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Article

Bioassay arenas for studying the biology and ecology of adult western corn rootworm

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The western corn rootworm (*Diabrotica virigifera virgifera* LeConte; Coleoptera: Chrysomelidae) is one of the most destructive maize pests in the USA (Levine & Oloumi, 1991; Meinke et al., 2009). It has been in an invasive phase in Europe since the early 1980s (Guillemaud et al., 2010; Szalai et al., 2011), and is currently causing considerable economic problems in maize production of Central and South-eastern Europe, as well as northern Italy (Dillen et al., 2010; Wesseler et al., 2010).

Diabrotica v. virgifera also belongs to one of the best-studied insect pests (Levine & Chan 1990). Approximately 1440 grey and peer reviewed papers or conference proceedings have been published on this pest between 1910 and 2011 (CAB Abstracts 2012). There is considerable knowledge on the management of its larvae and adults (Levine & Oloumi, 1991; Vidal et al., 2005). This includes, for example, preventive control measures such as crop rotation or the choice of tolerant or transgenic maize hybrids (Ivezic et al., 2009), as well as direct control measures, such as biological control (Toepfer et al., 2009) or chemical control (Rozen et al., 2010). Also the pest`s behaviour, ecology and population dynamics are well understood (Spencer et al., 2009; Meinke et al., 2009).

To support studies on this pest, several methods had been established for mass rearing *D. v. virgifera* adults, larvae and eggs (Branson et al., 1975; George & Ortman, 1965; Guss et al., 1976; Krysan & Miller, 1986). Consequently a number of different rearing cages are proposed, mainly based on different set-ups of gauze cages for adults and plastic trays with germinated maize only or with germinated maize in sterilised soil for the larvae.

However, there are few publications that include bioassay arenas. For example, Knolhoff, (2006; 2009) used cylindrical bioassay arenas with different crop plants and an inverted funnel to force adult beetles to search for the food. This was, for studying the searching and feeding behaviour of rotation tolerant rootworm populations. For a comparable research question, O`Neal et al. (2004) used two-chamber emigration arenas to assess the propensity of adult populations to leave maize during or after

anthesis. In another assay, they used a side-arm olfactometer to test whether the relative attraction of adult beetles to other crops such as soybean was influenced by maize phenology. Toepfer et al. (2005) used metal stands on an inverted plastic funnel, surrounded by a water filled petri dish, in a gauze cage to evaluate fight take-off responses of differently marked and unmarked *D. v. virgifera* adults. As similar device was used by Li et al. (2010) to study activity difference between *D. v. virgifera* populations. A vertical tube apparatus was used to investigate the crawling activity of *D. v. virgifera* adults in response do different diets (Mabry et al., 2004). Strnad and Dunn (1990) used square glass plates (930 cm^2) with moist filter paper to record the searching behaviour of neonate *D. v. virgifera* larvae for maize roots and other potential host plants. Xie et al. (1992) modified this method by using sealed 90 mm plastic petridishes to test the behavioural responses of *D. v. virgifera* larvae to hydroxamic acids applied to maize roots on filter paper. However, Knutson et al. (1999) concluded from comparable behavioural trials, that such bioassays are little useful for differentiating hosts or orientation cues of *D. v. virgifera* larvae. Nowatzki et al. (2006, 2008) developed feeding-behaviour and lethal-time bioassays for adult beetles based on 90 mm plastic petridishes, filter paper, and toxin treated agars or cellulose membrane disks. These assays allowed assessing *D. virgifera* resistances to toxins.

Altogether, however, it appeared that there is no standardised bioassay arena available for *D. v. virgifera*. We therefore propose two bioassay arenas, one for studying single *D. v. virgifera* adults or pairs of *D. v. virgifera*, and one for studying a small standardised populations of up to 20 pairs. The first was used for studying phenotypic traits of individual beetles from different *D. v. virgifera* populations under standardised conditions (Li et al., 2009; 2010); but can be used for many other standardised studies on the biology, ecology and behaviour of single or paired *D. v. virgifera* adults. The second was used for standardised crossings and rearing of differentiated small populations of *D. v. virgifera* (Li et al., in prep.), but can be used for many other standardised studies on the biology and ecology of small populations of *D. v. virgifera* adults.

Small size bioassay arena

The bioassay arena consists of two ca. 140 to 200 cm^3 transparent plastic cups (e.g. urinalysis cups of 48 mm diameter, 80 mm high, 145 cm^3, Fig. 1), stacked one inside the other (herein referred to as the upper and lower cups). The upper cup has a lid with a mesh-covered hole of 10 to 15 mm diameter to provide adequate ventilation and to prevent the beetles from escaping the arena. The upper cup also has a 10 mm hole in the bottom to allow the female to gain access to a lower, soil-filled cup for egg-laying. The bottom of the upper cup and the lower 20 mm of the wall of the lower cup are painted black to provide a dark environment for oviposition (Sherwood et al., 1993).

For most research questions, it might be advisable to work with newly emerged male–female pairs. Emerged adults can be sexed according to antenna length (Kuhar & Youngman, 1995; Staetz et al., 1976) and tarsus characteristics (Hammack

& French, 2007). Males that have emerged before females (see protandry in *Diabrotica sp*. in Branson, 1987) can be excluded from a study, when similar age of males and females is required in an experiment. Then, males and females emerging the same day can be transferred as single male–female pairs into the rearing arenas. Each individual adult can receive a unique identification number. Abundant food can be provided into each arena depending on the aim of the experiment. Li et al. (2009), for example, provided unlimited food, i.e. of two soft unripe kernels from organically produced sweet maize, one 13 x 13 x 13 mm cube of zucchini flesh, one 13 x 13 x 13 mm cube of pumpkin flesh, and 5 x 5 x 5 mm of artificial pollen diet (Branson & Jackson, 1988; Singh & Moore, 1999). A 10 x 5 x 5 mm cube of 15% aqueous agar served as a water source for the adults. In this case, food and agar are changed every 5–7 days. Whatever the aim of the experiments is, the pairs of *D. v. virgifera* in the bioassays can be provided with the same amount of food, as *D. v. virgifera* vigour and behaviour is known to be influenced by an individual's recent diet experience (Mabry & Spencer, 2003; Mabry et al., 2004). After 10 days of maturation (Branson & Johnson, 1973; Hill, 1975) two teaspoons, i.e. a 5 to 10 mm layer, of moist, sterile black and non-sticky field soil (sieved at 0.15 mm; 25–35 w% moisture) can be placed into the lower cup of the bioassay arena. *Diabrotica v. virgifera* prefers dark soil for oviposition, but sieved sand can be also used depending on the aim of the experiment. The lower cup of the bioassay arena (containing soil and eggs) can be regularly removed (e.g. every 7 or 14 days), and replaced with a new one to collect the soil for assessing adult egg laying. Care has to be taken that females are not removed through this process as they like to sit in the soil for oviposition. Collected soil can be sieved through a 0.2-mm sieve, and recovered eggs (they are of 0.4 to 0.6 mm size) can be counted. With this approach, the age-specific and cumulative fecundity and realized lifetime fecundity can be assessed for each individual female. Moreover, individual adult life span can be assessed by regularly checking the bioassay arena, i.e. the lower and upper cups for live and dead adults. The date of death can be recorded and dead adults removed.

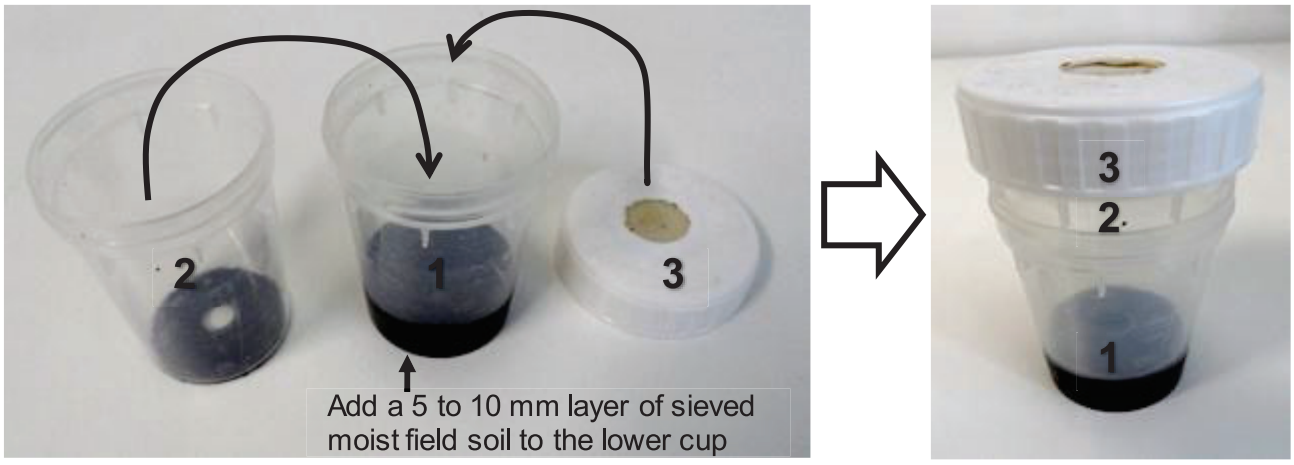


Fig. Small size bioassay arena for investigating and/or rearing single pairs of *Diabrotica v .virgifera* under standardised laboratory conditions.

Medium size bioassay arena

The bioassay arena consists of one ca. 1000 to 2000 cm^3 transparent plastic cylinder (e.g. a food cylinder of 110 mm diameter, 155 mm high, 1500 cm^3, Fig. 2). The cylinder has a lid with a 60 to 80 mm mesh-covered hole to provide ventilation and to prevent the beetles from escaping the arena. A well-closing, soil-filled petridish is added into the bottom of the cylinder for egg laying (e.g. a 90 mm diameter petridish, 20 mm high, 160 cm^3). The petridish has three 10 mm holes in the top to allow the females to gain access to the soil. The petridish contains four tablespoons i.e. a 10 mm layer, of moist, sterile, black and non-sticky field soil (sieved at 0.15 mm; 25–35 w% moisture). *Diabrotica v. virgifera* prefers dark soil for oviposition, but sieved sand can also be used depending on the aim of the experiment. Both, the bottom and the lower 25 mm of the cylinder, as well as the petridish lid are painted black to provide a dark environment for oviposition (Sherwood et al., 1993).

For most research questions, it might be advisable to work with newly emerged male–female pairs (see above). About 10 to 20 pairs of males and females, emerging the same day, can be transferred into the arenas. Food can be provided into each cylinder depending on the aim of the experiment. One could multiply the diet amounts proposed by Li et al. (2009) for single adult pairs (as above) with the number of pairs transferred into the arena. Food and agar should be changed every 5–7 days. All groups of *D. v. virgifera* pairs in the bioassay arenas can be provided with the same amount of food, and held under similar conditions. Every 7 or 14 days, the beetles are shortly removed with an aspirator device, and the petridish (containing soil and eggs) exchanged for a new one. Then beetles are returned to the arena. Soil can be sieved through a 0.2-mm sieve, and recovered eggs counted. With this approach, the age-specific and cumulative fecundity and realized lifetime fecundity can be assessed for a group of adults. Adult life span can be assessed by regularly checking the bioassay arenas for live and dead adults. Moreover, the transparency of the cylinder allows direct observations of adult behaviour. The medium size arena is also useful to conduct standardised crossings of males and females from different *D. virgifera* populations, and to collect their hybrid-offspring-eggs.

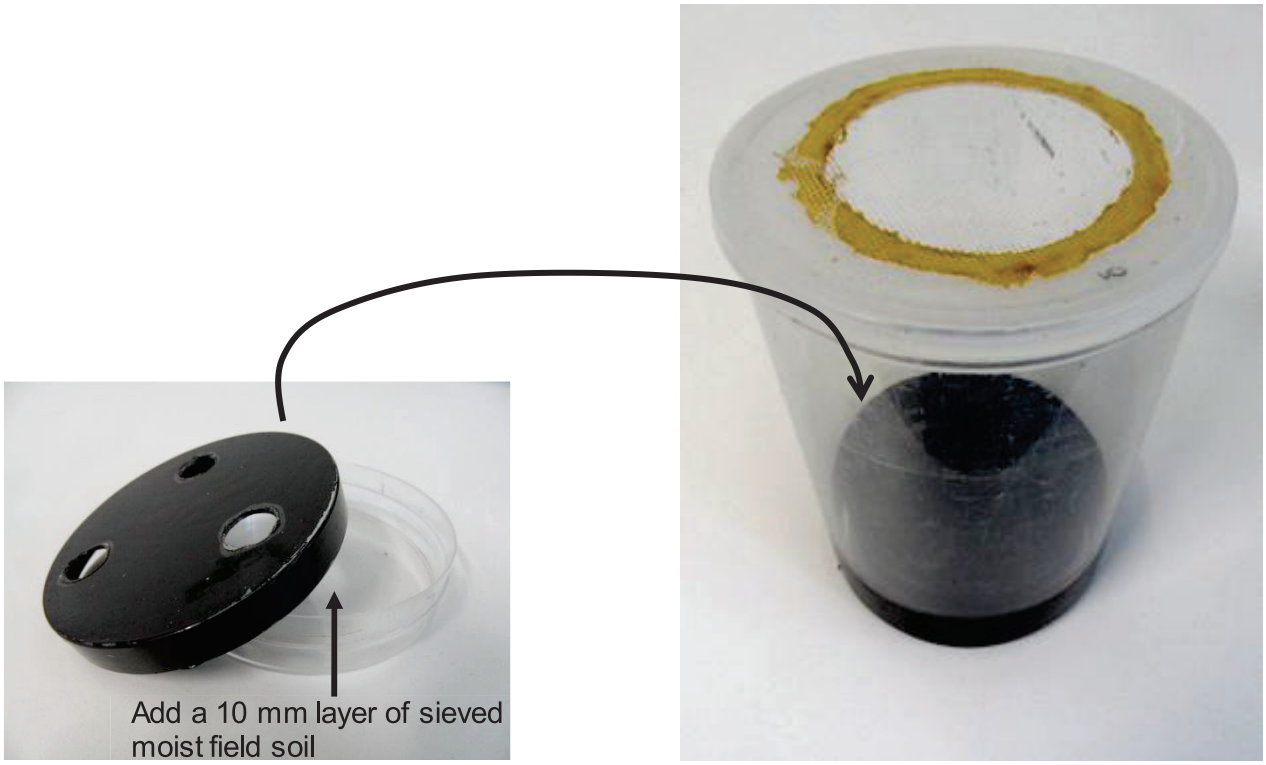


Fig. Medium size bioassay arena for investigating and/or rearing multiple pairs of *Diabrotica v. virgifera* under standardised laboratory conditions.

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