# Autoradiographic Detection of Lesser Cornstalk Borer (Lepidoptera: Pyralidae) Eggs in Soil Environments[1]

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**ABSTRACT** A nondestructive method of detecting lesser cornstalk borer, *Elasmopalpus lignosellus* (Zeller), eggs in soil is outlined. Fourth instar larvae were fed a diet containing radioactive phosphorus (4 μ Ci/g), and eggs laid by females that emerged from these larvae contained sufficient radioactivity to expose X-ray film. Fecundity, oviposition rates, and longevity were similar for $^{32}$P-labeled adult females and unlabeled adult females. In greenhouse tests using corn planted in simulations of conventional tillage and no-tillage conditions, eggs were autoradiographically detected in soil and on plant tissue.

**KEY WORDS** Autoradiography, $^{32}$P, lesser cornstalk borer, oviposition.

The inability to visually observe lesser cornstalk borer (LCB), *Elasmopalpus lignosellus* (Zeller), eggs in soil has hampered research on LCB oviposition behavior. Laboratory studies have suggested that increased LCB oviposition rates at high temperatures strongly influence the development of outbreak populations (Mack and Backman 1984). The development of LCB populations also appears to vary with different crop management tactics (All and Gallaher 1977). A procedure has been developed for extracting LCB eggs from soil (Smith et al. 1981). However, studies on the impact of environmental conditions and cropping procedures on distributions of individual LCB eggs, dispersal of ovipositing females, and predation of LCB eggs have not been possible without disturbing the habitat. Ovipositing LCB females attach oval white eggs (ca. 0.7 by 0.4 mm diameter) to the substrate with a mucilaginous secretion, and as embryonic development progresses, the eggs turn to a deep crimson color (Luganbill and Ainslie 1917). A majority of the eggs are deposited in the soil (Smith et al. 1981). They are extremely difficult to locate because of their small size, variable color, and property of adhering to soil particles. This research was conducted with the objectives of developing and validating a method of detecting individual LCB eggs in soil.

## Materials and Methods

Fourth instar larvae (< 12 h-old) were placed in rearing cups (30 ml) that contained 2 g $^{32}$P-labeled diet (4 μ Ci/g) and 8 ml vermiculite (Cheshire and All 1979). Each cup was retained in a growth chamber (28°C, 14:10, light: dark) until the adult emerged, when individual $^{32}$P-labeled adult females were paired with non-radioactive males. Plastic rearing cages (7 × 7×22 cm) containing a food source (10% sucrose) and an oviposition substrate (blue paper towel) were used to

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maintain adults in the same growth chamber as larvae. Thirty randomly located eggs were removed from the paper towels and the radioactivity was measured with a liquid scintillation counter.

The use of no-screen medical X-ray film (DuPont Chronex®) for detecting $^{32}P$-labeled LCB eggs was evaluated by exposing eggs (<12 h-old) attached to paper towels from 4 cages. The towels were each placed with a sheet of film (25 by 30 cm) between two glass plates, and were retained in total darkness for 48 h. The X-ray film was processed according to standard developing procedures. The size of each egg and its corresponding exposure was measured with a dissecting microscope equipped with a micrometer grid.

Effect of $^{32}P$ on fecundity and longevity were evaluated on 100 adult females reared on diet containing $^{32}P$ (4 μ Ci/g) and females reared on unlabeled diet. Each $^{32}P$-labeled female and unlabeled female was paired with an unlabeled male, and individual mating pairs were retained (28°C) in rearing cages until the female died. The pupal stage duration, adult longevity, and oviposition rate were recorded daily for each LCB female.

Autoradiographic detection of $^{32}P$-labeled eggs in soil and on plants was evaluated in cage tests in a greenhouse. The cage (inside dimensions: 32 × 47 × 58 cm high consisting of a wooden frame covered with 1.5 mm saran mesh) was placed in a greenhouse flat (35 × 50 × 10 cm deep) containing sifted (2.5 mm mesh) Appling sandy loam soil, which was smoothed with a straightedge. All LCB females selected for study began ovipositing during the night before their release. The insects were retained in the test cages for 48 h.

In one experiment, corn seedlings (3 leaf stage) were planted in 2 rows (12 cm row spacing) of 8 plants (5 cm plant spacing). Four $^{32}P$-labeled LCB females and 4 non-radioactive males were released in each of 4 cages. In a second experiment, 5 flats were equally divided into simulations of conventional tillage (CT) and no-tillage (NT). CT simulations consisted of bare soil, whereas, NT simulations had 100 × 10 cm sections of wheat straw distributed evenly on the soil surface. Four 2-3 leaf corn seedlings were transplanted in a square grid (12 cm plant spacing) in each CT and NT simulation. One $^{32}P$-labeled LCB female and 1 unlabeled male were released in each cage.

After the 48 h oviposition period, the LCB adults were removed. The plants were cut at the soil surface. The greenhouse flats and excised plants were then placed in an incubator at 70°C for 12 h to desiccate the soil and plant tissue to prevent condensation on the X-ray film.

A sheet of X-ray film (32 × 43 cm) was placed over the undisturbed soil in each greenhouse flat. Contact between the film and soil was maintained by weighting the film with shell vials (9.5 × 2 cm diam.) that were taped together in 3 rows of 20. All excised plants and wheat straw sections were placed in a sheet of X-ray film (21 by 35 cm) between glass plates. Plants and wheat straw sections were not allowed to overlap. A sheet of Albanene® paper was placed on each side of the X-ray film to protect the film from scratches. All components of the simulations were retained in total darkness for 5 d. The X-ray film was processed according to standard developing procedures. After processing the film, corn seedlings and wheat straw sections were examined under a dissecting microscope to verify that each exposure resulted from a radiolabeled egg. A section of soil (10 × 10 × 2 mm deep) from each of 4 greenhouse flats was sorted with forceps under a dissecting microscope, and the locations and approximate depths of eggs in soil were compared with the corresponding exposures on film. The diameter of each image on film was measured.

## Results and Discussion

Data were analyzed using PROC GLM (SAS Institute 1985). One-way ANOVA was conducted for dimensions of eggs and their corresponding images from the preliminary exposures, for measurements of longevity, fecundity, and oviposition rates of LCB females that emerged from $^{32}$P-labeled and unlabeled diet, and for distances of eggs from plants in the NT and CT simulations.

The measurements conducted with liquid scintillation counter showed that the radioactivity of 30 eggs was 2,000 disintegrations per minute. Eggs oviposited on the paper towels appeared on the x-ray film as a solid black circle with an indistinct halo. In each of the 4 exposures, every black circle on the exposed film corresponded to the exact location of an egg. The mean ($\pm$ SD) diam. of the images (0.78 mm $\pm$ 0.21; n = 170) was larger ($F = 47.11$; df = 1, 338, $P < 0.0001$) than the mean ($\pm$ SD) diam. of the radiographed eggs (0.67 mm $\pm$ 0.09; n = 170). The emission of radiation in all directions from the spherical eggs accounted for both the difference between the actual sizes of the eggs and their corresponding images.

No significant differences ($P < 0.10$) in longevity, fecundity or oviposition were detected between LCB females that emerged from the $^{32}$-P-labeled diet and LCB females that emerged from the unlabeled diet. Means ($\pm$ SD) for the time required for 4th instar larvae to develop to adult females were 15.2 $\pm$ 3.5 d with the $^{32}$P-labeled diet (n = 46) and 15.6 $\pm$ 3.8 d with the unlabeled diet (n = 41). Means ($\pm$ SD) for longevity after emergence were 13.8 $\pm$ 7.4 d for the $^{32}$P-labeled females and 13.7 $\pm$ 7.3 d for the unlabeled females. Percentages of $^{32}$P-labeled females and unlabeled females that produced eggs (74% and 67%) were similar to those of Berberet et al. (1982). Mean ($\pm$ SD) numbers of eggs per ovipositing female were 199.0 $\pm$ 110.4 for $^{32}$P-labeled females (n = 28) and 206.8 $\pm$ 110.7 for unlabeled females (n = 26). Peak oviposition rates of $^{32}$P-labeled females and unlabeled females occurred 4.8 $\pm$ 2.2 d and 4.9 $\pm$ 1.4 d ($\bar{x} \pm$ SD) after adult emergence. $^{32}$P-labeled females and unlabeled females oviposited for 10.3 $\pm$ 2.2 d and 9.7 $\pm$ 2.3 d ($x \pm$ SD). No visual differences in appearance or behavior were observed between $^{32}$P-labeled females and unlabeled females.

In the cage test, $^{32}$P-labeled eggs that were laid in the soil exposed X-ray film with images that were identical in shape and color to the exposures of eggs on the paper towels (Fig. 1), but the sizes of the images ($\bar{x} \pm$ SD diam. = 0.51 $\pm$ 0.31; n = 608) were more variable. Each spot exposed on the film corresponded with an egg from the four areas that were examined in each replicate. No eggs were found in the soil adjacent to unexposed film. Although the exact depth of the eggs in these samples could not be measured, a decrease in diameter of images with an increase in depth of eggs was apparant. Thirty-one clusters of eggs were detected in soil, and individual eggs in images of these clusters were difficult to distinguish (Fig. 1A). Five egg clusters were examined under magnification, and these clusters consisted of up to 12 eggs laid both side by side and on top of each other. Exposures of adjacent eggs were often connected by a narrow dark band that apparently was a secretion produced by ovipositing LCB females (Fig. 1B), suggesting that the eggs were laid in close succession.

Exposures of eggs were easily distinguished from other marks on the film. Soil particles that penetrated the protective layer of paper caused irregularly shaped marks with distinct edges, and pressure from cut corn stalks resulted in mottled gray marks (Fig. 1B). Fecal material deposited by radioactive moths (10 occasions) resulted in large circular exposures (ca. 5 mm diam.) that could be distinguished from smaller irregularly shaped egg clusters and individual eggs.

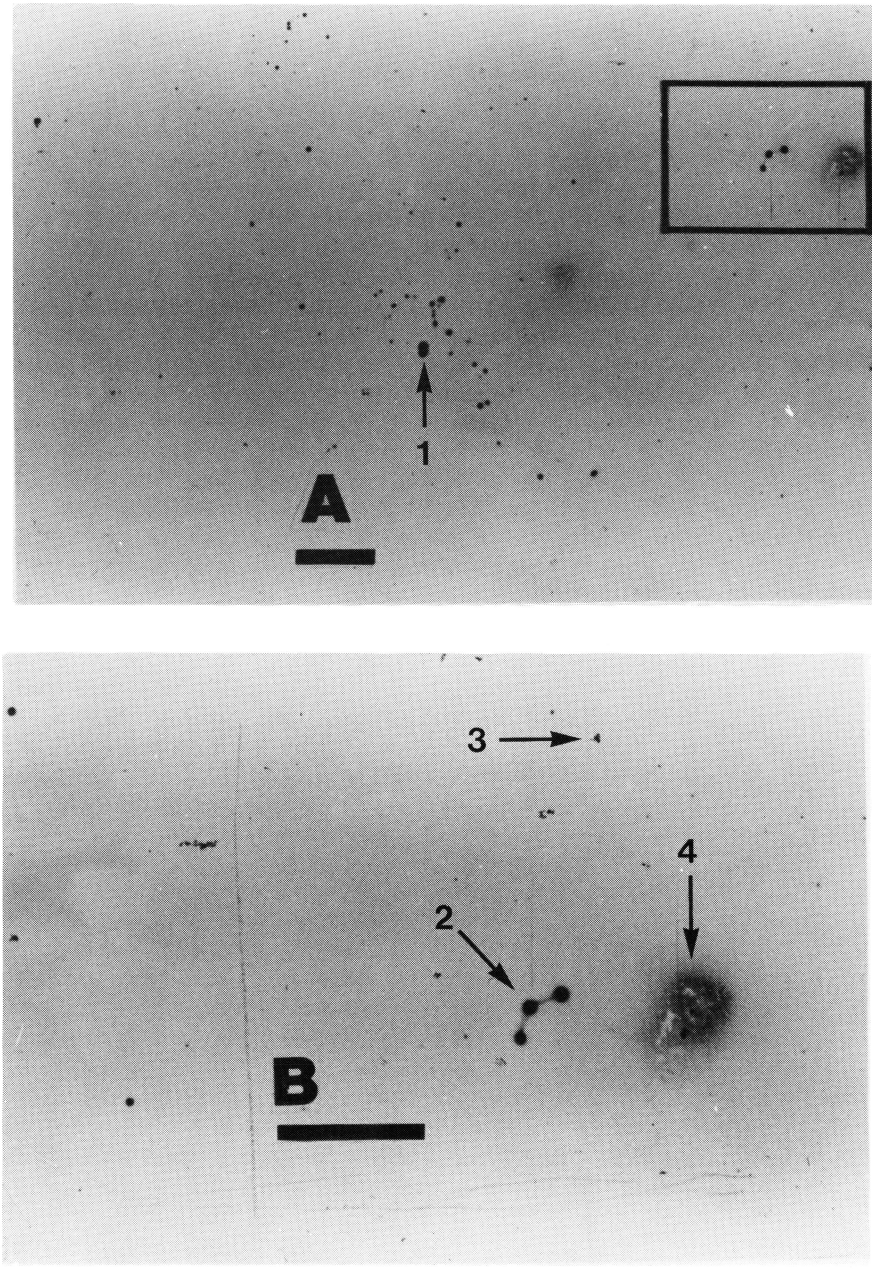


Fig. 1. Radiograph of LCB eggs in soil (A) and an enlargement of the enclosed area (B). (1) egg cluster; (2) eggs apparently laid in close succession; (3) scratch on film from soil particle; (4) artifact from cut corn stalk. Reference bars = 5 mm.

All of the eggs observed on either side of the corn seedlings and wheat straw sections exposed the X-ray film with characteristic black circles surrounded by an indistinct border (halo). Mean ± SD diameters of exposures from plants and wheat straw were 0.61 ± 0.31 (n = 85) and 0.56 ± 0.58 (n = 53). Images of eggs in immediate contact with the film were larger than images of eggs on the side of corn seedlings and wheat straw sections that was opposite the film.

A total of 558 eggs were detected in soil and on plants in the 4 greenhouse flats with corn seedlings and bare soil. Percentages of eggs laid on plants and in soil were 14 and 86%, respectively. The mean ± SD distance of eggs in soil from the nearest plant was 3.2 ± 1.3 cm (n = 482). In the CT simulations, 73 eggs were detected in soil and no eggs were detected on plants. Percentages of the 90 eggs imaged from plants, wheat straw, and soil in the NT portions of the flats were 10, 59, and 31%, respectively. The mean ± SD distance of eggs in soil from the nearest plant was larger ($F = 7.17$; $df = 1, 99$; $P < 0.005$) in NT (4.6 ± 2.6 cm; n = 28) than in CT (3.7 ± 1.7; n = 73). These results suggest that wheat straw is a suitable LCB oviposition substrate, and that the presence of dead wheat residues may influence the distribution of LCB eggs in NT corn systems.

This study demonstrated that LCB eggs can be labeled by rearing immature females on a $^{32}P$-labeled diet, and that radiolabeled eggs deposited in soil or on plant tissue can be imaged by autoradiograpy. Although the majority of LCB eggs in soil are impossible to see from above the soil surface, this method provides a nondestructive means of detecting the locations of individual eggs.

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