Impact of Cultivation and Subsequent Burial on *Cydia pomonella* (Lepidoptera: Tortricidae) and *Conotrachelus nenuphar* (Coleoptera: Curculionidae)

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ABSTRACT We assessed the efficacy of cultivation as a potential management strategy for codling moth, *Cydia pomonella* L. (Lepidoptera: Tortricidae), and plum curculio, *Conotrachelus nenuphar* Herbst (Coleoptera: Curculionidae) in apple orchards. Cocooned codling moth pupae and thinning apples infested with plum curculio larvae were cultivated over in the field. Emergence, percent burial, damage to buried fruit, and depth of burial was recorded. In the laboratory, both insects were buried at variable depths in sand and potting soil and emergence was measured. A greater proportion of plum curculio larvae buried in infested fruit under laboratory conditions survived to adulthood compared with unburied infested fruit, down to 15 cm. No codling moth adults emerged from under 1 cm or more of sand. Buried codling moth larvae experienced drastically reduced survival to adulthood compared with unburied larvae. These results indicate that strip cultivation may negatively impact codling moth diapausing larvae and pupae on the ground, but not likely to negatively impact plum curculio in infested dropped apples.

KEY WORDS strip cultivation, codling moth, *Cydia pomonella*, plum curculio, *Conotrachelus nenuphar*

Strip cultivation is a weed management strategy used in perennial fruit crops wherein drive rows are in grass or mixed vegetation and mowed, while a strip on each side of the tree rows is cultivated (Weibel 2002). When accomplished using a shallow, ground-driven implement, strip cultivation is used to reduce weed competition with the crop, while allowing weeds to regrow and retain soil nutrients and organic matter (Peck et al. 2011, Zoppolo et al. 2011). Strip tillage can also bury inocula of apple scab (*Venturia inaequalis* Cooke) in orchards, reducing disease pressure (Gomez et al. 2005).

Strip cultivation may also reduce insect pest populations. For example, in annual crops, the presence of bare soil at least once a year affords an opportunity to affect pest populations through soil disturbance (Stinner and House 1990). In cotton, tillage is a commonly used management strategy for pink bollworms, *Pectinophora gossypiella* Saunders (Lepidoptera: Gelichiidae), as well as white grubs, termites, and scale insects (Chu et al. 1996, Russell 2004). In maize, plowing under crop residue has been shown to reduce spring oviposition sites for black cutworm, *Agrotis ipsilon* Hufnagel (Lepidoptera: Noctuidae) (Johnson et al. 1984, Willson and Eisley 1992). In both peanut and sweet potato trials, tillage reduced wireworm (Coleoptera: Elateridae) abundance and damage (Seal et al. 1992, Scarpellini et al. 2004).

There is limited research on cultivation for arthropod pest control in perennial fruit crops. In grapes, throwing up a furrow onto the vinerows was at one time recommended as a means of grape berry moth (*Paralobesia viteana* Clemens, Lepidoptera: Tortricidae) control, with some rudimentary trials indicating that emerging adults are unable to dig upwards through 2.5–5 cm of soil (Isely 1917). When codling moth-infested fruit were buried in orchards, larvae were able to navigate to the soil surface from 15-cm depth (Steiner 1929). Mounding soil to cover exposed apple rootstock was found to be as effective as insecticidal trunk sprays in controlling dogwood borer, *Synanthedon scitula* Harris (Lepidoptera: Sesiidae) (Gut et al. 2005). Unfortunately, research on cultural control tactics, including strip cultivation, lacks the economic incentive offered by research centered on product development, thus proportionately less research is done to address it (Dent 2000).

Apples have a diverse community of pest insects (Slingerland and Crosby 1914). Two economically important pests of eastern United States apple production are codling moth, *Cydia pomonella* L. (Lepidoptera: Tortricidae), and the plum curculio, *Conotrachelus nenuphar* Herbst (Coleoptera: Curculionidae). The life history of both pests provides the opportunity for management through cultivation. Codling moth lay eggs on the surface of developing fruit and on nearby leaves in

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the spring. Larvae enter and feed inside fruit and exit fruit to seek a pupation site either on the tree, or, if no sheltered cracks are available from bark or pruning wounds, in the leaf litter on the ground (Geier 1963, Blomefield and Giliomee 2012). Second-generation codling moth diapause as larvae in the fall, at which time those that have chosen a diapause site on the ground could be targeted by cultivation. The ground-diapausing portion of the population is greater in orchards with smoother-barked trees (Slingerland 1898, Crosby and Leonard 1914). Plum curculio adults feed and lay eggs in developing apples; larvae feed within fruit, and exit fruit to pupate in soil after June drop. Upon completion of pupation, adults emerge from soil in August to feed on mature fruit and overwinter (Quaintance and Jenne 1912, Paradis 1957, Lan et al. 2004). Plum curculio could be targeted with a cultivator when the larvae are still feeding in excised apples on the ground, and after larvae bury themselves to pupate.

Insufficient research has been done to investigate the potential for cultivation as an insect pest management tactic in perennial fruit. Thus, we set out to determine the direct impact of strip cultivation on codling moth and plum curculio, important insect pests of apple production. Specifically, this study focused on how the application of strip cultivation affects the survivorship of codling moth and plum curculio. Our two experimental objectives were to determine: 1) the percent burial, mechanical damage, and burial depth of sentinel plum curculio-infested apples and cocooned codling moth in apple orchards, and 2) the survival of plum curculio and codling moth buried at variable depths in a controlled laboratory environment.

Materials and Methods

Insect Culture. Adult plum curculio were collected from a colony maintained by the Pesticide Alternatives Laboratory, Michigan State University, East Lansing, MI. Weevils for this colony were collected yearly from Benzie, Ionia, Leelanau, and Manistee Counties, Michigan. Colony adults were held in cages containing thinning apples treated with pyriproxyfen (Esteem 35 WP, Valent Corporation, Walnut Creek, CA; 750.0 mg/liter water) in order to break the obligate diapause, inducing oviposition (Hoffman et al. 2007). After exposure to plum curculio adults for 5 d, apples were transferred to mesh racks on trays lined with moistened paper towel. Larvae were collected daily and added to jars containing a moistened soil medium for pupation and development to adulthood.

Four female and two male freshly emerged adults were placed in 35- by 122-cm mesh bags of black pet screen (Phifer Incorporated, Tuscaloosa, AL) along with 50 thinning apples. Thinning apples were a mix of Fuji and Honeycrisp picked near Wenatchee, WA. Plum curculio were sexed using the shape of the first ventral abdominal segment under a Leica KL200 (Leica Microsystems, Mannheim, Germany) dissecting microscope, according to Thomson (1932). After 20 d, plum curculio infestation was assessed by removing five apples from each mesh bag and dissecting them under the microscope. Upon confirmation that the plum curculio were completing their development as larvae, we began experiments. In rearing for the field experiments, we began with 50 of the mesh bags containing plum curculio adults and thinned apples, and the 25 bags containing the greatest number of plum curculio larvae in the assessed apples were used for the experiments. To rear plum curculio for the laboratory experiments, we set up 10 of the same mesh bags and used the five bags containing the greatest number of larvae for the experiments.

Codling moth pupae for the field experiments were ordered and shipped from the USDA Yakima Agricultural Research Laboratory, Wapato, WA. For lab experiments, codling moth eggs were acquired from the colony at the Michigan State University Trevor Nichols Research Station, Fennville, MI. The eggs acquired were laid on a piece of wax paper and submerged in a 200:1 dilution of H_2O:sodium hypochlorite for 30 s to prevent fungal growth. As neonates began to hatch several days later, they were removed one at a time with a small wet paintbrush and placed individually into 59 ml plastic cups from Gordon Food Service containing recently poured and cooled codling moth diet. Each batch of diet consisted of 213 g organic pinto bean powder, 20 g agar, 3.2 g ascorbic acid, 32 g brewers' yeast, 1 g Fabco, 2 g methyl paraben, 1 g sorbic acid, and 4 Vanderzandt Vitamin, after Shorey and Hale (1965). All ingredients were acquired from Bioserve (Beltsville, MD), except the pinto beans, which were obtained from a grocer. Diet cups containing larvae were kept at 25°C and 45% humidity with a photoperiod of 16:8 (L:D) h for the first experiment and 20°C and 75% humidity with a photoperiod of 16:8 (L:D) h for the second experiment.

Field Experiments. Field experiments were conducted on Crosier-Williamstown loam at a commercial organic apple orchard with 30-yr-old trees, located near Flushing, MI (N 43.0274, W 83.9133). The orchard is situated on flat land with heavy clay soil. Our experimental plots consisted of a 1- by 2-m section within the cultivated area of the orchard floor, positioned 15 m away from each other within rows. Two separate experiments with completely randomized design were performed, one with codling moth and the other with plum curculio.

For the codling moth experiment, a simulated leaf litter containing cocooned codling moth was constructed by carefully placing pupae within a 1.5- by 2.0-cm tube of colored TimeMed label tape (PDC Healthcare, Valencia, CA) containing a Zinc #8-32 nut (Fig. 1). The label tape served as an artificial pupation site and both the color and nut helped in retrieval after cultivation. Sixty cocooned codling moth were spread out evenly on the ground within each experimental plot. Eight plots received the cultivation treatment, and eight received the control treatment, and the experiments were conducted in July 2013. For the plum curculio experiment, 50 infested apples (obtained from the previously described method) were spread evenly over each plot with two apples picked at random

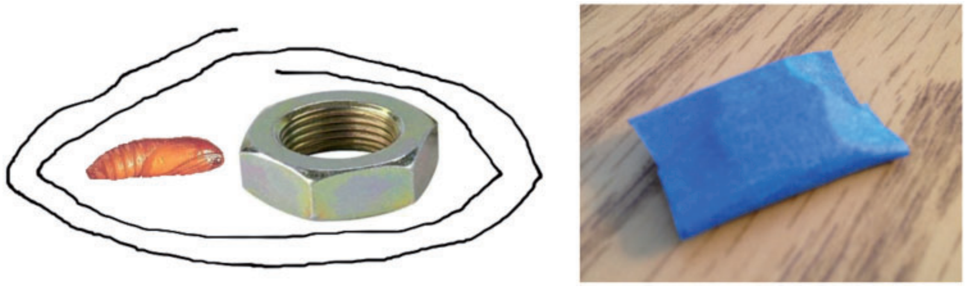

Fig. 1. Schematic and photograph of simulated codling moth leaf litter. As depicted, each codling moth pupa was rolled together with a zinc nut using a piece of colored tape.

from each of the 25 rearing bags. Ten plots received the cultivation treatment and 10 received the control treatment, and the experiments were conducted in October 2012.

In both experiments, our grower collaborator made two passes at 3–5 mph in the cultivation treatments with a custom-built Lilliston style (spider tines) cultivator. After cultivation, the apples and tape rolls were immediately retrieved from both treatments by hand and occasionally using a rake. In the codling moth experiment, an Ace 350 metal detector (Garrett Metal Detectors, Garland, TX) was used to locate the zinc nut attached to the simulated leaf litter. Percent recovered, percent damaged, and percent buried cocooned codling moth and plum curculio-infested apples were recorded, and their depth of burial estimated visually. Retrieved codling moth were evaluated in the laboratory for mechanical injury.

Laboratory Experiments. Survival and development after burial was assessed at a range of depths in custom-built burial arenas. One experiment was performed with plum curculio and three experiments were performed with codling moth. The experimental arenas consisted of 15-cm-diameter PVC tubes purchased from Home Depot, oriented vertically. They were cut to lengths that provided 7.5 cm of soil medium below the buried insects and 7.5 cm of air space above the level of soil. The total height of arenas used for plum curculio were—15, 17.5, 22.5, 30, and 60 cm—with 0, 2.5, 7.5, 15, and 45 cm corresponding burial depths (Fig. 2a). The total heights of arenas for the codling moth experiment were—15, 16, 17.5, 22.5, and 37.5 cm—with 0, 1, 2.5, 7.5 and 22.5 cm corresponding burial depths (Fig. 2b). Commercial sheer fabric was attached across arena bottoms with Surebonder hot glue. Burial proceeded as follows: we added 7.5 cm of soil medium to the arena, spread the insects out in a layer, and covered them with the treatment depth of soil medium.

For all four laboratory burial experiments, each burial depth was replicated over five burial arenas. For the plum curculio experiment, five infested apples, one from each larval rearing bag, were buried in each arena in rinsed and dried Quikcrete sand. In the first codling moth experiment, the contents of diet cups—codling moth pupae within the small plug of food medium—were buried in sand 30 d after eggs hatched while they

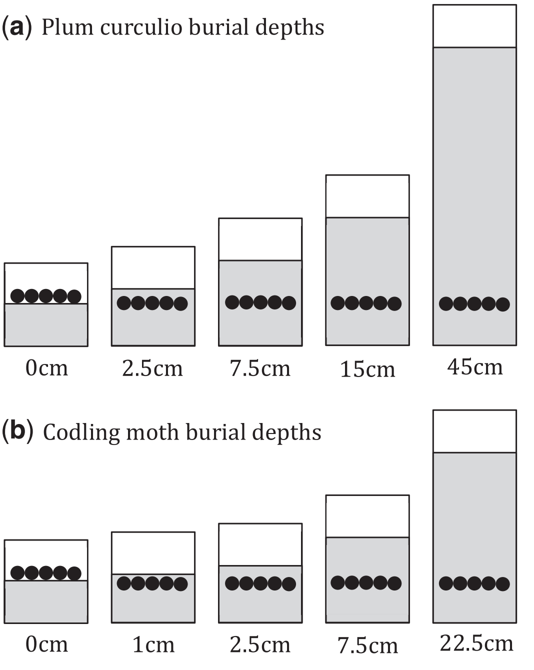

Fig. 2. Laboratory insect burial diagram. Burial depth treatments are listed beneath each burial. (a) Codling moth burial depths, (b) Plum curculio burial depths. Gray area represents soil medium, and black circles indicate buried insects.

were pupae. In the second codling moth experiment, they were buried while codling moth were larvae, in sand. In the third codling moth experiment, they were buried while codling moth were larvae, in Vigoro All-Purpose potting soil. Commercially available media were used rather than soil collected from orchard environments for easy standardization with future experiments.

The arena was then covered with a piece of shear fabric affixed using a rubber band so that any emerging insects would not be able to escape. We brought the soil medium to field capacity by setting the tubes in a tray of water until moisture appeared at the surface of the medium. Tubes were removed from the water and set in a dry environment at 18°C and a photoperiod of 16:8 (L:D) h for the duration of the experiment. They were checked every 4–5 d for emergence. In the plum curculio experiment and the third codling moth experiment, larvae and pupae found to have emerged were not removed from the arenas, to assess whether they would complete their development. Adults were removed when counted and returned to respective colonies.

Data Analysis. All analysis was done using R statistical computing software. Means and standard errors were calculated for field data. Means and standard errors were computed for percent buried and damaged from field experiments. Data from laboratory experiments were nonnormal. Therefore Kruskal–Wallis models of emergence against burial depth for the plum curculio experiment (5 levels) and the second codling moth experiment (5 levels), and a Kruskal–Wallis model of emergence against burial depth and life stage for the second codling moth emergence (5×2 levels) were used followed by Wilcoxon pairwise comparisons when the overall model was significant.

Results

Field Experiments. Recovery after cultivation was $91.7 \pm 3.1\%$ from plum curculio-infested thinning apples and $86.8 \pm 2.2\%$ from codling moth litter (Fig. 3). Field burial with the Lilliston-style cultivator was $61.3 \pm 1.4\%$ for plum curculio and $83.1 \pm 2.9\%$ for codling moth. Mechanical injury to codling moth was $7.5 \pm 1.6\%$, and damage to infested apples was $14.9 \pm 1.9\%$. Depth of burial ranged between 0 and 7 cm for both apples and artificial litter.

Lab Experiments. The effect of depth of burial on emergence of plum curculio was highly significant ($\chi^2 = 15.73$, df = 4, $p < 0.01$; Fig. 4). Significantly more *C. nenuphar* adults emerged from the 2.5-cm burial than from the surface control ($p = 0.04$), and numerically but not significantly more emerged from 7.5- and 15-cm burial ($p = 0.13$ and $p = 0.17$, respectively) than from the surface control. None emerged from 45-cm burial.

In the first codling moth experiment, where pupae were buried in sand, no *C. pomonella* emerged from any of the burial depths, whereas an average of 50% emerged from the soil surface treatment (Fig. 5a). In the second experiment, where larvae were buried in sand, 3.8 ± 0.7 per tube emerged from the surface, 1.8 ± 0.6 from the 1-cm treatment, and 0 from all deeper treatments (Fig. 5b). The effect of depth on emergence was significant ($\chi^2 = 20.3$, df = 4, $p < 0.01$). In the third experiment, where larvae were buried in potting mix, and left in the arenas to complete their development, emergence of larvae occurred from various depths, and the effect of depth on emergence was significant ($\chi^2 = 12.58$, df = 4, $p = 0.01$). Significantly more codling moth larvae emerged from the soil surface treatment than the 7.5- and 22.5-cm depths ($p = 0.049$ and $p = 0.007$, respectively) but not the 1- or 2.5-cm burial depth ($p = 0.086$ and $p = 0.52$, respectively; Fig. 5c). There were much fewer adults that emerged than larvae (Fig. 5c), and the depth effect was significant for adults ($\chi^2 = 17.17$, df = 4, $p < 0.01$). The number of larvae and adults recorded for the surface control and 7.5-cm depth were very similar (surface $p = 0.38$; 7.5 cm $p = 0.42$), whereas fewer adults emerged than larvae unburrowing from the 1-cm and 2.5-cm depths (1 cm $p = 0.07$; 2.5 cm $p = 0.09$).

Discussion

Two passes with a Lilliston cultivator resulted in 8 and 15% of sentinel plum curculio and codling moth larvae, respectively, being mechanically damaged. However, half or more of sentinel larvae in the area were buried up to 7 cm deep. This is consistent with previous studies in that cultivation-mediated mortality more typically is attributed to the alteration of habitat versus direct mechanical destruction of pests (Stinner and House 1990). The added weight of the zinc nut used to maximize recovery may also have altered the percent burial in the codling moth experiment. However, considering the size of the cultivating implement and the weight of the surrounding soil, it is unlikely to

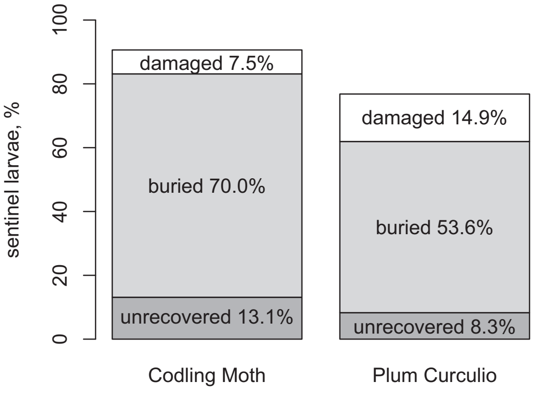

Fig. 3. Field insect burial results. Percent unrecovered, buried, and physically damaged codling moth litter (left) and plum curculio-infested apples (right) after two passes with a Lilliston cultivator. Data reflect totals across all replicates.

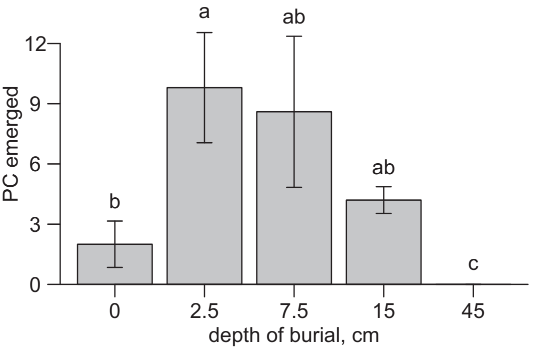

Fig. 4. Lab plum curculio burial results. Mean (±SEM) emergence per chamber, by depth of burial in wetted sand. Differing letters indicate a statistical difference ($\alpha = 0.05$) in a Wilcoxon rank sum test.

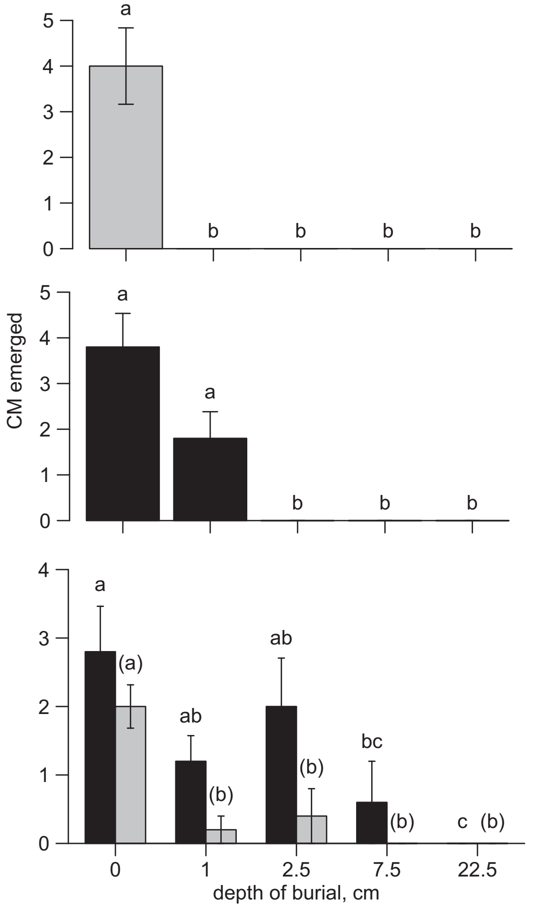

Fig. 5. Lab codling moth burial results. Mean (±SEM) emergence per chamber, by depth of burial. Adults are in gray and larvae in black. Differing letters indicate a statistical difference in Wilcoxon rank sum tests ($\alpha = 0.05$). (a) Emergence of adult codling moth from sand. (b) Emergence of larval codling moth from sand. (c) Emergence of larval codling moth from potting soil and development of adult codling moth from emerged larvae.

have affected the outcome by more than a few percentage points.

In wetted sand, more *C. nenuphar* were able to emerge from the buried apple treatment than from the surface treatment. These results are somewhat intuitive: plum curculio larvae bury themselves before pupating (Lan et al. 2004), so it is unsurprising that artificially buried larvae would be more likely to recruit successfully into the next generation. These results suggest that mechanical burial is unlikely to provide management of plum curculio. This agrees with previous work with weevil pest species such as *Hypera postica* Gyllenhal (Coleoptera: Curculionidae) and *Sitona hispidula* F. (Coleoptera: Curculionidae) in alfalfa, which are unaffected by tillage (Stinner and House 1990). Deeper burial (up to 45 cm) did result in mortality of plum curculio in our study. Similarly, moldboard plow reduced emergence of sunflower seed weevils (*Smicronyx fulvus* LeConte) by 29–56% in the field (Gednalske and Walgenbach 1984). The moldboard plow is designed to run at a 25–30 cm depth, which makes its use impractical in the orchard environment.

The increase in adult plum curculio emergence from burial that was observed in the laboratory may or may not also apply to burial by a cultivator in the field; field efforts are needed, possibly using emergence cages over cultivated versus uncultivated patches. If adult emergence is found to increase through burial in the field, then apple growers using strip cultivation shortly after June drop as a weed management strategy would be at risk of worsening plum curculio pressure. Further, our lab experiments only addressed mortality by burial alone; field burial may also make insects vulnerable to fungal disease, nematodes, or natural enemy predation (Dent 2000).

In wetted sand, no codling moth adults were able to emerge from burial, whereas larvae were able to emerge from up to 1 cm. Larvae that emerged from burial in potting soil were largely unable to complete their development to the adult stage, which may be due to cuticular injury while burrowing upwards or the metabolic cost of excavation. Abrasive soils may damage insect cuticles and reduce survivorship and movement. Our results are in contrast to those of Steiner (1929), who reported emergence of codling moth from up to 15 cm of orchard soil. Our results suggest that larval or pupal burial resulting from cultivation events could be a potentially useful management tool for codling moth, as soil mounding is with dogwood borer (Gut et al. 2005). The ability of the insects to survive burial in the field will vary with soil type. Field trials addressing the impact of strip cultivation would be necessary in any case before making recommendations to growers.

The timing and phenology of the two codling moth generations is likely to affect the utility of cultivation as a management tool. The first generation of codling moth pupates over several summer weeks with individuals emerging throughout this period (Riedl and Croft 1978). In contrast, the development of the second generation is arrested as they enter diapause. Thus, if cultivation can reduce codling moth populations, treatments targeting codling moth on the orchard floor are likely to be more effective when focusing on the second generation with passes made in the late fall or early spring. Cultivation is more likely to impact codling moth in orchards of smoother-barked trees, where suitable diapausing sites on trees and posts are rare, forcing codling moth to pupate in the less favorable environment of the leaf litter.

In summary, there is evidence to suggest that strip cultivation is a potential management tool for codling moth but not plum curculio. This is most practical for growers already running a cultivator through their orchard; accomplishing some weed and pest control with a single tool is desirable. The plum curculio is not negatively affected by burial alone, and in a sterile-sand environment burial actually results in higher percentage recruitment into the next generation for plum curculio. Codling moth adults, on the other hand, can be killed by burial alone, even under as little as 1 cm of sand. Codling moth larvae are also sensitive to burial, able to burrow out from both sand and potting soil but largely unable to complete their development after emerging from the soil. The results from both of these insects merit further study in field environments. Among potential questions to be addressed are: Is burial also a boon to plum curculio in the field? Is it as effective at reducing populations of codling moth in the field? To what degree do these outcomes vary by soil type? Further work is needed before management recommendations can be made to tree fruit growers.

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