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Optimizing Egg Recovery From Wild Northern Corn Rootworm Beetles (Coleoptera: Chrysomelidae)

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Abstract

The northern corn rootworm, *Diabrotica barberi* Smith & Lawrence (Coleoptera: Chrysomelidae), is one of the most important insect pests in the U.S. Corn Belt. Efforts to obtain eggs from wild northern corn rootworm populations using techniques developed for other rootworm species have been unsuccessful due to lack of oviposition. In 2016, we evaluated four oviposition media in choice tests within each of three female densities in 30.5 × 30.5 × 30.5 cm BugDorm cages. The number of eggs laid per female was significantly affected by female density and the interaction of female density × oviposition media, but oviposition was relatively poor in all oviposition media (1.2 eggs per female when averaging the three female densities and all oviposition media). Single females were also evaluated in nonchoice assays in 6 cm × 6 cm × 8 cm clear plastic boxes and averaged up to 108 eggs per female depending on the oviposition media. In 2017, the cumulative number of eggs laid per female in boxes with one female was not significantly different from the number of eggs laid per female in boxes with 3 females. In 2018, the cumulative number of eggs laid per female was not significantly different between female densities of 1, 3, 5, or 10 females per box. Total egg production per box therefore increased as female density increased. More than 27,000 wild northern corn rootworm eggs were collected from just 190 females when collected relatively early in the field season. We now have an efficient and robust system for obtaining eggs from wild northern corn rootworm females.

Key words: *Diabrotica barberi*, corn rootworm, rearing, oviposition, resistance management

Corn rootworms, including northern, *Diabrotica barberi* Smith & Lawrence (Coleoptera: Chrysomelidae), and western corn rootworms, *Diabrotica virgifera virgifera* LeConte, are the most economically damaging pests of maize (*Zea mays* L.) in the U.S. Corn Belt (Krysan 1986, Levine and Oloumi-Sadeghi 1991) with yield loss and control costs estimated to be $2 billion dollars per year (Wechsler and Smith 2018). Damage is caused primarily by larvae that feed on the roots and affect water and nutrient uptake by corn plants (Krysan 1986, Urías-López and Meinke 2001). Plant lodging can result in additional yield losses (Spike and Tollefson 1991) and is exacerbated by wind, rain, and high western corn rootworm larval numbers reducing root biomass (Chiang 1973, Levine and Oloumi-Sadeghi 1991). Crop rotation with unsuitable larval hosts, such as soybean, *Glycine max* (L.), has been the most effective management tactic for the northern corn rootworm and

western corn rootworm. However, for varying reasons, not every grower can utilize this management practice. The use of corn hybrids expressing *Bacillus thuringiensis* (Bt) Berliner has been widely used against corn rootworms since 2003 (Vaughn et al. 2005) and resistance evolution to commercially available Bt traits by the western corn rootworm has been documented in the field (Gassmann et al. 2011, Wangila et al. 2015, Jakka et al. 2016, Zukoff et al. 2016, Ludwick et al. 2017, Reinders et al. 2018). Recently, field-evolved resistance of northern corn rootworm to Bt corn has been reported (Calles-Torrez et al. 2018, 2019). Both western corn rootworm and northern corn rootworm have a history of adaptation to control measures and have independently evolved biotypes resistant to crop rotation (Krysan et al. 1986, Levine et al. 2002, Gray et al. 2009) and cyclodiene soil insecticides (Ball and Weekman 1962, Bigger 1963, Hamilton 1965) in addition to Bt corn.

To slow the evolution of resistant insect pest populations to Bt crops, the U.S. Environmental Protection Agency (EPA) has mandated that an insect resistance management program must be in place prior to registration of any Bt product. As part of the 2010 Bt corn registration extensions, the EPA required all registrants of corn rootworm targeted products to develop and implement a resistance management plan for northern corn rootworm (EPA 2010a) in addition to western corn rootworm. To achieve this goal, the EPA anticipated the need to investigate novel techniques for rearing and conducting bioassays with northern corn rootworm. For example, the Biopesticide Registration Action Document for Cry3Bb1 (EPA 2010b) included the following data requirement: 'Continuation of baseline susceptibility studies currently underway for WCR and initiation for NCR and monitoring techniques, such as discriminating dose concentration assays, as well as investigation of their feasibility as resistance monitoring tools'. Similar EPA data requirements were included in the registration process for other Bt proteins targeting corn rootworms, but according to the EPA, due to 'little success with NCR, population monitoring currently centers around the WCR'. In fact, members of ABSTC (Agricultural Biotechnology Stewardship Committee) were in agreement that even when sufficient northern corn rootworm populations were collected, obtaining sufficient eggs for use in bioassays has been challenging (M. Carroll [Bayer], T. Burd [Syngenta], personal communication). Northern corn rootworm simply did not lay eggs in sufficient quantities using techniques that had been developed for western corn rootworm. The recent documentation of Bt resistance in North Dakota northern corn rootworm populations (Calles-Torrez et al. 2018, 2019) increases the importance of obtaining sufficient egg densities from wild populations for Bt resistance monitoring. Therefore, the goal of the current work was to increase wild northern corn rootworm oviposition under laboratory conditions. Toward this goal, we evaluated the effect of female beetle density and oviposition media on northern corn rootworm egg production. Although not directly compared, two container sizes (6 cm × 6 cm × 8 cm boxes and 30.5 cm × 30.5 cm × 30.5 cm BugDorm cages) were used in these experiments.

Materials and Methods

Insects

First-year cornfields in northwest Missouri were found to have significant northern corn rootworm populations in 2016 (Today's Farmer Online 2016). Over 4,000 beetles were collected in 2016 (Table 1). Field-collected northern corn rootworms were brought to the laboratory and kept in 30.5 × 30.5 × 30.5 cm BugDorm cages (MegaView Science Co., Ltd., Taichung, Taiwan). Beetles were provided a diet of zucchini (*Cucurbita pepo* L.), dry artificial diet (AG_F9766B-M1, Frontier Agricultural Sciences, Newark, DE), and 0.9% agar with 0.1% sorbic acid as a water source until bioassays were initiated within 2 d. In 2017, more than 750 northern corn rootworm beetles were collected (Table 1). Adults

were maintained as previously described prior to use in bioassays. In 2018, northern corn rootworm field populations from four different locations were collected (Table 1).

Bioassays

In 2016, the effect of four oviposition media on northern corn rootworm oviposition in a no-choice and separate choice experiment was evaluated. Single female northern corn rootworm were held in 6 cm × 6 cm × 8 cm (288 cm³) clear plastic boxes (Althor Products LLC, Windsor Locks, CT; herein referred to as 'box') or multiple females were held in 30.5 × 30.5 × 30.5 cm (28,372 cm³) BugDorm cages (herein referred to as 'cages') during the no-choice and choice experiments, respectively. Each box or cage was considered a replicate. Oviposition media treatments for both experiments were as follows: 1) 60-mesh sieved soil that was wetted (~30% volume by weight) and stirred (Geisert and Meinke 2013); 2) wetted and stirred sieved soil with four germinated wheat seeds; 3) unsieved soil with ~1.0 to 1.5 cm diameter clumps of soil; and 4) unsieved soil with ~1.0 to 1.5 cm diameter clumps of soil with simulated wheat stubble (straw) on the soil surface. In the no-choice experiment, the four oviposition media were in a completely random design. Each box had a single female, so oviposition media was the only factor evaluated.

The choice experiment was designed as a split plot in space in which the treatments were arranged 3 × 4 factorial. The main plot was density (25, 100, or 500+ females) and the subplot was oviposition media and the interaction of female density × oviposition media. Females in the choice experiment were exposed to all four oviposition media simultaneously to evaluate potential media preference. The four types of oviposition media were set up in the same 6 cm × 6 cm × 8 cm small boxes used for the no-choice tests, but with their top off and placed in the cages. Replication varied among oviposition media treatments in the no-choice experiment; number of replicates per treatment included sieved soil (25 replicates), sieved soil+wheat (10 replicates), clumped soil (25 replicates), and clumped soil+straw (10 replicates). Those with 10 replications were started a day later. Number of replicates also varied across female densities in the choice experiment, with three replications of both 25 and 100 females per cage and four replications of 500+ females per cage. Adult food in each experiment included miniature field corn ears and their pieces, sweet corn ears and pieces, silk, and/or pieces of zucchini, and 0.9% agar with 0.1% sorbic acid as water source, replaced every Monday and Thursday. In the no-choice assays, beetles were transferred to new boxes on a weekly basis until all were dead (a maximum of 4 wk). In the choice assay, boxes with oviposition media were changed weekly until all were dead (a maximum of 8 wk). After removal from the cages, boxes containing eggs were left at room temperature for 2 wk, moved to 13°C for two additional weeks, and finally kept at 8.5°C for 6 mo to simulate fall and winter soil temperatures (Geisert and Meinke 2013). After 6 mo, the boxes were removed from refrigeration to evaluate oviposition. This procedure was adopted in the same way as for the boxes from the no-choice

Table 1. Northern corn rootworm beetle collections

Date collected	Beetles collected	Collection county	State	Experimental location
4 Aug. 2016	>4,000	Nodaway	MO	Columbia, MO
28 July 2017	>750	Nodaway	MO	Columbia, MO
26 July 2018	>600	Nodaway	MO	Columbia, MO
7 Aug. 2018	>500	Floyd	IA	Columbia, MO
13 Aug. 2018	>500	Webster	IA	Polk City, IA
15 Aug. 2018	>500	Cuming	NE	Lincoln, NE

assay. While not ideal, different egg recovery techniques were necessary for the different media types. For boxes with sieved soil, eggs were extracted by washing the contents with tap water through a 60-mesh sieve and recovered eggs were then counted individually for each oviposition box. For boxes with clumped soil, eggs were extracted from soil via salt flotation in solution. The solution was 2 M magnesium sulfate (Epsom salt U.S.P., Hy-Vee, Inc, West Des Moines, IA) and was used in a 2-liter graduated cylinder (Števo and Cagáň 2012). Eggs and debris were removed from the solution's surface, placed into separate vials, and then eggs were counted individually for each oviposition box. We recognize that differences in egg production observed are confounded with potential differences in egg recovery techniques, but the point of this work was to compare the number of eggs recovered and available for future work and this what is reported. When eggs per female were analyzed, total eggs were divided by the total number of females present at the beginning of the experiment.

In 2017, two female densities (1 and 3 females per box) were evaluated using wetted and stirred 60-mesh sieved soil oviposition media in the 6 cm × 6 cm × 8 cm boxes. Boxes and adult food and water sources were as described for 2016 experiments. Females were moved to a new box each week as in 2016. Twelve replicates with both one and three females per box were evaluated. Adult food and water sources were as described for 2016 experiments. Rather than overwintering prior to egg washing, eggs were washed from the sieved soil oviposition media through a 60-mesh sieve and counted weekly until adult mortality occurred or egg-laying ceased.

In 2018, the four northern corn rootworm populations collected from Missouri, Iowa (two populations) and Nebraska sites were evaluated for oviposition in four different densities: 1, 3, 5, or 10 females per box as previously described, except beetles collected in Webster Co, IA were placed in 3 cm × 3 cm × 6.5 cm boxes (all

with 10 replications). Assays of the Missouri and Floyd Co, Iowa populations were conducted at the Plant Genetics Research Unit, USDA–ARS in Columbia, MO; the Webster Co, IA population was assayed at the DM Crop Research Group Inc., facility, Polk City, IA; and the Cuming Co, NE population was assayed at the University of Nebraska, Lincoln. Each lab followed the same assay protocol. A single oviposition media (wetted and stirred sieved soil, previously described) was used for all assays, and food and water were provided as previously described. Ten replicates of each female density were initiated shortly after collecting each northern corn rootworm population. Each week, remaining living females were transferred to new boxes, and eggs were washed through a 60-mesh sieve and then counted until all adults were dead (a maximum of 8 wk for the Missouri population).

Statistical Analysis

All data were analyzed using SAS 9.4 software (SAS Institute, Cary, NC). In 2016, the larger cage choice experiment was designed and analyzed as a split plot in space in which the treatments were arranged 3 × 4 factorial. The main plot was female density (25, 100, or 500+ females within a cage). The denominator of the main plot was cage within density. The subplot contained the effect of the oviposition media (clumps, clumps + straw, sieved soil, or sieved soil + wheat seedlings as described) and the interaction of female density × oviposition media. The residual mean square was used as the denominator of the F for the subplot effects. Because Bartlett's test for unequal variance was significant for the untransformed data, we used square root $(x + 0.5)$ transformation prior to analysis, but show the untransformed data in Fig. 1. The no-choice experiment with single females in boxes was set up and analyzed as a completely randomized design to compare oviposition in nonchoice
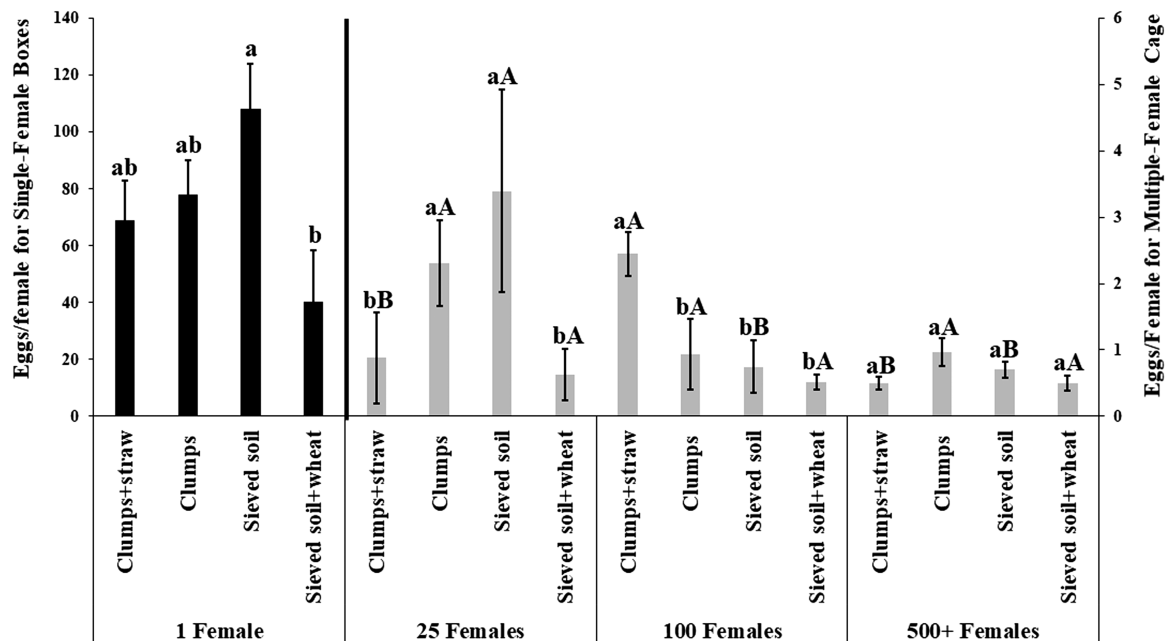

Fig. 1. Average cumulative number of eggs per female (±SE) from wild northern corn rootworm collected in 2016 using 6 × 6 × 8 cm boxes containing one female (black bars, left y-axis) or using 30.5 × 30.5 × 30.5 cm BugDorm cages with three different densities (25, 100, or >500 females per cage; gray bars, right y-axis). Each experiment had four different oviposition media. The single female per box experiment was a no-choice test. Oviposition in the multiple female cage experiment involved choice tests with all four media in each cage. Two y-axis were used due to large differences in oviposition. Bars followed by different lowercase letters are significantly different within each female density. Bars followed by different uppercase letters are significantly different between female densities (LSMeans using PROC GLIMMIX in SAS 9.4, at $P < 0.05$).

tests in the four types of oviposition media. Bartlett's test was run first to verify equality of variance. Then the glimmix analysis was followed by an LSMeans statement in SAS to run a Fisher's protected multiple range test to determine significant differences among means. In 2017, there were only two treatments, so a simple *t*-test was run. Because a check for unequal variance indicated that the variances were unequal, Cochran's *t*-test was used to compare the two means. In 2018, the cumulative number of eggs per female and the total number of eggs per cage were analyzed separately. Bartlett's test for unequal variance was significant for the untransformed data, sqrt(*x* + 0.5), and log(*x* + 1) data for both the cumulative number of eggs per female and the total number of eggs per cage, so all data were rank transformed prior to analysis as outlined by Conover and Iman (1981). The data were then analyzed as a split plot in which the treatments were arranged 4 × 4 factorial. The main plot was location (Nodaway Co, MO; Floyd Co, IA; Webster Co, IA; and Cuming Co, NE), and the subplot was female density (1, 3, 5, or 10 females) and the interaction of female density × location. The denominator of *F* for the main plot was replication within location. The denominator of *F* for density and density × location was the residual mean square.

Results

In 2016, a significant effect of oviposition media on northern corn rootworm oviposition in the no-choice experiment (one female per box) was observed ($F = 2.88$; df = 3, 66; $P = 0.0427$). For this, the mean eggs per female laid in sieved soil were significantly greater than the mean in sieved soil with wheat seedlings, but other differences were not significant (Fig. 1). However, in choice tests using multiple northern corn rootworm females, the main effect of oviposition media was not significant ($F = 2.86$; df = 3, 21; $P = 0.0614$), whereas the main effect of female density was significant ($F = 4.84$; df = 2, 7; $P = 0.0480$) and the interaction of female density × oviposition media type was also significant ($F = 3.7$; df = 6, 21; $P = 0.0114$). Although significant differences in eggs per female were observed among treatments at higher female densities in the 30.5 × 30.5 × 30.5 cm cages (Fig. 1), elaborating on these would detract from the take-home message because the total number of eggs per female was relatively small compared with females in the smaller boxes. Averaging oviposition across the four media, an average of 74.0 ± 15.0 eggs were laid per female in the 6 cm × 6 cm × 8 cm boxes (including 108.3 ± 15.7 for the sieved soil treatment), whereas 1.8 ± 0.81, 1.2 ± 0.34, and 0.67 ± 0.13 eggs per female, respectively, were laid by 25, 100, or 500+ female treatments when also averaging across the four oviposition media. Total oviposition numbers per female from the larger number of females in larger cages cannot be statistically compared with total oviposition numbers from 6 cm × 6 cm × 8 cm clear plastic boxes with a single female. However, given the nearly 100-fold difference, we used the smaller plastic boxes for 2017 and 2018 experiments.

In 2017, when oviposition in the one female and three female per box treatments was compared, there was not a significant difference (Cochran $t = 0.24$; df = 11; $P = 0.8139$) between cumulative number of eggs laid per female (Fig. 2). In 2018, the effect of location (confounded with beetle collection date differences) was highly significant ($F = 19.63$; df = 3, 36; $P < 0.0001$), but the effects of female density ($F = 1.26$; df = 3, 108; $P = 0.2924$) and location × female density ($F = 0.75$; df = 9, 108; $P = 0.6595$) were not significant for eggs per female. The population collected first (Missouri) had significantly more eggs per female than all other populations,
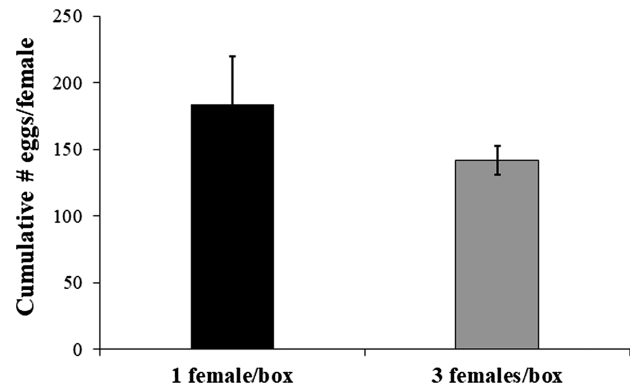

Fig. 2. Average cumulative number of eggs per wild northern corn rootworm female (±SE) from 6 × 6 × 8 cm boxes with one or three females. Beetles were collected in 2017 in Northwest of Missouri. No significant differences existed in the cumulative number of eggs between treatments (Cochran's *t*-test in SAS 9.4, at *P* < 0.05).

and the population collected second (Floyd Co., IA) had significantly more eggs per female than the last two populations. The last two populations, collected only 2 d, apart were not significantly different (Fig. 3). When analyzing total eggs per box the effect of population (again confounded with beetle collection date differences) was highly significant ($F = 24.33$; df = 3, 36; $P < 0.0001$), as was the effect of female density ($F = 102.53$; df = 3, 108; $P < 0.0001$). However, the interaction of location × female density ($F = 1.54$; df = 9, 108; $P = 0.1444$) was not significant. Similar to the eggs per female results, the population collected first (Missouri) had significantly more eggs per box than all other populations, the population collected second (Floyd Co., IA) had significantly more eggs per box than the last two population, and the last two population, collected only 2 d apart were not significantly different (Fig. 3). The 10 females per box treatment produced more total eggs compared with all other densities, the five females per box treatment produced more than the three or one female per box treatments, and the three females per box treatment produced more total eggs than the treatment with just one female per box (Fig. 3).

Discussion

We now have an efficient and robust system for obtaining eggs from wild northern corn rootworm females after the addition of the 2018 data from four different northern corn rootworm populations including oviposition data collected in three different laboratories. The 2016 study clearly documented that relatively few eggs per female were laid when northern corn rootworms were in the 30.5 × 30.5 × 30.5 cm BugDorm cages often used to maintain other corn rootworm species (Meihls et al. 2008, 2012; Pereira et al. 2019). For unknown reasons, northern corn rootworm oviposition in the small plastic boxes was much higher (Fig. 1), but these differences could not be compared statistically. In 2016, this difference was confounded with differences in beetle density and suggested the possibility of an oviposition-deterring pheromone because single females laid nearly 100-fold more eggs (depending on the comparisons evaluated) than females at higher densities (Fig. 1). The 2017 experiment was focused on supporting or refuting an oviposition-deterring pheromone hypothesis. Because there was no significant difference in the number of eggs laid per female between one or three females per box (Fig. 2), data did not support our initial hypothesis of an oviposition-deterring pheromone altering egg laying.
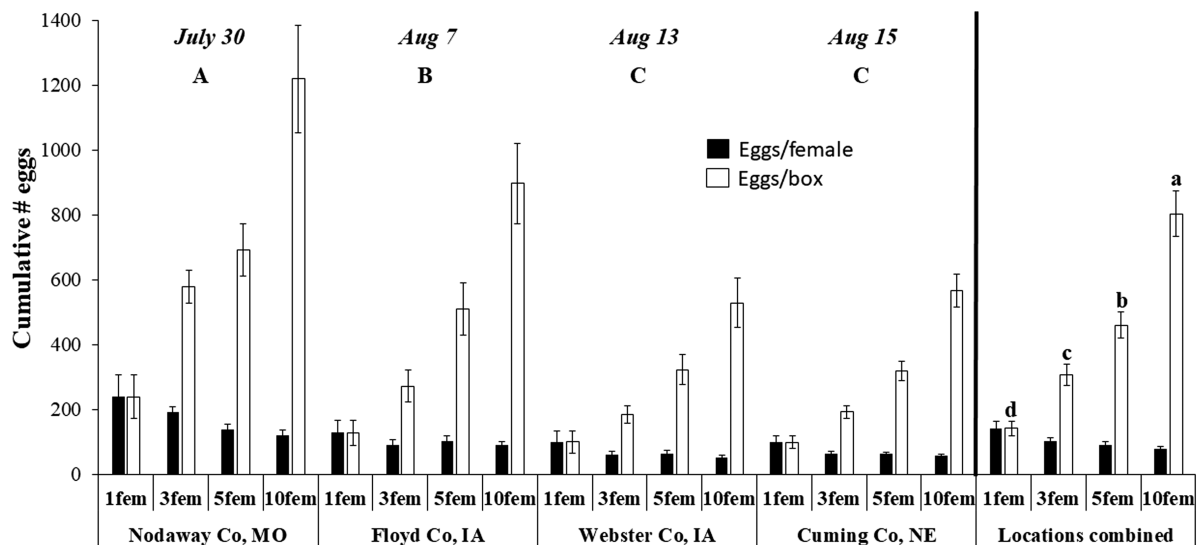
Fig. 3. Cumulative number of eggs per female (±SE; black bars) and total eggs per box (white bars) at four different wild northern corn rootworm female densities per 6 × 6 × 8 cm box laid by females collected in Missouri, Nebraska, and Iowa (two different sites) in 2018. Collection dates for each population indicated above the bars. Significant differences between locations (both for total eggs laid per box and eggs per female) are noted by different uppercase letters. Different lowercase letters indicate significant differences between female densities in the overall analysis (LSMeans using PROC GLIMMIX in SAS 9.4, at $P < 0.05$).

Results of the 2018 assays indicate that at least 10 females can be placed in the smaller boxes without significantly reducing the number of eggs laid per female. This provides an efficient system for obtaining eggs from wild northern corn rootworm. Although the collection of wild northern corn rootworm eggs is not novel (Naranjo and Sawyer 1987, Boetel and Fuller 1997, Campbell and Meinke 2010, Geisert and Meinke 2013), the efficiency of the method demonstrated here will greatly aid in obtaining the large amounts of eggs needed for monitoring resistance to Bt traits. For example, an average of 1,219 ± 165.4 eggs per small box from 10 females was obtained from the Missouri population in the 2018 experiment. Including all female densities from Missouri, 27,284 wild northern corn rootworm eggs were obtained from the 190 field-collected females. An additional ~20,000 wild northern corn rootworm eggs were obtained from 150 females at 5 females per box density from the Missouri location. That is more than 47,000 eggs from 340 females from just the Missouri location. Based on our field collections, we collected approximately 66% females from the field, overall. Therefore, nearly 50,000 eggs from a typical collection of about 500 wild northern corn rootworm beetles could be expected based on results of this study if beetles are caught relatively early in the oviposition period. Although a number of small boxes must still be maintained, the fact that at least 10 females can be placed in an individual box reduces the work load considerably versus previous practice of one female per box (Campbell and Meinke 2010, Geisert and Meinke 2013) and increases efficiency. If fewer northern corn rootworm are collected, fewer females per cage can be added and this will increase the number of eggs laid per female (Fig. 3). In fact, the number of eggs laid per female from one female in a box was nearly significantly greater than the number of eggs laid per female when 10 females were in a box ($t = 1.85$; df = 108; $P = 0.0666$) when this unprotected comparison is made. However, when plenty of females are available, grouping them together in boxes will increase efficiency.

Extended diapause can potentially help delay resistance evolution (Geisert and Meinke 2013) but would also be another possible constraint to Bt resistance monitoring. Varying levels of extended diapause exist among populations (Geisert and Meinke 2013), so it will be important to document level of extended diapause to determine egg cohort sizes that will be needed for future Bt bioassays after each simulated winter. Extended diapause was not previously known from Missouri (Today's Farmer Online 2016), and we found that from beetles collected in Missouri, approximately 40–45% of eggs hatched in the first year and between 12 and 20% hatched in the second year (data not shown).

From each of the four locations collected in 2018, a total of 190 females were evaluated for oviposition with 1, 3, 5, or 10 females per box. Overall, fewer total eggs were recovered when adult collections took place later in the field season. The 190 females from the 30 July 2018 collection (Nodaway Co, MO) yielded 27,284 wild northern corn rootworm eggs, the August 8 collection (Webster Co, IA) yielded 18,073 eggs, the August 13 collection (Floyd Co, IA) yielded 11,367 eggs, and the August 15 collection (Cuming Co, NE) yielded 11,784 eggs. Our data fit well with the only available literature on this topic which reports that females collected later in the season lay fewer eggs and die earlier compared with females collected in early season (Boetel and Fuller 1997). Although northern corn rootworm females have been reported to oviposit for at least 12 wk, ~50% of northern corn rootworm oviposition occurred within the first 4 wk of adult emergence (French and Hammack 2010), and oviposition declined gradually each week from a maximum of around 75 eggs in week 2 to less than 15 eggs in week 12. In fact, the laboratory northern corn rootworm colony from USDA–ARS in Brookings, SD, discards colonies after their third week of survival with only 2 wk of possible oviposition. Compared with western corn rootworm, duration of northern corn rootworm oviposition is 20–28 d shorter, which makes northern corn rootworm oviposition a limiting factor in terms of performing bioassays (Naranjo and Sawyer 1987). Clearly, if the peak portion of the oviposition window is missed because of later adult collections, egg yield per female is reduced. Overall, the highest number of eggs recovered in a single female box in 80-mesh sieved soil was 239 ± 66.8 from the Missouri collection in 2018 (Fig. 3). The 2018 total oviposition per female were similar to other studies in other locations of the U.S. Corn Belt that utilized

similar oviposition media (Naranjo and Sawyer 1987, Boetel and Fuller 1997, Geisert and Meinke 2013).

One impetus for this research was the concern of northern corn rootworm evolving resistance to Bt corn hybrids and reducing the longevity of these products in the market. Compounding this concern, it is known that there is cross-resistance among three of the four Bt toxins commercially available for western corn rootworm management (Gassmann et al. 2014, Wangila et al. 2015, Zukoff et al. 2016). The recent reports of northern corn rootworm resistance evolution to Bt corn hybrids in North Dakota (Calles-Torrez et al. 2018, 2019) highlight the need for testing susceptibility of northern corn rootworm field populations from the U.S. Corn Belt to Bt proteins as soon as possible.

In conclusion, this study shows that female density (up to 10) in the boxes does not significantly affect individual northern corn rootworm fecundity, but the cumulative number of northern corn rootworm eggs was significantly higher with 10 females per box than for other female densities evaluated in this study (Fig. 3). Although experimental design did not allow direct comparisons, we conclude that the size of the cage is very important, and that small, clear plastic boxes were superior to 30.5 × 30.5 × 30.5 cm Bugdorm cages for northern corn rootworm egg production (Fig. 1). Oviposition media played a statistically significant, but relatively minor role in fecundity (Fig. 1). We recommend sieved soil because egg washing is much easier than from clumped soil. Additional trials with other densities (i.e., more than 10 females per box compared with lower female densities) and/or leaving females in the same box for more than 1 wk may be desired when large numbers of beetles are collected to obtain an optimal density relative to effort with maximum oviposition by northern corn rootworm females in mind.

Acknowledgments

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