

Methods for assessing infestations of sunflower stem weevil (Coleoptera: Curculionidae) in sunflower stems

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Abstract—The sunflower stem weevil, *Cylindrocopturus adspersus* LeConte (Coleoptera: Curculionidae), reduces sunflower, *Helianthus annuus* Linnaeus (Asteraceae), yields by spreading pathogens, damaging vascular tissues, and promoting lodging of sunflower plants. To assess weevil populations for host plant resistance and insecticide field trials, larvae are dissected out of stems and counted; a process that is slow and limits experimental designs. To improve efficiency of sunflower stem weevil sample processing, field-collected sunflower samples were used to evaluate whether digital radiographs (X-rays) of stem sections or population estimates from rearing out overwintering stem weevils are suitable substitutes for dissection of complete stems. Digital X-rays of small stem pieces (15 cm above soil level) split longitudinally were found to explain over 75% of the variation in numbers of weevil larvae from dissected stem samples (50 cm), but required less than one-fifth the time of manual dissection. Use of small emergence boxes to estimate weevil populations was similarly time efficient, but may not be easily related to weevils per plant because of parasitism and other mortality. Results suggest for large field trials with sunflower stem weevils, digital X-rays provide much more time-efficient larval population estimates.

Résumé—Le charançon de la tige du tournesol, *Cylindrocopturus adspersus* LeConte (Coleoptera: Curculionidae), réduit les rendements du tournesol, *Helianthus annuus* Linnaeus (Asteraceae), en transmettant des pathogènes, en endommageant les tissus vasculaires et en favorisant la verse chez les plants de tournesol. Afin d'évaluer les populations de charançons pour des essais sur la résistance des plantes et les insecticides, on retire les larves des tiges par dissection et on les énumère, un processus long qui impose des restrictions aux plans d'expérience. Afin d'améliorer l'efficacité du traitement des échantillons de charançons de la tige du tournesol, nous avons utilisé des échantillons de tournesol récoltés sur le terrain pour évaluer si des radiographies digitales (rayons X) de sections de tiges ou des estimations de populations obtenues par élevage de charançons après l'hibernation constituent des méthodes adéquates de remplacement de la dissection des tiges complètes. Des radiographies digitales au X-rayons X de petites sections de tiges (15 cm au-dessus du niveau du sol) fendues longitudinalement expliquent plus de 75% de la variation des nombres de larves de charançons trouvées dans les échantillons provenant de tiges disséquées (50 cm), mais requièrent moins d'un cinquième du temps nécessaire pour les dissections manuelles. L'utilisation de petites boîtes d'émergence pour estimer les populations de charançons permet aussi une économie de temps, mais la relation avec le nombre de charançons par plant est moins facile à établir à cause du parasitisme et des autres causes de mortalité. Nos résultats indiquent que pour de grandes expériences en nature avec les charançons de la tige du tournesol, la radiographie-X digitale fournit des estimations des populations de larves qui permettent une plus grande économie de temps.

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The sunflower stem weevil, *Cylindrocopturus adspersus* LeConte (Coleoptera: Curculionidae), is a pest of cultivated sunflowers, *Helianthus annuus* Linnaeus (Asteraceae), in North America. One generation occurs each year, with most adults appearing between April and June. Weevils enter sunflower fields to feed on leaf and stem tissue, later laying eggs just beneath the stem surface, ~4–20 cm above the first node (Charlet 1983a). Larvae feed on vascular tissue and pith until fall, at which point mature larvae enter diapause and overwinter in stems, usually 4–6 cm above soil level (Charlet 1983b).

Sunflower stem weevils may affect sunflowers in multiple ways. Infestations can introduce and spread stem pathogens such as charcoal rot (Yang *et al.* 1983). Larval feeding on vascular tissues also can reduce seed yield and oil content, but only with very high densities of weevil larvae (60–80 larvae per stem; Rogers and Jones 1979). Perhaps the most significant risk is that feeding may weaken stems and promote lodging, reducing the number of plants that can be mechanically harvested. Relative to direct losses, lodging may occur with lower densities (<40 larvae per stem; Charlet *et al.* 1985).

To limit future losses by stem weevils, considerable effort has been made to identify sources of host plant resistance in sunflower (Charlet *et al.* 2009). Due to the difficulty in quantifying damage from internal feeding by weevil larvae, resistance has primarily been assessed by dissection of plants to estimate per-stem weevil densities. However, the time needed to dissect stems (\approx 45 minutes each; based on dissection of several hundred infested stems) suggests other techniques could improve evaluation efficiency of sunflower stem weevil infestations. Because radiographic (X-ray) techniques have been successfully used to detect insect-damaged sunflower seeds (Peng and Brewer 1995), field-collected sunflower samples were used to determine if digital X-rays of stem sections are suitable substitutes for dissection of complete stems. Also, because X-ray equipment might be unavailable or cost-prohibitive in some cases, the use of small, inexpensive cages to rear and capture adult weevils also was examined.

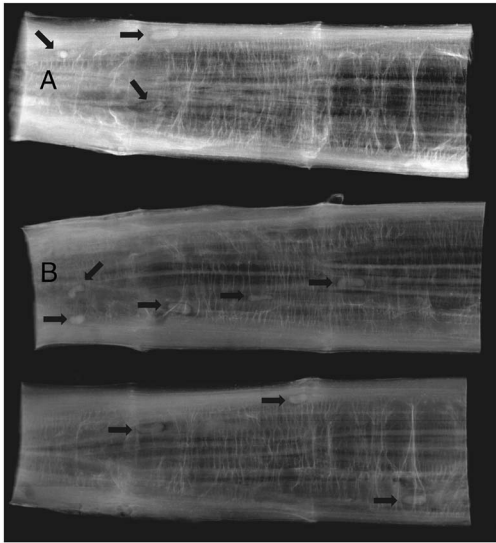
Insect-damaged sunflower stems were collected from Colby, Kansas, United States of America (2011–2012) and Scottsbluff, Nebraska, United

States of America (2011). In 2011, samples from Kansas ($n = 49$ stems, 4–5 per plot) consisted of diverse entries planted into a large resistance screening trial. After harvesting of stems (with primary root attached) and storage under diapause conditions (6°C for at least 120 days), stems were cut into three pieces; root, lowest 8 cm of stem above ground level, and remaining stem (\approx 30 cm). Stem pieces were returned to cold storage until X-ray images (10 second exposure, 28 kV) were taken using a specimen radiography system (MX-20; Faxitron Bioptics LLC, Tucson, Arizona, United States of America) configured with a digital camera. For each stem, one image was captured of the 8 cm section of stem intact, followed by an image of the 8 cm section cut longitudinally into two pieces. Subsequently, a single experienced technician dissected all pieces of each stem, and the numbers of *C. adspersus* were recorded. Samples from Nebraska ($n = 96$ stems, 12 per plot) consisted of a single commercial hybrid. Six stems from each plot in Nebraska were cut and subjected to digital radiography, and dissected as described above; the remaining six stems per plot were cut into 25 cm sections and placed into polypropylene emergence boxes (25 × 15 × 7 cm) ventilated though holes covered with brass cloth and stored in a heated greenhouse until emergence of overwintering stem insects was complete.

Methods in 2012 were adjusted based on 2011 results. Additional stems from Kansas ($n = 180$ stems, 4–5 per plot) were used with a slightly different system to accommodate longer stem samples. Radiographs used the same X-ray source, but acquired images on a phosphor storage plate, which was used to transfer images using a computed radiography reader (ScanX CR; Faxitron Bioptics LLC). Stem pieces for X-ray were 15 cm long and imaged only after being cut longitudinally. After digital images were captured, entire stems were dissected as previously described for half ($n = 90$) of the samples, while stems from the other half were placed individually into emergence boxes.

To test the relative effectiveness of different methods (X-ray, dissection, emergence boxes) for obtaining stem insect population estimates, comparisons of the sunflower stem weevil population estimates were made between samples of equal size. For example, paired sample *t*-tests were

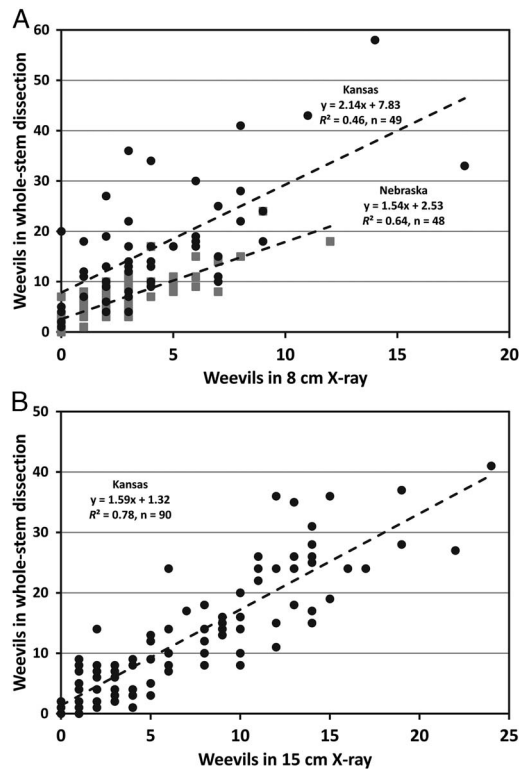
Fig. 1. Digital X-ray images of an 8 cm section of sunflower stem before (A) and after (B) being cut longitudinally. Visible weevils indicated by arrows. Note the greater number of weevil larvae visible in the stem after cutting.



used to compare larval counts from radiograph images of 8 cm stem sections versus later dissection of the same 8 cm pieces. Independent sample *t*-tests were used to compare the larval counts of whole plant dissections versus the numbers of adults (weevils and solitary weevil parasitoids) from stems placed into emergence boxes. To determine whether partial stem X-rays are predictive of data on whole stems (using dissection or emergence boxes), linear regression was used.

Digital X-rays of stems collected in 2011 showed more weevil larvae when 8 cm sections were split into two pieces rather than imaged as a single, intact piece for both Kansas ([mean \pm SE] 4.35 ± 0.52 and 2.04 ± 0.36 ; $t = 7.04$, $df = 48$, $P < 0.001$) and Nebraska (3.17 ± 0.37 and 2.04 ± 0.23 ; $t = 4.61$, $df = 47$, $P < 0.001$) samples (Fig. 1). When images of split 8 cm stem sections were compared to manual dissections, more weevils were found using dissection for Kansas samples (5.33 ± 0.62 and 4.35 ± 0.52 ; $t = 4.18$, $df = 48$, $P < 0.001$), while no difference was found for Nebraska. When whole stems were cut and placed in ventilated boxes, the emergence of *Nealiolus curculionis* (Fitch) (Hymenoptera: Braconidae) ($n = 50$) and *Neocatolaccus tylodermae*

Fig. 2. Scatter plots and linear regressions between number of weevils found in (A) 8 cm sections or (B) 15 cm sections of stems subjected to X-ray imaging and the complete individual stem and root pieces (≈ 50 cm) later manually dissected to count weevil larvae in samples from Kansas and Nebraska, United States of America in 2011–2012.



(Ashmead) (Hymenoptera: Pteromalidae) ($n = 18$) suggested weevil estimates would be reduced by parasitism. As a result, weevils and parasitoids were summed for emergence boxes to compare with whole-stem dissection. Still, higher estimates of stem weevil infestations were provided by dissection (7.40 ± 0.70) relative to emergence boxes (4.85 ± 0.57) ($t = 2.81$, $df = 94$, $P = 0.006$).

Regression indicated that weevil counts from X-ray images of the first 8 cm of stem above ground level (split before imaging) in 2011 were predictive of stem weevil infestation in the stem and primary root using dissection, but that the X-ray data accounted for just 46–64% of the variation in total weevil numbers (Fig. 2A). Using X-rays of larger (15 cm) stem sections in

2012 also predicted total weevil explained more of the variation (78%) in total weevil counts (Fig. 2B). Additional X-rays of an equal set ($n = 90$) of stems accounted for only 49% of the variation in subsequent emergence data, and total emergence (weevils and parasitoids) was less than half the number of larvae found in X-ray images (data not shown).

Acoustics (Pearson *et al.* 2007), near-infrared hyperspectral imaging (Singh *et al.* 2009), electrical conductance (Pearson and Brabec 2007), and digital X-ray imaging (Toews *et al.* 2006) are some of the non-invasive properties or techniques capable of detecting insect infestation of plant samples. Such technology-aided methods generally possess speed or accuracy not possible using visual inspection or dissection. On the other hand, considerable effort may be needed to adapt a technology to a specific purpose or the initial cost of technology may be prohibitive.

Relative to manual dissection, inspection of samples for sunflower stem weevils using X-ray images was nearly one order of magnitude faster (≈ 6 minutes). Images of small (8 cm), intact stem sections detected stem weevil larvae, but did not provide acceptable quantitative estimates of weevil numbers relative to dissection of the same piece unless longitudinally split before imaging. Subsequent tests showed images of larger (15 cm) sections were better at approximating the contents of a whole stem sample; most of the discrepancy between the weevil population estimates from X-rays and dissections were from weevils located below or above the section on which X-ray images were taken. Though the cost of digital X-ray equipment ($\geq \$50\,000$ USD) is high, it is a long-term investment for projects that annually hire semi-skilled labour to dissect samples and is flexible enough in use to be shared by several research projects. The X-ray data arguably suggest that dissection of partial (15 cm) sections of stem may also provide a method to speed up dissections at no cost; while this is correct, the benefit of such a modification will be limited, as most of the time spent dissecting stems is in careful examination of the base of the stem, where the thicker wood on stems helps conceal overwintering weevils.

As an alternative, the use of small emergence boxes to assess stem weevil populations had low

initial (equipment) and labor costs, taking only ≈ 4 minutes per stem. In 2011, the sum of emerged weevil adults and solitary parasitoids suggests about two-thirds of weevils detectable by dissection could be found using emergence boxes, and additional data on parasitoids is obtained at no added cost. However, data from 2012 provided less support for using emergence boxes, as overwintering survival appeared substantially lower than in the previous year.

Results with alternative methods suggest that use of dissection should be limited to validation or occasional small-scale projects. For research on host-plant resistance or insecticide efficacy, where large numbers of stems need to be assessed, digital X-rays or emergence boxes both provide much more time-efficient larval population estimates.

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