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REPORT

Cell lines derived from the western corn rootworm larvae, *Diabrotica virgifera virgifera* (Chrysomelidae: Coleoptera)

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Insect cell lines serve as valuable tools in screening potential insecticides, the production of recombinant proteins, and the understanding of insect biology (Smagghe et al. 2009; Smagghe and Swevers 2012; Zitzmann et al. 2017). Our lab is focusing on the establishment of "next generation" insect cell lines that are generated from specific tissues or from under-represented species (Reall et al. 2019; Zhou et al. 2019). To date, no cell lines have been established from the western corn rootworm (WCR), *Diabrotica virgifera virgifera*. This insect is a major pest of maize and occurs very broadly, including North America and Europe (Gray et al. 2009, Wesseler and Fall 2010, Marchioro and Krechemer 2018, https://www.cabi.org/isc/datasheet/18637#toDistributionMaps). Larvae consume maize roots and adults feed on silks, pollen, leaves, and kernels. Crop losses due to WCR, and associated control costs, exceeded $2 billion worldwide (Wechsler and Smith 2018). Additionally, the WCR has become resistant to control technologies, including insecticides, crop rotation, and insecticidal toxin-producing maize (Paolino and Gassmann 2017). The only *Diabrotica* spp. cell line currently available is from the southern corn rootworm, *Diabrotica undecimpunctata* (Lynn and Stoppleworth 1984), a pest with a much different plant host range (Marchioro and Krechemer 2018). Availability of WCR cell lines will enable species-specific testing of biologicals as well as in-depth investigations into genomic and physiological characteristics.

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We report here on three cell lines established from *D. virgifera virgifera* larvae. Insects were obtained from the Plant Genetics Research Lab, USDA-ARS laboratories (Man Huynh, B. Hibbard) in Columbia, MO, and reared on artificial diet in 96-well-plates in complete darkness at 25°C (Huynh et al. 2017). Third instar larvae were surfaced sterilized using 95% ethanol (to anesthetize, 3 min), 0.525% sodium hypochlorite (5 min), and 70% ethanol (5 min), rinsed with media, and gently crushed with a plastic pestle in 0.5-mL medium, then minced with micro scissors. The tissue suspension was centrifuged (800×*g*, 5 min, 4°C) and the resulting pellet was washed three times in CMF-PBS (calcium magnesium-free phosphate buffered saline). The final pellet was brought up in EX-CELL 420 + 9% FBS containing 50 U/mL penicillin and 0.05 mg/mL streptomycin (Sigma-Aldrich, St. Louis, MO) and transferred into three T_{25} flasks (Falcon, Fisher Scientific, Waltham, MA). Cultures were maintained at 28°C, and 1/2 media was replaced approximately biweekly, then weekly, then full media replacement. Cultures were initiated on June 17, 2017, and first passages were generated on February 12, 2018, for DvWL1 (via pipetting), October 23, 2017, for DvWL2 (using 0.5% trypsin-EDTA, Sigma-Aldrich), and October 2, 2018, for DvWL3 (using trypsin). Actively growing cultures are currently passaged using trypsin (3 min, DvWL1; 4–5 min, DvWL2 and DvWL3). All three lines have each been passaged over 40 times.

The cell lines consist of well-attached cells with a variety of morphologies, including spherical, flattened multi-sided (epithelial-like), and elongated cells (fibroblast-like) (Fig. 1). The spherical cells ranged in diameter from 7 to 50 μm (DvWL1 and 2) and 8–27 μm (DvWL3). The multi-sided and elongated cells ranged from 10 × 8 to 85 × 10 μm (DvWL1), 16 × 16 to 77 × 27 μm (DvWL2), and 16 × 16 to 166 × 14 μm (DvWL3). The DvWL3 line also contains networked cells

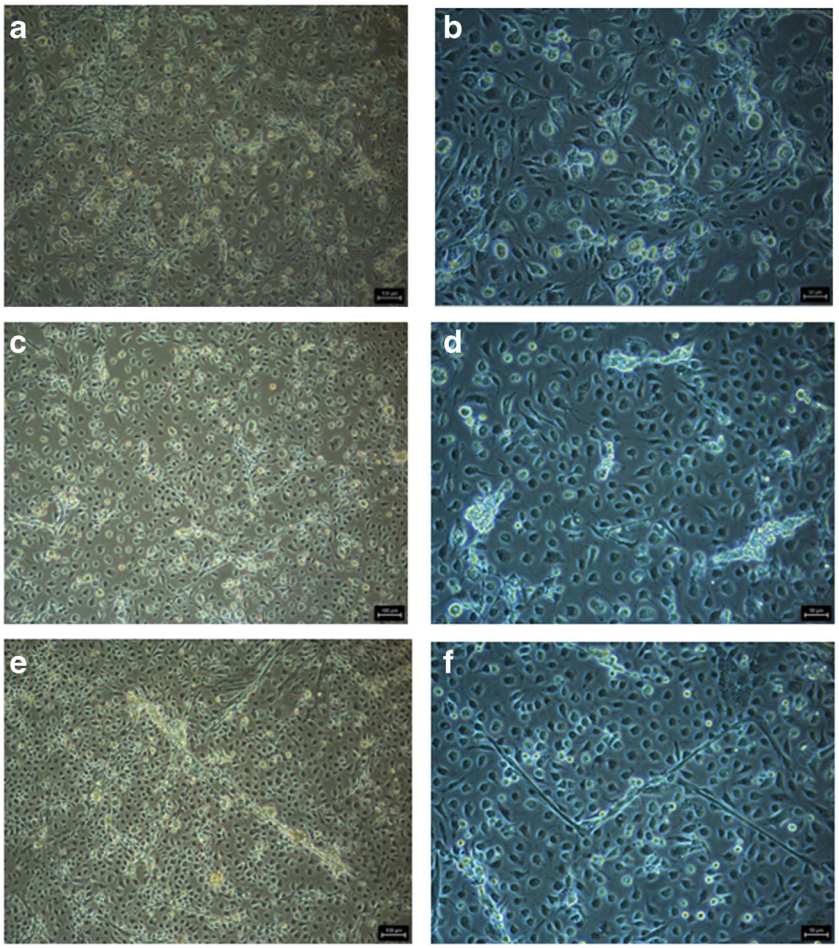

Fig. 1. Representative images of WCR cell lines from whole larvae at two magnifications (× 100, × 200). (*a*, *b*) DvWL1, P37. (*c*, *d*) DvWL2, P41. (*e*, *f*) DvWL3 (with networked cells that are contracting), P18

that continued to contract even up to the 18th passage (Fig. 1, e and f).

The identities of the three cell lines were authenticated using DNA fingerprinting (DAF-PCR) with the methods described by McIntosh et al. (1996) and modified by Reall et al. (2019). Primers used were mammalian aldolase, prolactin, and interleukin (Fig. 2). The resulting DNA patterns from the DvWL cell lines were similar to one another and to the larval tissue, but different from cell lines from other species and orders.

Growth curves were generated by seeding 1×10^5 cell/mL (5 mL/flask) into T_{25} flasks (Corning, Corning, NY), and cell counts were determined using a live cell imager after optimizing the protocol for each cell line (Lionheart FX, BioTek, Winooski, VT). Thirty-six areas of each flask were counted and combined to generate the final cell count per flask per time point. Cells were initially fed the day after set up, to ensure medium was free from debris that could impact the imaging process. Cells were monitored until they had completed their log growth phase (Fig. 3). If this lasted beyond a week, cells were fed 7 d after the last feeding. The linear portion of the growth curves were used to determine the doubling times (http://www.doubling-time.com/compute.php), 102 h for DvWL1, 51 h for DvWL2, and 67 h for DvWL3.

The cell lines reported here are the first established from *D. virgifera virgifera*. A variety of lines have been established from other coleopterans. Cellosaurus, the online database https://web.expasy.org/cellosaurus/ (accessed on 2/3/2020), shows 36 cell lines based on searches using the keywords "Coleoptera" or "beetle". Other cell lines from whole larvae include RIRI-BR1, from *Blaps rhynchoptera* (Zhang et al. 2014). This line exhibits a diversity of morphologies with diameters for spherical cells ranging from 8 to 20 μm to dimensions of spindle-shaped cells ranging from 10 to 30 μm × 5–12 μm. These cells have a doubling time of 79.5 h,

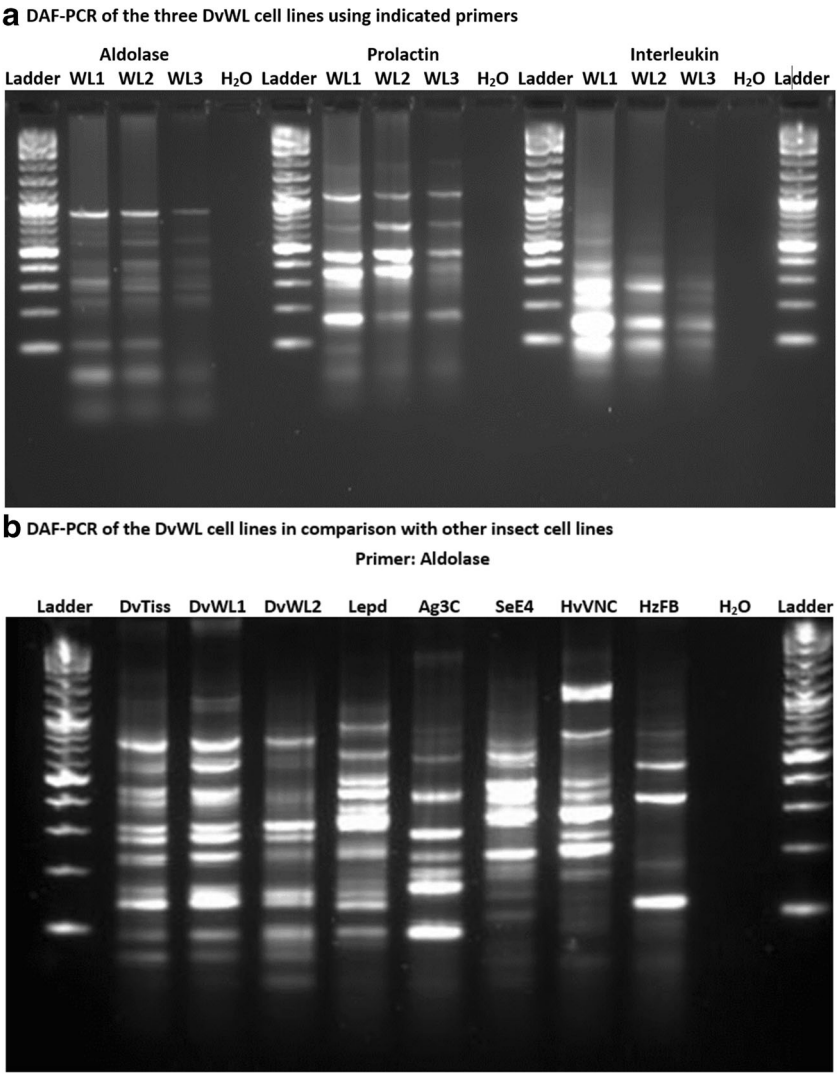

Fig. 2. DAF-PCR authentication of the DvWL cell lines using three primers. (*a*) Comparison of the DNA patterns for the DvWL cell lines: DvWL1 cell line, P40; DvWL2 cell line, P43; DvWL3, P22. (*b*) Comparison of the DNA patterns of the DvWL1, P10, and DvWL2, P12, cell lines with the tissue of origin (larval tissue, DvTiss) and selected insect cell lines: Colorado potato beetle pupal cell line, BCIRL-Lepd-SL1, P39 (Lepd); cotton boll weevil embryonic cell line, BRL-Ag3C, P69 (Ag3C); beet armyworm embryonic cell line, BCIRL/AmCy-SeE4, P79 (SeE4); tobacco budworm ventral nerve cord cell line, BCIRL/RP-HvVNC-WG3, P159 (HvVNC); corn earworm fat body cell line, BCIRL-HzFB33, P353 (HzFB). Water: negative control. Ladder: DNA ladder (Invitrogen™ 10068013, Fisher Scientific).

approximately between that of DvWL3 and DvWL1. Wen et al. (2015) generated a cell line, NRCAN-Tb521, from whole larvae of the ash and privet borer, *Tylonotus bimaculatus*. This line exhibits a branched, fibroblast-like morphology with dimensions ranging from 11.2 to 37.6 μm (diameter) and 31.2–79 μm (length) and a doubling time of 255 h, much longer than the DvWL cell lines. NRCAN-Tb521 cells attach firmly to flask surfaces and require trypsin for detachment, similar to the DvWL lines. Two cell lines were generated from *D. undecimpunctata* embryos and have doubling times higher than the DvWL lines (~5 d) , but also require trypsin for passaging (Lynn and Stoppleworth 1984). Many other coleopteran cell lines have long doubling times, such as ~6 d (from *Heteronychus arator*, Crawford 1982), ~4.5 d (from *Anomala cuprea*, Mitsuhashi 2003), ~5 d (from *Xylotrechus pyrrhoderus*, Iwabuchi 1999), 83.6–105.3 h

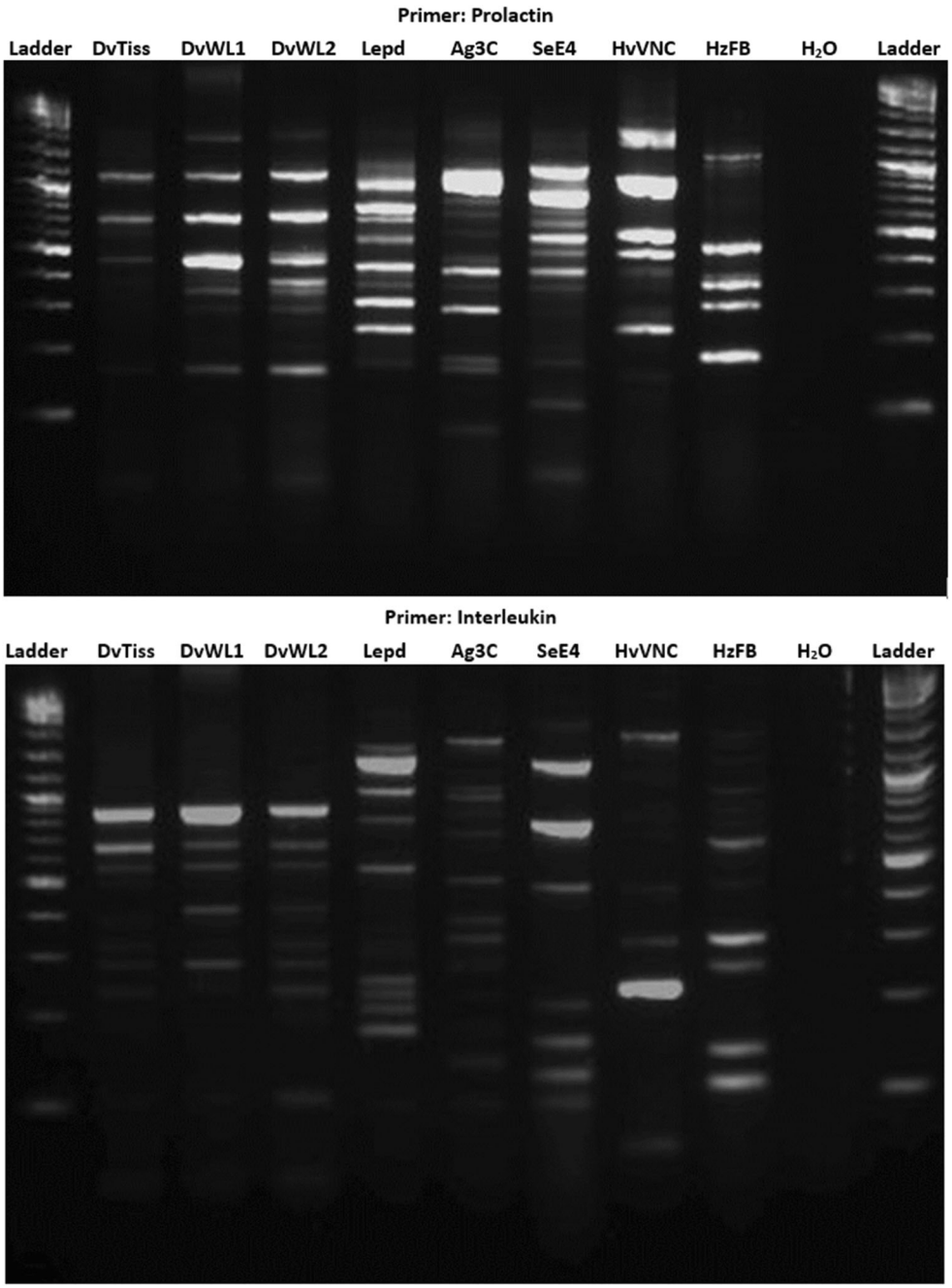

Fig. 2 continued.

(from *Holotrichia oblita*, Zheng et al. 2014), and 155. 2 h (from *Tribolium castaneum*, Goodman et al. 2012).

Our three cell lines from *D. virgifera virgifera* whole larvae will be useful in studying basic biological traits of this insect as well as in developing effective control agents. These lines are now available to researchers worldwide upon request.

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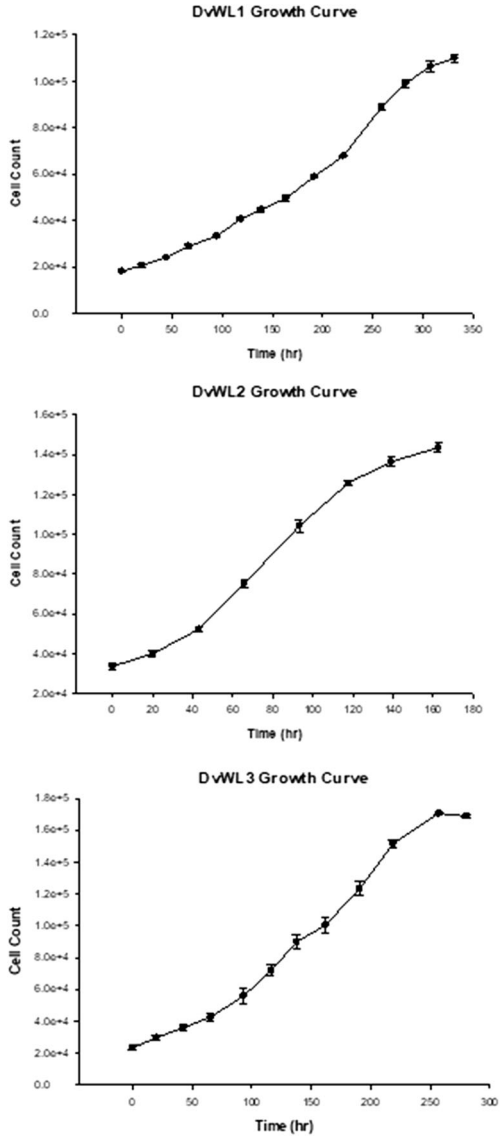

Fig. 3. Growth curves of the DvWL cell lines generated using a live cell imager. Each point represents the mean (± S.E.) of the cell counts per flask (3 replications/cell line).

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