with ~400 plants per plot (~50 plants per row). Border rows and alleyways measured 3 meters between plots, and were planted with the conventional corn. The treatments were randomized in each block and site.

### Sampling methods

In the years 2007 and 2008, *O. insidiosus* nymphs and adults were monitored using visual observations, and yellow sticky cards. Destructive sampling technique was used in 2008 to validate the actual nymph and adult counts. Voucher specimen of both nymphs and adults of *O. insidiosus* were properly preserved using 85 percent alcohol, and some adult specimen were properly pinned and labeled. Specimens were identified using diagnostic key references, field guides, data bases and on-line information.

### Visual observations

The sample unit consisted of 20 randomly selected plants from rows 2 and 3 in each plot. R2 (blister) samplings were conducted on 7/25, 7/23, and 7/27 in 2007 while 8/04, 8/05, 8/06 in 2008 at Mead, Clay Center and Concord, respectively. R1 (silking) samplings were conducted at Mead (8/12/08), Clay Center (8/13/08), and Concord (8/11/08). Nymphs and adults of *O. insidiosus* were observed visually on maize ears, and tapping of silks was done with clean sheet of bond paper underneath to quantify the number of nymphs and adults. The mean nymph, adult, and nymph plus adult count per plant were used for the analysis.



**Fig. 1**  
Location map of the three experimental field research sites in Nebraska.

### Yellow sticky cards

The sample unit consisted of two yellow sticky cards measuring  $23 \times 28$  cm and sticky on one side only (Pherocon® AM, Trécé Inc., Adair, OK) (Musser *et al.*, 2004; Pilcher *et al.*, 2005). Two wooden stakes ( $2.5 \times 2.1 \times 244$  cm) were positioned between rows 5, 6, and 7 of each plot at the seedling stage (V3). At the reproductive stage (R1-silking), the cards were folded and clipped with 2 binder clips around the wooded stake facing the maize rows just above the maize ears on 7/30, 8/01 and 8/03 in 2007 while 8/12, 8/13 and 8/11 in 2008 at Mead, Clay Center and Concord, respectively. After approximately 7 days, the yellow sticky cards were collected, sealed in plastic bag, and brought to the Department of Entomology Laboratory at the University of Nebraska-Lincoln for quantification. *Orius insidiosus* adults were counted with the aid of a dissecting microscope. The adult counts from the 2 yellow sticky cards were pooled, and mean adult counts per card per day were used for the analysis.

### Destructive sampling

The sample unit consisted of five randomly selected sample maize ears from row 4 of each of the treatment in each block per site. Samplings were conducted at R2 (blister) sampling period in Mead (8/12/08), Clay Center (8/05/08), Concord (8/06/08). Each randomly selected sample maize ear was covered with a properly labeled plastic bag (ziplock), separated from the stalk using a knife, sealed the plastic bag, and brought to the University of Nebraska Lincoln-Department of Entomology for quantification. Adults and nymphs of *O. insidiosus* were counted using dissecting microscope. Mean nymphs, adults, and nymphs plus adults counts of five sample maize ears were pooled for the analysis.

### Data analysis

The data were analyzed by analysis of variance (ANOVA) using SAS software (SAS 2003) by PROC MIXED. Mean separations were determined with Fisher's protected least significant differences (LSD).

## RESULTS

### Visual observations

During 2007 and 2008, visual observations revealed significant differences in mean adult counts of *O. insidiosus* between transgenic and conventional maize at R2 (blister) sampling period. In 2008, sampling at R1 and R2 stages revealed highly significant numbers of minute pirate bug between transgenic and conventional maize (Table 1). There were fewer adult counts on CP4 EPSPS maize with insecticide application when compared with transgenic, and the conventional maize in both years. Similarly, CP4 EPSPS maize with insecticide application yielded significantly less mean nymphs, adults, and nymph plus adult counts of *O. nubilalis* at both R1 (silking) and R2 (blister) sampling periods in 2008. In 2007, Cry1Ab x Cry3Bb1 x CP4 EPSPS maize obtained significant higher mean adult counts per plant (0.5667) at R2 (blister) sampling period. Similarly, significantly higher nymphal and adult mean densities of *O. insidiosus* at R1 and R2 stages were observed during 2008 (Table 1).

For visual observations, significant differences were recorded in mean adult counts of *O. insidiosus* at R1 (silking) sampling period in 2007 among all three sites, with 0.7375, 0.5625 and 0.2400 adults/plant for Concord, Mead and Clay Center, respectively. A significant amount of variations in nymphal and adult counts of *O. insidiosus* were found at R1 (silking) sampling period in 2008. The mean counts of *O. insidiosus* were significantly higher variations in Clay Center at R2 (blister) sampling period in 2008, with 0.9458 nymphs/plant, 0.5833 adults/plant, and 1.5292 nymphs plus adults/plant.

### Yellow sticky cards

*Orius insidiosus* mean adult counts using yellow sticky cards at R2 (blister) sampling period recorded significant differences in 2007, and no significant differences in 2008 (Table 2). CP4 EPSPS maize plus an insecticide application for control of first generation *O. nubilalis* yielded significantly fewer mean adult counts of 0.4583 adults/trap/day in the yellow sticky card at R2 (blister) sampling period in 2007. Conventional maize, CP4 EPSPS

**Table 1**

Mean nymph, adult, and nymph plus adult counts of minute pirate bug [*Orius insidiosus* (Say)] on different transgenic and non-transgenic maize at R1 (silking) (80-85 DAP) and R2 (blister) (90-95 DAP) sampling periods using visual observations in Nebraska in 2007 and 2008.

Particular	<i>Orius insidiosus</i> mean counts <sup>c</sup>						
	2007		2008		90-95 DAP		
	90-95 DAP	80-85 DAP	80-85 DAP	Adult	Nymph + Adult	Nymph	Adult
(a) Treatments							
Cry1Ab maize	-	0.6000 b	0.9292 b	1.5292 b	0.3750 bc	0.4208 b	0.7958 b
Cry1Ab x CP4 EPSPS maize	0.6125 a	-	-	-	-	-	-
CP4 EPSPS maize	0.5417 a	0.5917 b	1.0833 ab	1.6750 b	0.5792 a	0.4417 b	1.0208 a
CP4 EPSPS maize + Insecticide-1 <sup>a</sup>	0.3417 b	0.2333 c	0.5083 c	0.7417 c	0.3292 c	0.2500 c	0.5792 c
CP4 EPSPS maize + Insecticide-2 <sup>b</sup>	-	0.6958 b	1.0833 ab	1.7792 ab	0.4958 ab	0.4833 ab	0.9792 ab
Cry1Ab x Cry3Bb1 x CP4 EPSPS maize	0.5667 a	0.8208 a	1.2333 a	2.0542 a	0.5625 a	0.6125 a	1.1750 a
Conventional maize	0.5042 a	0.7375 ab	0.9500 b	1.6875 b	0.4583 ab	0.4958 ab	0.9542 b
	<i>P-value</i>	0.0145	0.0001	0.0001	0.0001	0.0012	0.0011
	<i>Significance</i>	*	**	**	**	**	**
(b) Sites							
Mead	0.5625 b	0.8437 a	1.6854 a	2.5292 a	0.3063 b	0.4292 b	0.7354 b
Clay Center	0.2400 c	0.8833 a	1.0646 b	1.9479 b	0.9458 a	0.5833 a	1.5292 a
Concord	0.7375 a	0.1125 b	0.1437 b	0.2562 c	0.1479 c	0.3396 b	0.4875 c
	<i>P-value</i>	0.0001	0.0001	0.0001	0.0001	0.0002	0.0001
	<i>Significance</i>	**	**	**	**	**	**

<sup>a</sup> CP4 EPSPS maize + Insecticide-1 = CP4 EPSPS maize applied with permethrin (Pounce® 1.5G) to control the first generation of *Ostrinia nubilalis*.

<sup>b</sup> CP4 EPSPS maize + Insecticide-2 = CP4 EPSPS maize sprayed with bifenthrin (Capture® 2 EC) to control the second generation of *Ostrinia nubilalis*.

<sup>c</sup> Numbers in a column for treatment and site followed by the same letter are not significantly different according to Fisher's protected LSD test for mean separation. \* - significant at P≤0.05, \*\* - highly significant at P≤0.0001

**Table 2**

Mean adult counts of minute pirate bug [*Orius insidiosus* (Say)] on different transgenic and non-transgenic maize at R1 (silking) (80-85 DAP) to R2 (blister) (90-95 DAP) sampling periods using yellow sticky card in Nebraska in 2007 and 2008.

Particular	<i>Orius insidiosus</i> mean counts <sup>c</sup>	
	2007	2008
(a) Treatments		
Cry1Ab maize	-	0.0655
Cry1Ab x CP4 EPSPS maize	0.8095 a	-
CP4 EPSPS maize	0.8393 a	0.0762
CP4 EPSPS maize + Insecticide-1 <sup>a</sup>	0.4583 b	0.0881
CP4 EPSPS maize + Insecticide-2 <sup>b</sup>	-	0.0881
Cry1Ab x Cry3Bb1 x CP4 EPSPS maize	0.7679 a	0.0917
Conventional maize	0.8452 a	0.0714
	<i>P-value</i>	0.0046
	<i>Significance</i>	*
(b) Sites		
Mead	1.0357 a	0.0554 b
Clay Center	0.5964 b	0.0720 a
Concord	0.6000 b	0.7917 a
	<i>P-value</i>	0.0001
	<i>Significance</i>	**

<sup>a</sup> CP4 EPSPS maize + Insecticide-1 = CP4 EPSPS maize applied with permethrin (Pounce® 1.5G) to control the first generation of *Ostrinia nubilalis*.

<sup>b</sup> CP4 EPSPS maize + Insecticide-2 = CP4 EPSPS maize sprayed with bifenthrin (Capture® 2 EC) to control the second generation of *Ostrinia nubilalis*.

<sup>c</sup> Numbers in a column for treatment and site followed by the same letter are not significantly different according to Fisher's protected LSD test for mean separation. NS - not significant, \* - significant at P≤0.05, \*\* - highly significant at P≤0.0001

maize, Cry1Ab x CP4 EPSPS maize, and Cry1Ab x Cry3Bb1 x CP4 EPSPS maize had recorded significantly comparable *O. insidiosus* mean adult counts per yellow sticky cards per day at R2 (blister) sampling period in 2007.

Significant variations were recorded on mean adult counts of *O. insidiosus* among the three sites using yellow sticky cards at R2 (blister) sampling period in 2007 and 2008. Mead had yielded significantly higher mean adult counts of *O.*

*insidiosus* of 1.0357 adults/trap/day in 2007, and significantly obtained fewest mean adult counts of 0.0554 adults/trap/day in 2008.

*Destructive sampling* In 2008, destructive sampling revealed significant differences among the mean counts on nymph, adult, and nymph plus adult of *O. insidiosus* between transgenic and non-transgenic maize at R2 (blister) sampling period. CP4 EPSPS maize plus an insecticide application for

control of first generation *O. nubilalis* yielded significantly fewer *O. insidiosus* mean counts per plant of nymphs, adults, and nymphs plus adults per plant (1.4667, 0.8833 and 2.3500, respectively). In contrast, Cry1Ab x Cry3Bb1 x CP4 EPSPS maize yielded significantly higher mean counts of *O. insidiosus* (1.100 nymphs/plant, 0.0.800 adults/plant and 1.900 nymphs plus adults/plant).

All of the three sites revealed significant variations on the nymph, adult, and nymph plus adult mean counts per plant of *O. insidiosus* at R2 (blister) sampling period in 2008. Significantly higher mean count variations of *O. insidiosus* nymph, adult, and nymph plus adult were recorded at Clay Center (1.8833 nymphs/plant), Mead (1.0500 adults/plant) and Clay Center (2.9333 nymphs plus adults/plant), respectively.

## DISCUSSION

Similar trend of mean adult counts of *O. insidiosus* on CP4 EPSPS maize treated with insecticide at R2 (blister) sampling periods were observed for visual observations in both years, yellow sticky cards in 2007 and destructive sampling in 2008. Additionally, visual observations recorded significantly fewer *O. insidiosus* nymph, adult, and nymph plus adult mean counts per plant at R1 (silking) and R2 (blister) sampling periods in 2008 on CP4 EPSPS maize with insecticide application. Same trend was observed using destructive sampling method on CP4 EPSPS maize plus an insecticide application at R2 (blister) sampling period in 2008.

Cry1Ab x Cry3Bb1 x CP4 EPSPS maize yielded significantly higher mean adult counts at R2 (blister) sampling periods using visual observations in 2007 (0.5667 adults/plant) and 2008 (0.6125 adults/plant), using yellow sticky cards in 2007 (0.7679 adults/card/day), and using destructive sampling in 2008 (0.8833 adults/plant). The same trends of *O. insidiosus* nymph, adult, and nymph plus adult mean counts were recorded using visual observations at R1 (silking) and R2

(blister) sampling periods in 2008. The *O. insidiosus* mean nymph, and nymph plus adult counts using destructive sampling supports the same trend at R2 (blister) sampling period in 2008.

Cry1Ab maize, Cry1Ab x CP4 EPSPS maize, CP4 EPSPS maize, CP4 EPSPS maize plus an insecticide application for control of second generation *O. nubilalis*, and conventional maize had revealed either no significant differences or significantly comparable nymphs, adults, and nymphs plus adults mean counts of *O. insidiosus* at two sampling periods for all sampling methods used. The populations of *O. insidiosus* varied significantly among three locations (Mead, Clay Center and Concord) using three sampling techniques either at R1 (silking) and R2 (blister) sampling periods in 2007 and or 2008.

The variation of *O. insidiosus* population abundance among the sites may be due to abiotic and biotic factors such as temperature and precipitation (Head *et al.* 2005). Consequently, development of *O. insidiosus* is very dependent on temperature and availability of the food supply (Sabelis and van Rijin, 1997, Copping 2004).

The results suggest that the sampling techniques used are effective in monitoring the population abundance of *O. insidiosus*. These results support some ecological field studies on non-target arthropods of transgenic maize. Al-deeb *et al.* (2001) reported that visual counts of *O. insidiosus* were made on *Bt* and non-*Bt* maize field at three locations in Kansas, and found out that *Bt* maize does not have significant effects on *O. insidiosus*. Musser *et al.* (2004) suggested the use of field counts of immature and predators because these counts are accurate, have no associated supply costs, and can be made quickly. On the other hand, Pilcher *et al.* (2005) used the same brand of yellow sticky cards (Phercon AM non-baited) that were used in the study to assess the impact of transgenic *Bt* maize including *O. insidiosus*. Their results showed that significantly higher numbers of adult *O. insidiosus* preferred the early planting date with both *Bt* events when analyses

**Table 3**

Mean adult counts of minute pirate bug [*Orius insidiosus* (Say)] on different transgenic and non-transgenic maize at R2 (blister) sampling period (90-95 DAP) using destructive sampling in Nebraska in 2008.

Particular	<i>Orius insidiosus</i> mean counts		
	Nymph	Adult	Nymph + Adult
(a) Treatments			
Cry1Ab maize	0.8667 c	0.7833 a	1.6500 b
Cry1Ab x CP4 EPSPS maize	0.9667 bc	0.9000 a	1.8667 b
CP4 EPSPS maize	0.7833 c	0.2667 b	1.0500 c
CP4 EPSPS maize + Insecticide-1 <sup>a</sup>	1.3000 ab	0.6833 a	1.9833 ab
CP4 EPSPS maize + Insecticide-2 <sup>b</sup>	1.4667 a	0.8833 a	2.3500 a
Cry1Ab x Cry3Bb1 x CP4 EPSPS maize	1.1000 bc	0.8000 a	1.9000 ab
	<i>P-value</i>	0.0040	0.0009
	<i>Significance</i>	**	**
(b) Sites			
Mead	1.0167 b	1.0500 a	1.8083 b
Clay Center	1.8833 a	0.3167 c	2.9333 a
Concord	0.3417 c	0.7917 b	0.6583 c
	<i>P-value</i>	0.0001	0.0001
	<i>Significance</i>	**	**

<sup>a</sup> CP4 EPSPS maize + Insecticide-1 = CP4 EPSPS maize applied with permethrin (Pounce® 1.5G) to control the first generation of *Ostrinia nubilalis*.

<sup>b</sup> CP4 EPSPS maize + Insecticide-2 = CP4 EPSPS maize sprayed with bifenthrin (Capture® 2 EC) to control the second generation of *Ostrinia nubilalis*.

<sup>c</sup>Numbers in a column for treatment and site followed by the same letter are not significantly different according to Fisher's protected LSD test for mean separation. \*\* highly significant at  $P \leq 0.0001$

were run across all locations, and years during the first *O. nubilalis* generation.

Transgenic maize (Cry1Ab or Cry3Bb1) had no observable effects on populations of *O. insidiosus* as measured by all three sampling methods. *Orius insidiosus* nymphs and or adults were fewer on insecticide treated CP4 EPSPS maize. These findings importantly support previous ecological field studies on non-target predators that transgenic maize does not have a significant effect on the predator *O. insidiosus* regardless of the sampling method (Pilcher *et al.*, 2005; Fernandes *et al.*, 2007).

Prasifka *et al.* (2005) suggested not to use small plots (width <9 m) for ecological studies on transgenic crops. With the significant differences recorded among the treatments of three sampling techniques, the use of experimental plot measuring 60 square meters (10 x 6 m) for transgenic maize is suggested for validation.

## CONCLUSIONS

Results from this research show that Cry1Ab maize, Cry1Ab x CP4 EPSPS maize, Cry1Ab x Cry3Bb1 x CP4 EPSPS maize, and CP4 EPSPS maize had no significant effect on *O. insidiosus* population abundance compared to CP4 EPSPS maize treated with insecticides. Nymph and adult populations of *O. insidiosus* is present in different transgenic and conventional maize at R1-R2 growth stages (silking-blister) in varying numbers across three sites at Mead, Clay Center and Concord Nebraska. Visual observations, yellow sticky cards, and destructive samplings are effective in monitoring techniques of *O. insidiosus* in field maize adult populations for a plot size area of 60 square meters. Findings importantly supports and strengthens non-target arthropod ecological field studies that Cry1Ab x CP4 EPSPS maize, Cry1Ab x Cry3Bb1 x CP4 EPSPS maize, and CP4 EPSPS maize have no impact to *O. insidiosus* populations.

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