Evaluation of Tag Entanglement as a Factor in Harmonic Radar Studies of Insect Dispersal

G. BOITEAU,[1] C. VINCENT,[2] F. MELOCHE,[3] T. C. LESKEY,[4] AND B. G. COLPITTS[5]

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ABSTRACT The observation of insects and other small organisms entangled in the habitat after the addition of vertical or trailing electronic tags to their body has generated concerns on the suitability of harmonic radars to track the dispersal of insects. This study compared the walking behavior of adult Colorado potato beetle (*Leptinotarsa decemlineata* (Say) Chrysomelidae), plum curculio (*Conotrachelus nenuphar* (Herbst) Curculionidae), and western corn rootworm (*Diabrotica virgifera virgifera* (LeConte) Chrysomelidae) with and without vertical and or trailing tags in field plots or arenas. The frequency of the larger Colorado potato beetles crossing bare ground or grassy plots was unaffected by the presence of an 8 cm trailing harmonic radar tag. However, plum curculios and western corn rootworms, were either unable to walk with a 4 cm trailing tag (plum curculio) or displayed a reduced ability to successfully cross a bare ground arena. Our results revealed the significant impact of vegetation on successful insect dispersal, whether tagged or not. The vertical movement of these insects on stems, stalks, and tubes was also unaffected by the presence of vertical tags. Trailing tags had a significant negative effect on the vertical movement of the western corn rootworm. Results show that harmonic radar technology is a suitable method for studying the walking paths of the three insects with appropriate tag type and size. The nuisance factor generated by appropriately sized tags was small relative to that of vegetation.

KEY WORDS Electronic tag, entanglement, harmonic radar, insect tracking, insect walking

Our knowledge of the dispersal ecology and behavior of many invertebrates is limited by the difficulty of visually detecting the organisms within dense vegetation. In temperate habitats, most invertebrates are small (frequently <3 cm long) (Price 1997) and many are cryptic (Ruxton et al. 2004). Capture–recapture of marked invertebrates is a useful method but the quality of the data are often affected by low recapture rates (Hall and Hadfield 2009, Boiteau 2005, Hagler and Jackson 2001). In theory, the recently developed radar tracking systems (Riley and Smith 2002, Boiteau and Colpitts 2004) should make possible the collection of quality data on invertebrate dispersal. However, regardless of the mark or tag selected, their potential impact on the parameters measured must be minimal and should be determined before their use in experimental studies.

Essentially, harmonic radar systems track insects by illuminating an electronic tag attached to the target insect with a high power microwave source and recapturing the second harmonic of the incidental signal reradiated from the tag (e.g., Colpitts and Boiteau 2004). The movement of the tagged insect can be tracked through this exchange of signals between the radar and the tagged insect. Different harmonic radar systems to track insects have been developed around the same concept but they all require tags to be affixed on individual insects (Riley and Smith 2002). The size and weight of the tags must be small enough not to alter normal movement of flying (Riley et al. 1996, Roland et al. 1996) or walking (O'Neal et al. 2004, Boiteau et al. 2010) insects.

In planning the utilization of harmonic radar systems to analyze the foraging strategy of insects across host and nonhost habitats, it is necessary to ensure that information gained on walking time, speed, number, and duration of rest periods and frequency of dispersal bouts are only affected by the normal interplay of habitat structure and insect behavior and not by tag entanglement in the vegetation or the soil.

Both the design of the tags and the particular composition of each habitat will determine if a tag can be a nuisance to the movement of the insects. O'Neal et al. (2004) reported that walking movements of ground beetles had been hindered by occasional entanglement of the 8 cm trailing tags on plant debris but

[1] Corresponding author: Agriculture and Agri-food Canada, Potato Research Centre, 850 Lincoln Road, PO Box 20280, Fredericton, NB, E3B 4Z7 Canada (e-mail: gilles.boiteau@agr.gc.ca.

[2] Agriculture and Agri-Food Canada, Horticulture Research and Development Centre, 430 Gouin Blvd., Saint-Jean-Sur-Richelieu, QC, J3B 3E6 Canada.

[3] Agriculture and Agri-Food Canada, Eastern Cereal and Oilseed Research Centre, K.W. Neatby Building, 960 Carling Ave., Ottawa, ON, K1A 0C6 Canada.

[4] USDA–ARS Appalachian Fruit Research Station, 2217 Wiltshire Road, Kearneysville, WV, 25430-2771.

[5] Department of Electrical and Computer Engineering, University of New Brunswick, 15 Dineen Drive, Fredericton, NB, E3B 5A3 Canada.

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Dahlquist (2008) (also using 8 cm trailing tags) reported no hindering tracking banana weevils. The 15-cm dipole tags used by Williams et al. (2004) were occasionally found broken or twisted, suggesting that they had been caught in the ground or tree vegetation. It is not clear how much of an impediment tag entanglement is to the collection of sound data and how frequent it is. Here we question if the use of electronic tags can exert an entanglement effect such that it has a significant impact on quality of the data collected with harmonic radar technology. The study was carried out on three economically important pest species of three different agricultural habitats: potato (Colorado potato beetle (*Leptinotarsa decemlineata* (Say) Chrysomelidae)), corn (western corn rootworm (*Diabrotica virgifera virgifera* (LeConte) Chrysomelidae)), and orchards (plum curculio (*Conotrachelus nenuphar* (Herbst) Curculionidae)). The harmonic radar system would provide much needed information on Colorado potato beetle (Boiteau et al. 2003, Hoy et al. 2008), western corn rootworm (Meloche et al. 2001, Meloche and Hermans 2004), and plum curculio (Leskey and Wright 2004, Vincent et al. 1999, Chen et al. 2006) movement in real time and over a range of distances and locations. Boiteau et al. (2009, 2010) documented the effect of glue used for tag adhesion and tag weight requirements for these three insects.

The objective of the study was to obtain a quantitative assessment of the impact of random entanglements on insect movement. If entanglement on the vegetation or the soil occurs frequently enough to impede the walking movement of tagged insects, the percentage of insects with tags completing any given walk should be lower than the percentage of insects without tags completing the same walk. The average time taken to move upward on a given substrate/host or through a field section should be longer than that for insects without tags. The predictions were tested by comparing the success of two types of tagged and untagged insects at dispersing on a vertical structure as well as through bare ground and grassland plots.

Materials and Methods

Insects

There are two general types of dipole tags for harmonic radar tracking: vertical and trailing. The two types were tested because they each provide different opportunities for nuisance or entanglement. It is presumed that vertical tags would be an obstacle to digging and that trailing tags would snag on objects. Vertical tags used in this study have been previously used with the three insect species and had no impact on their ability to walk in controlled arenas although they had marginally reduced travel speed in some instances (Boiteau et al. 2010). The trailing tags had been described in O'Neal et al. (2004) but had not been previously tested on these three insects.

Colorado Potato Beetle. Adult Colorado potato beetles used in the tests were collected from a pesticide-free potato field at the Agriculture and Agri-Food Canada (AAFC) Potato Research Centre in Fredericton, NB. Approximately 18–24 h before each test, adults were sexed according to Rivnay (1928), prepared and separated into treatment groups of 14 individuals (sex ratio 1:1) for the horizontal tests and three individuals for the vertical tests. The three treatments were: (1) individuals with vertical tag, (2) individuals with trailing tag, and (3) individuals without tags (control). The vertical dipole tag consisting of a 2 mm proximal pole followed by a 1 mm loop and a 6 mm pole made of AWG #34 copper wire weighed ≈2.1 mg and was attached to the pronotum of adults using a drop of Crazy Glue (Elmer's Products Canada, Corp., Scarborough, Ontario, Canada) (Colpitts and Boiteau 2004, Boiteau et al. 2009). The trailing tag consisting of an 8 cm long section of a flexible fine gauge (0.07 mm diameter) Teflon-coated aluminum wire (product #TFIR-003-50, Omega Engineering, Stamford, CT) similar to the one used with Carabidae by O'Neal et al. (2004), weighed ≈2.8 mg and was attached to the pronotum of the beetle with hot-melt glue (Mastercraft, Canadian Tire Corporation, Toronto, Canada). It laid horizontally against and beyond the elytra of the insect. Based on preliminary results, a fine drop of hot glue was applied along the elytral margin of all individuals to prevent the loss of a considerable number of tagged and untagged beetles to flight in the field tests. In addition, beetles used in the field test were individually marked (Unruh and Chauvin 1993) to differentiate them from the colonizers that occasionally found their way into the traps from other test plots or from fields outside the plots. Single sex groups of adults were then placed in ventilated 1 liter plastic container provided with potato leaflets until released the following morning.

Plum Curculio. Adult multivoltine plum curculios obtained from a laboratory colony maintained in Kearneysville, WV, and reared in the laboratory according to Amis and Snow (1985), were used as test subjects. Immediately after emergence, adults were held in groups of 100 for ≈2 wk at 25°C and 14L:10D in an environmental chamber to reach sexual maturity. Adults were sexed according to the methods of Thomson (1932) and were returned to the chamber in single sex groups of ≈40 individuals held in wax-coated cups (473 ml) with a clear plastic lid and provided with a source of water (wetted cotton dental wick) and a green thinning applets as a food source.

Approximately 18–24 h before each release, adults of each sex were prepared and separated into three treatment groups of ≈10 individuals for the horizontal tests and ≈20 individuals for the vertical tests. The three treatments were: (1) tagged individuals, (2) untagged individuals with glued elytra, and (3) individuals without tags or glue (control). Trailing tags were not included because of difficulty attaching trailing tags to the irregular surface of the plum curculio elytra (there was no site large and smooth enough) and the obvious impairment they created when adults tried to walk even short distances. Preliminary tests with 8 and 4 cm trailing tags showed that plum curculios were unable to maintain their balance. For

tagged individuals, a vertical dipole tag, similar to those used with the Colorado potato beetle (see above), was attached to the pronotum of adults using a drop of Bowman FSA adhesive (Barnes Group, Cleveland, OH). Tag attachment was aided by use of a Nikon SMZ 1500 (7.5–112.5 ×) stereomicroscope and forceps to permit precise placement of the tag in a drop of glue until dryness. For untagged adults with glued elytra, Bowman adhesive was applied with an insect pin along the entire length of the elytral margin to prevent flight. Adults of the control group were not manipulated in any way. Single sex groups of adults were then placed in 150 (outer diameter) × 15 mm petri dishes lined with moistened filter paper until release the following morning.

Western Corn Rootworm. Adult western corn rootworms were collected at the AAFC Eastern Cereal and Oilseed Research Centre in Ottawa, ON, during August 2009 on grain and cereal corn. Newly collected wild adults were brought to the laboratory in the morning and sexed according to White (1977). Beetles were kept separate in 150 (outer diameter) × 15 mm petri dishes until release in the afternoon. The three treatments were: (1) individuals with vertical tag, (2) individuals with trailing tag, and (3) individuals without tags (control). Western corn rootworm are very mobile and take off frequently so a fine drop of hot melt glue was applied along the elytral margin of all individuals with a 15 watts hot glue gun (Stanley model GR10) to prevent the flight of tagged and untagged beetles during the field tests. The vertical dipole tag consisted of a 2 mm proximal pole followed by a 1 mm diameter loop and a 6 mm pole made of AWG #41 silver plated (6%) copper wire (MWS Wire Industry). The tag weighed ≈1.2 mg and was attached to the pronotum of adults with hot glue. The trailing tag consisted of a 4 cm long section of a flexible Teflon-coated aluminum wire (product #TFIR-003-50, Omega Engineering), similar to the one used for the Colorado potato beetle (see above). The tag weighed ≈1.4 mg and was attached to the pronotum of the beetle with hot glue and laid horizontally against and beyond the elytra of the insect. It had been determined in preliminary tests that the 8 cm trailing tag (similar to the one used for the Colorado potato beetle) was too long and prevented western corn rootworm walking and climbing so trailing tags were shortened to 4 cm.

Vertical Walking

The nuisance of electronic tags to the vertical mobility of adult Colorado potato beetles, and multivoltine plum curculio was evaluated in the laboratory. Their nuisance to western corn rootworm was evaluated in the field.

Colorado Potato Beetle. Tests were carried out at the AAFC Potato Research Centre in Fredericton, NB, at room temperature. The experimental setup consisted of three potato stems placed ≈0.4 m apart from each other with lighting centered above the plant material to facilitate vertical movement by the test insects. Each stem was held vertically into a 250 ml Erlenmeyer filled with water and the array of stems was placed against a white wall. The height of the potato stems above the Erlenmeyer averaged 30.8 ± 0.45 cm and was similar between treatments (F = 0.276; df = 2, 84; P = 0.759).

For each replicate, one beetle with vertical tag, one beetle with trailing tag, and one beetle without tag were placed at the base of the stems, facing the stems to favor climbing behavior. Beetles started climbing almost immediately and the time taken to climb was recorded using digital stopwatches. Five beetles accidentally dislodged from the plants by the observer were not considered in the analysis. Observations were carried out simultaneously on the three plants over a period of 20 min or until the top or bottom of the plant was reached. Because beetles that reached the top were not inclined to climb down or would spend considerable time exploring the leaves instead of continuing their downward climb, beetles were transferred from the leafy stems to a bare stem for the downward test. The parameters observed were: (1) numbers successfully reaching the top or the bottom, (2) time spent traveling upward or downward, and (3) distance traveled. Fifteen replicates of each treatment were carried out for each sex.

The total distance traveled and the height reached on the plant by male and female beetles with and without tags were each compared using two-way analysis of variance (ANOVA) on untransformed data. The time taken to reach the top of the plants and the time spent walking downward by beetles with and without tags were compared using an ANOVA on ln-transformed data. Chi-square tests were used to compare the number of beetles reaching the top of the plants or traveling down the stems for the different treatments.

Plum Curculio. Tests were carried out at the USDA-ARS Appalachian Fruit Research Station in Kearneysville, WV. Each adult was placed inside at the base of a 30 cm tall clear Plexiglas cylinder with an inner diameter of 7 cm. To aid in detection of test subjects and facilitate upward movement by adults, trials were conducted in a darkened room, and cylinders were illuminated from above using a bank of fluorescent lights 25 cm above the top of the cylinders. The duration of each trial was 5 min and each test subject was evaluated in three separate trials conducted consecutively. If a test subject reached a height of 20 cm, it was gently prodded to induce it to drop to the cylinder floor to allow for further climbing during the test period. Recorded parameters for each test subject included detection of upward movement as well as the total distance moved and the number of times it reached the top of the cylinder.

Fifteen males and 15 females were evaluated for each experimental treatment. Data were subsequently analyzed using the GLM procedure (SAS Institute 2003) to construct ANOVA tables for the total distance moved (centimeter). The models included sex and treatment as class variables. Dependent variable data were not transformed as homogeneity-of-vari-

ance assumptions were not violated according to Levene's Test. To determine if the likelihood of movement by a particular adult was related to sex or treatment, a logistic regression model was constructed using a stepwise procedure.

Western Corn Rootworm. The experiment was conducted at the edge of a corn field at the AAFC Eastern Cereal and Oilseed Research Centre, Ottawa, ON. Fresh adults from the field were brought to the laboratory in the morning, sexed according to White (1977) and prepared for the afternoon test. Beetles were kept in 150 (outer diameter) × 15 mm petri dishes until release in the afternoon. The three treatments included: (1) individuals with vertical dipole tag, (2) individuals with 4 cm trailing tag, and (3) individuals without tags. The leaves of five corn plants were removed from the ground up to the corn ear.

Fifty adults (25 males: 25 females) were placed one at a time between two nodes on a random corn plant 15 cm above ground and the time to climb up 20 cm recorded using a digital chronometer. The mean time taken to climb up 20 cm on the corn stalk by each sex and for each treatment was analyzed using two-way ANOVA on untransformed data (SPSS, version 3.1). Chi-square tests were used to compare the number of beetles climbing the 20 cm vertical distance on the stalk for the different treatments. Most western corn rootworm beetles started immediately to climb the plant and few fell off. Beetles that fell down were not considered in the analysis.

Horizontal Walking

The impact of radar tags on the horizontal mobility of adult Colorado potato beetles and multivoltine plum curculios was evaluated in the field using six plots with low likelihood for entanglement (bare ground) and six plots with high likelihood for entanglement (grass). The impact of trailing tags and vertical tags on the horizontal mobility of the western corn rootworm was compared in bare ground plots only.

Colorado Potato Beetle. Experimental plots were established in a small timothy pasture surrounded by hedgerows on two sides, another pasture on one side and a potato field on the last side at the AAFC Potato Research Centre in Fredericton, NB. A grassy surface was established in three plots, while a bare soil surface was established in the remaining three plots by removing the sod and infilling with similar soil. Grass was maintained ≈10 cm tall and bare ground was typical of fallow ground surrounding potato fields with unevenness, clods, rocks, etc. Each plot measured 1.35 × 0.65 m and was surrounded by pitfall traps consisting of four sections of plastic rain gutter (10.0 cm width) glued together at the four corners using silicone. Each pitfall trap was buried in the soil such that the upper lip was flush with soil surface. Pitfall traps were filled with a 3:1 water:ethylene glycol solution. Plots were randomly distributed on the site.

On each test day, groups of 14 test adults (1:1 sex ratio) were carefully released at 10:00 a.m. in the middle of each plot and collected from the traps every hour until 4:00 p.m. If, after a 6 h, period some beetles had not been found in the traps, the plot was then searched. The mean number of tagged and untagged beetles trapped over the 6 h period was used as an indicator of the level of entanglement in relation to habitat and tag type. Proportional trapping data were arcsine transformed as required and data were analyzed using the GLM procedure (SAS Institute 2001) to construct ANOVA tables. The model included the following class variables: plot type (grassy versus bare) and Colorado potato beetle treatment (vertical tags, trailing tags, or no tags), and sex. If the model indicated significant differences, multiple comparisons were calculated using Student-Newman-Keuls test ($P = 0.05$). The trap catch for each treatment was calculated hourly, cumulated over the daily observation period and compared graphically.

Plum Curculio. Six experimental plots were established in a grassy margin of an area planted in mixed fescue and surrounded by hedgerows, small wood lots, and apple orchard blocks at the USDA-ARS Appalachian Fruit Research Station in Kearneysville, WV. A 15 cm tall grassy surface was established in three plots, while a bare soil surface using top soil and sand was established in the remaining three plots. Each plot measured 1 × 1 m and was surrounded by a square pitfall trap comprised of schedule 40 PVC pipe (10.2 cm outer diameter) pieces used to create an open trough and joined at the four corners by PVC elbows attached with heavy-duty PVC cement and filled halfway with a 1:3 mixture of glycol and water.

Seven males and seven females from each treatment group were gently placed in the center of a grassy and a bare soil plot at 9:00 a.m. Twenty-four hours later, the pitfall trap surrounding each plot was visually examined for captured adults. A particulate skimmer was also used to recover captured adults. Three consecutive releases comprised a single replicate and three release replicates were conducted.

Proportional recapture data were arcsine transformed and data were analyzed using the GLM procedure (SAS Institute 2003) to construct ANOVA tables. The model included the following class variables: plot type (grassy versus bare), plum curculio treatment (tagged, glued elytra, or control), and sex. If a class variable was not significant, it was dropped from the model. If the model indicated significant differences, multiple comparisons were calculated using Student-Newman-Keuls test ($P = 0.05$).

Western Corn Rootworm. The field experiment was conducted in the plowed area at the edge of a com field at the AAFC Eastern Cereal and Oilseed Research Centre in Ottawa, ON, using a 1 × 1 m arena consisting of bare sandy loam. Adults were collected in the morning and sexed according to White (1977). Ninety adults (sex ratio 1:1) were separated in three equal treatment-group. Ten adults of each group were individually placed in the middle of the arena and a digital chronometer was used to measure the time required for each to reach the arena edge (≥50 cm) during each 5 min period. The test was repeated three

Table 1. Percentage of adult Colorado potato beetles ($n = 30$), multivoltine plum curculios ($n = 30$), and western corn rootworms ($n = 50$) with and without tags successfully reaching the top of potato stems, a 20 cm vertical cylinder or a 20 cm section of corn stalk, respectively

Treatment	Colorado potato beetle	Plum curculio	Western corn rootworm
Vertical tag	44a[c]	16.7a	83a
Trailing tag	47a[b]	—	59b[a]
No tag (glue)	—	20.0a	95a
No tag (no glue)	32a	30.0a	—
	$\chi^2 = 1.84$	$\chi^2 = 1.67$	$\chi^2 = 7.50$
	$P = 0.175$	$P = 0.433$	$P < 0.05$

Period of observation: 20 min for Colorado potato beetle; 5 min for PC; 3 min for western corn rootworm.

[a] A 4 cm trailing tag; [b] 8 cm trailing tag.

[c] Means followed by the same letter are not significantly different.

times. The difference in mean travel time to reach the arena edge for each sex and treatments were analyzed using two-way ANOVA (SPSS software version 3.1). The number of entanglement and the number of beetles crossing the edge was also recorded. Chi-square test was used to compare the number of beetles crossing the arena edges for the different treatments. The number of entanglement and the number of beetles crossing the edge was also recorded.

Results

Vertical Walking

Colorado Potato Beetle. The mean total distance traveled (centimeters) (up and down a stem) by beetles with trailing tags (56.50 ± 3.05), beetles with a vertical tag (62.96 ± 4.18), and beetles without tags (61.07 ± 3.59) did not differ significantly ($F = 0.866$; df = 2, 82; $P = 0.424$). The mean total distance traveled by male (58 ± 3.13) and female (61.40 ± 2.76) beetles did not differ significantly ($F = 0.42$; df = 1, 84; $P = 0.519$) and there was no interaction between sex and tag treatment ($F = 0.57$; df = 2, 84; $P = 0.568$). The percent of beetles tested with a trailing tag or a vertical tag that successfully completed the climb up to the top of the potato stems was actually slightly higher than that of beetles without a tag although not significantly (Table 1). The average time (seconds) taken to reach the top of the potato plant stems by beetles with a trailing tag, with a vertical tag or without a tag did not differ significantly ($F = 1.411$; df = 2, 34; $P = 0.258$; Table 2). The mean height (centimeters) achieved by beetles with a trailing tag (24 ± 1.40), beetles with a vertical tag (23.8 ± 1.54), or beetles without a tag (24.32 ± 1.44) did not differ significantly ($F = 0.158$; df = 2, 34; $P = 0.854$) either. The mean height achieved by male (24 ± 1.2) and female (24 ± 1.17) beetles did not differ ($F = 0.04$; df = 2, 84; $P = 0.842$) and there was no interaction between sex and tag treatment ($F = 0.16$; df = 2, 84; $P = 0.852$).

The proportion of beetles with trailing tags (7/30) that climbed down the stems within the observation period was significantly less than the proportion of beetles without tags (15/28) ($\chi^2 = 9.68$; df = 1; $P = 0.002$). The proportion of beetles with vertical tags (14/27) that climbed down the stems was similar to that without tags (15/28). However, the time (seconds) spent climbing down by beetles with trailing tags (165 ± 23) or with vertical tags (146 ± 21) did not differ significantly among themselves or from the beetles without tags (146 ± 23) ($F = 0.617$; df = 2, 35; $P = 0.546$).

Table 2. Time (seconds) required by adult Colorado potato beetles ($n = 30$) and western corn rootworms ($n = 50$) with and without tags to climb to the top of potato stems or a 20 cm section of corn stalk, respectively

Treatment	Colorado potato beetle	Western corn rootworm
Vertical tag	242 ± 68a[c]	26.3 ± 4.17b
Trailing tag	297 ± 79a[b]	61.3 ± 7.8a[a]
No tag (glue)	186 ± 79a	19.1 ± 2.62b

Period of observation: 20 min for Colorado potato beetle; 3 min for western corn rootworm.

[a] A 4 cm trailing tag; [b] 8 cm trailing tag.

[c] Means followed by the same letter within a column are not statistically different (Student-Newman-Keuls test, $P = 0.05$).

Plum Curculio. The model for mean total vertical distance traveled was not significant ($F = 1.89$; df = 3,86; $P = 0.14$) with no significant differences detected based on treatment or sex, although individuals in the control group moved numerically greater distances (18.53 ± 4.81 cm) compared with tagged (9.46 ± 3.07 cm) and glued (10.27 ± 4.18 cm) curculios. Similarly, the likelihood of movement by any of the test subjects was not explained by treatment or sex using logistic regression. For all treatments, >46% of individuals moved in vertical arenas, with 16.7–30.0% of the curculios reaching the top (Table 1).

Western Corn Rootworm. The mean time taken by male (41.4 ± 7.21 s) and female (30.0 ± 12.84 s) to climb the 20 cm distance on the corn stalk was not significantly different ($F = 3.6$; df = 1, 36; $P = 0.066$) and there was no interaction between sex and tag treatment ($F = 0.66$; df = 2, 36; $P = 0.523$). However, the mean time taken to climb up by beetles carrying a trailing tag was significantly higher ($F = 19.2$; df = 2,36; $P < 0.001$) than for beetles with a vertical tag or without a tag (but with elytra glued) (Table 2). Between 59 and 95% of the beetles successfully climbed the 20 cm distance on the corn stalk (Table 1). The proportion of corn rootworm with trailing tags that climbed the vertical stalk was significantly less than the proportion of beetles without tag or with vertical tag ($\chi^2 = 7.50$; df = 2; $P < 0.05$). Four males with trailing tag made frequent stops when climbing the stalk (none for males with vertical tag or without tags and none for females). Furthermore, five males and two females with trailing tag fell off the stalk compared with no falls for beetles with vertical tag or no tags.

Horizontal Walking

Colorado Potato Beetle. The percentage of beetles with trailing tags or with vertical tags walking out of bare ground and grassy plots over a 6 h period did not

Table 3. Mean (±SE) percentage of adult Colorado potato beetles (n = 42), plum curculios (n = 42), and corn rootworms (n = 30) with and without tags reaching the edge of an arena by walking over periods of 6 h, 24 h, and 5 min respectively

Treatment (tag)	Colorado potato beetle		Plum curculio		Western corn rootworm
	Bare ground	Grass	Bare ground	Grass	Bare ground
Vertical tag	78 ± 5.69a[c]	13 ± 6.69a	50.8 ± 12.7a	27.8 ± 4.1a	77.8 ± 8.1a
Trailing tag	75 ± 9.21a[b]	8 ± 1.0a[b]	—	—	58.8 ± 5.3b[a]
No tag (glue)	71 ± 9.33a	20 ± 4.1a	53.2 ± 16.7a	34.9 ± 22.2a	100.0 ± 0.0a
No tag (no glue)	—	—	40.5 ± 7.6a	24.6 ± 9.9a	—

[a] A 4 cm trailing tag; [b] 8 cm trailing tag.
[c] Means followed by the same letter within a column are not statistically different (Student-Newman-Keuls test, P = 0.05).

differ among themselves or from the beetles without tags (F = 0.3; df = 2, 12; P = 0.746). However, the percentage of beetles with and without tags walking out of the bare ground plots over a 6 h period was significantly higher (F = 106.2; df = 1, 12; $P < 0001$) than the percentage of beetles walking out of the grassy plots (Table 3; Fig. 1).

Plum Curculio. Plum curculio treatment (vertical tag, no tag with glued elytra and no tag with free elytra) and sex had no significant effect on the percentage of beetles walking out of the plots and were dropped from the model. Among the three experimental treatments, 44.4% (±9.0 SE) of individuals with glued elytra successfully walked from the center of experimental plots and were recaptured in the surrounding pitfall traps, followed by tagged individuals (39.2% ± 5.6 SE), and the control (32.5% ± 5.0 SE; Table 3). However in the case of the control group, it is likely that individuals also flew from plots based on their lack of tag on pronotum and adhesive on elytra. There also was no difference based on sex, with 38.8% (±6.1 SE) of females and 38.4% (±5.1 SE) of males being recaptured in surrounding pitfall traps.

The percentage of beetles walking out of the bare ground plots was significantly higher (F = 6.98; df = 1, 34; P = 0.012) than the percentage of beetles walking out of the grassy plots. In this case, 48.1% (±5.0 SE) of individuals released in bare soil plots were recaptured in surrounding pitfall traps, while only 29.1% (±5.1 SE) released in grassy plots were recovered from surrounding pitfall traps. Among individual treatments, the percentage that walked out of bare ground plots was higher than those in grassy plots (Table 3).

Western Corn Rootworm. Eighty-four of the 90 tagged and untagged rootworms successfully crossed the field arena within the observation period. Two rootworms with vertical tag and five with trailing tags were entangled on soil clumps. Another six rootworms with vertical tag and 10 with trailing tags were temporarily arrested by soil clumps but freed themselves and crossed the arena within the allotted time.

The mean time required to walk on bare ground from the center to the edge of the arena by male (62.7 ± 5.06 s) and female (51.9 ± 4.55 s) rootworm was not significantly different (F = 1.42; df = 1,78; P = 0.24) and there was no interaction between sex and treatment (F = 0.36; df = 2, 78; P = 0.30). However, the mean time required differed significantly according to treatment. (F = 4.56; df = 2, 78; P = 0.0172).

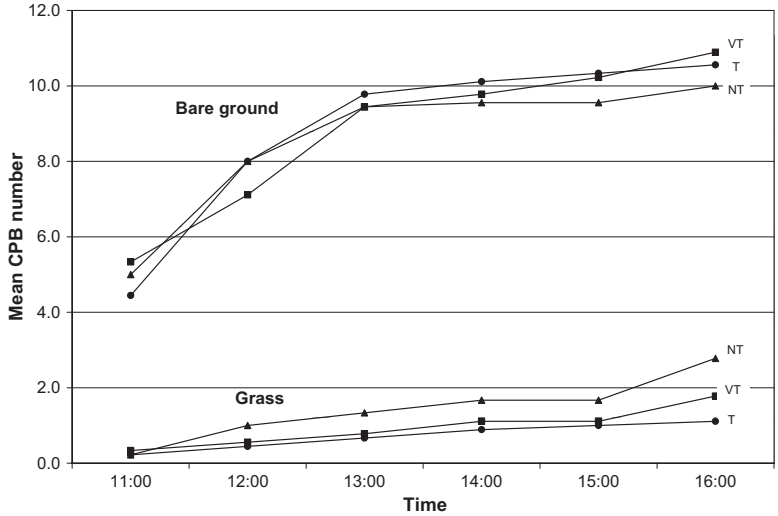

Fig. 1. Changes in the cumulative mean number of adult Colorado potato beetles with vertical (VT), trailing (T), or no (NT) harmonic radar tags walking out of bare ground or grassy field plots over a 6 h period.

Rootworms with trailing tag required a significantly higher mean time (75.7 ± 7.16 s) than rootworms with either vertical tag (53.1 ± 3.53 s), or no tag (43.2 ± 3.00 s) different (Student-Newman-Keuls test, $P < 0.05$). The proportion of corn rootworm that crossed the arena edge while pulling a trailing tag was significantly less than the proportion of rootworms with a vertical tag or without a tag ($\chi^2 = 15.81$; df = 2; $P < 0.001$).

Discussion

Vertical and trailing electronic tags used in the tests were occasionally observed entangled on vegetation or soil clumps. However, results showed that these entanglements had generally no significant impact on the number of insects reaching their destination or on the time taken to reach a destination. In cases when entanglements significantly reduced the number of insects reaching a destination or increased the time taken to reach a destination, the impact of the tag was minor relative to that of the many other factors (such as vegetation density) that affect such an outcome.

Vertical Walking. Colorado potato beetles with tags took the same time as beetles without tags to reach the top of a vertical structure and a similar number completed the climb. Results were similar for western corn rootworms with vertical tags but the short trailing tags slowed down the climb and reduced the number of successful climbs. Although reducing the length of the trailing tags to 4 cm had resulted in no obvious impairments in normal walking activity, the length of the tag remained a nuisance factor for western corn rootworms. As with the two other insects, there was no significant effect on vertical movement when a vertical tag was attached to an adult plum curculio. Trailing tags, however, were not evaluated because of the difficulty attaching them to the irregular surface of the elytra and the obvious impairment they imposed on the ability of plum curculio to walk.

In the case of Colorado potato beetles, the only evidence of tag nuisance came from a significantly lower number of beetles with trailing tags than with vertical tags or without tags walking down the stems. The mean time spent walking down the stems was also higher for beetles with trailing tags than for the other beetles but not significantly so. The position of the trailing tag in relation to the body of the insect did not seem to interfere with the walking behavior of the walking potato beetle.

Horizontal Walking. The bare ground plots and the grassy plots were chosen because they provided two highly contrasted habitats with low and high risk for tag entanglement, respectively. As expected, the plot landscape had a significant impact on the percentage of potato beetles and plum curculios leaving the plots over the monitoring period but, contrary to expectations, the presence of tags did not. The presence of a vertical or a trailing tag did not affect the percentage of Colorado potato beetles and the presence of vertical tags did not affect the percentage of plum curculios walking out of bare ground or grassy plots when compared with adults without tags. Colorado potato beetles with trailing tags were occasionally observed entangled to the vegetation and those with vertical tags were occasionally stopped by dried straw or soil clods but these incidents were of short duration and did not affect the overall count over the monitoring period. This was most clearly shown by the similar cumulative hourly rate at which tagged and untagged Colorado potato beetles left each type of habitat (Fig. 1). These results are in agreement with Dahlquist (2008) who reported no significant difference between the number of tagged and untagged banana weevils (*Cosmopolites sordidus* Germar), similar in weight to the Colorado potato beetle, finding the target baits set in their tropical trial plots.

The western corn rootworm was not tested in grass plots. However, the 4 cm trailing tag significantly limited the ability of male or female rootworms to cross the bare ground arena. The longer travel time recorded did not result from the weight of the trailing tag but from the nuisance caused by the trailing wire. The 4 cm long trailing tags weighed only 0.2 mg more than the vertical tags, which had no significant impact on the travel time in both tests.

Tag Entanglement and Use of Harmonic Radar. The visual observations of tag entanglements had suggested potential limitations to the use of harmonic tags to track the unimpaired movement of insects but results obtained here revealed only brief and infrequent interruptions caused by entanglement except for the short trailing tags attached to the corn rootworm. Furthermore, in the case of the Colorado potato beetle and the plum curculio, the impact on dispersal frequency was minimal compared with the substantial impact of vegetation in the habitat in which the insect is dispersing.

Walking plays a considerable role in the dispersal of the adult Colorado potato beetle throughout the season, from host colonization to overwintering site dispersal (Weber and Ferro 1994, Boiteau et al. 2003). This study confirms the suitability of vertical tags to track potato beetles (Boiteau et al. 2010) and extends it to trailing tags. Based on the results, although entanglements of vertical or trailing tags in the host plant or on soil clumps were very noticeable, they had no significant impact on the overall walking path of potato beetles and therefore should not limit the applicability of harmonic radar tracking to this species.

Although the weight of harmonic tags on plum curculio likely prevent flight, adults with tags were able to walk successfully across grassy terrain and bare soil in experiments reported here. These results correspond with previous laboratory-based trials in which vertical tags did not affect horizontal movement (Boiteau et al. 2010). The two terrain types used for these field-based trials represent the sort of landscape an adult likely will encounter when it leaves overwintering sites outside orchards and begins to move into orchard border areas. Plum curculio adult dispersal is limited primarily to movements to and from overwintering sites and is achieved mainly by walking in grasses when temperatures are <20°C (Lafleur and

Hill 1987, Lafleur et al. 1987, Prokopy et al. 1999). Daily movements from one tree to another are seldom observed, though movements between the tree and ground are frequent because of thanatosis (Chouinard et al. 1994). In addition, because adult plum curculios were able to move up vertical surfaces with attached tags in studies reported here with multivoltine plum curculios, and studies with univoltine adults, though movement of females was reduced to some degree (Boiteau et al. 2010), adults likely should be able to successfully negotiate the vertical surfaces of host tree trunks. Within host tree canopies, adults generally walk, traversing the upper and lower sides of limbs (Leskey and Prokopy 2002). Thus, our results indicate that a tagged plum curculio will be able to successfully colonize a host tree and therefore its movements during this process can be tracked with a harmonic radar system.

The western corn rootworm is the most challenging of the three species studied because of its rapid walking habit, high propensity for flight (Isard et al. 2000), sensitivity to glue and tag attachment problems (Boiteau et al. 2010). The species spends most of its time on the host plants (feeding, courtship, mating) or dispersing (feeding, mating) and the least time on or near the ground (weeds, grass, bare ground) for egg laying activity. Isolated $Bt_{rootworm}$ transgenic corn plots were providing an interesting method to measure western corn rootworm movement (Spencer et al. 2003) but the proliferation of corn hybrid with stacked gene (corn borer and corn rootworm) now limits the value of this technique. For this reason, the use of a harmonic radar system would help to study corn rootworm ground movement as well as local movement of flying adults. However, more research would be required to determine if shorter trailing tags could be used with the corn rootworm but the suitability of vertical tags (Boiteau et al. 2010) was confirmed. The nuisance of a trailing tag could have its largest impact on feeding activity because tags could get caught on sticky corn silk or in the clay soil when females enter cracks to deposit eggs.

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