

Response of adult Colorado potato beetles (Coleoptera: Chrysomelidae) to water in the landscape

Gilles Boiteau,¹ Pamela MacKinley

Abstract—This laboratory study confirmed that the strategy of adult terrestrial Colorado potato beetle (*Leptinotarsa decemlineata* (Say); Coleoptera Chrysomelidae) to survive the threat of drowning in water is based on avoidance of water crossings. It also showed that beetles at the surface of a body of water after failing to avoid it, long considered limited to passive floating and phoretic transport were in fact likely to rely on a complex fight or flee response. Beetles showed capacity to swim in a pattern similar to land foraging beetles. Beetles also tolerated submergence and walked underwater. These active behaviours should improve their probability of finding shore or refuge for longer survival. Results confirmed that Colorado potato beetles are likely to accumulate near water features in the potato agro-ecosystem landscape but suggest that successful crossings and colonisation of crops on the other side are more likely than previously expected. On a larger scale, new information provided by this study combined with our knowledge of dominant winds and currents should make it possible for future research to better predict the probability of surviving encounters with water and the orientation of invasive Colorado potato beetle colonisers dispersing at the surface of bodies of water.

Introduction

Our understanding of the behaviour of terrestrial insects near bodies of fresh and salt water is limited and often ambiguous. As with most other terrestrial arthropods, the adult Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae), encounters bodies of water randomly when dispersing by ground and by air (Hurst 1975). Although the Colorado potato beetle will seek water to satisfy their physiological requirement (Kaczmarek 1955), the species has an exceptional capacity to withstand effects of food and water deprivation (Feytaud 1938; Grison *et al.* 1954) except at two critical points in the life cycle: adults emerging from diapause die in a short time without access to water (Grison 1963; Le Berre and Louveaux 1980; MacQuarrie and Boiteau 2003) and those newly emerged from pupae require a period of feeding to survive (Termier *et al.* 1988; Weber and Ferro 1996; Alyokhin and Ferro 1999; Alyokhin *et al.* 1999). Adapted to the terrestrial environment, the Colorado potato beetle lacks the morphological

adaptations of aquatic insects to control its movement in the water. Overall, the entry in or landing on water of adult Colorado potato beetles has been considered accidental and lethal for most individuals (Hurst 1975). Historically, the ocean and bodies of fresh water have been considered direct barriers to the dispersal of the Colorado potato beetle and most terrestrial insects as well as indirect obstacles through their effect on the local climate (Gibson *et al.* 1925). Kaczmarek (1955), working in an arid area of Poland, reported that overwintered Colorado potato beetles moving towards a body of water to restore their water reserves did not attempt to cross it but accumulated in the nearby potato fields.

The abundance of foci of Colorado potato beetle colonisation near waterways could also indicate that bodies of water, in addition to their role as barriers, play an inadvertent key role in the distribution of the Colorado potato beetle (falling into the water) across the territory. According to Feytaud (1930), American researchers considered the swift current of rivers partially responsible for the rapid spread of the beetle

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G. Boiteau,¹ P. MacKinley, Agriculture and Agri-Food Canada, Potato Research Center, 850 Lincoln Road, PO Box 20280, Fredericton, New Brunswick, E3B 4Z7, Canada

¹Corresponding author (e-mail: gilles.boiteau@agr.gc.ca).

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across the United States of America. There are no reports of Colorado potato beetles swimming across bodies of water but there are multiple reports of floating beetles. Hurst (1975) suggested that potato beetles flying above bodies of water may encounter temperatures below those favourable for flight (threshold close to 25 °C) and be forced to land on the water. Colorado potato beetles in flight have actually been seen landing on the ocean and on boats (Dunn 1949; Thomas and Dunn 1951). Beetles approaching the water to drink have also been observed falling in or being drawn in by the back and forth movement of the water at the edge. Feytaud (1930) first described the flotation of adult Colorado potato beetles on water; the adult, the only stage able of flotation, floats on the ventral surface with most of the elytra and the stigmata above the water. According to Feytaud (1930), the Colorado potato beetle may occasionally try to walk on the surface but it is unable to swim. Feytaud (1930) suggested that the transport of the insect on water is entirely passive.

The wind and the tides can disperse large numbers of the floating beetles towards the shorelines (Hurst 1975). Records of live potato beetles washing upon the shores of United Kingdom and France provide evidence that such adults can survive exposure not only to fresh water but also to salt water (Dunn 1949; Bartlett 1981). Limited laboratory tests by Dunn (1949) demonstrated that adult Colorado potato beetles could remain alive for six to 10 days floating on sea water. One would expect beetles landing on boats (Dunn 1949; Thomas and Dunn 1951) or drifting on floating debris such as driftwood to have higher probability of survival than those floating on the water surface itself but lower than survival on land (in the absence of food). Groups of beetles may have an advantage over single beetles through a process known as rafting (Dunn 1949; Adams *et al.* 2011). It is also expected that surviving time on floating debris will be inversely proportional to the number of forays in the water.

Faced with global climate change, considerable transformations in hydrological events and the water landscape are expected (Pullin 1999). The frequency of encounters between terrestrial arthropods and the water landscape are likely to increase and affect their distribution and survival (Pullin 1999). It is important to increase our limited understanding of the role of the water

landscape on terrestrial insects as a threat or an opportunity to adapt our pest management strategies in accordance.

Whether the Colorado potato beetle finds itself in fresh water or the ocean as a result of a voluntary approach to drink or of an involuntary landing or fall, it has to respond to the potentially lethal situation. Vertebrates and invertebrate faced with such threats usually bring together a collection of fight and flee behaviours to ensure short-term survival. Considering that the established response of adult Colorado potato beetle to the threat of predation or sudden changes in their physical environment (*e.g.*, sudden changes in host plant oscillations) is made up of freezing (quiescence), feigning death, falling off plants, and walking away (Boiteau and Misener 1996; Acheampong and Mitchell 1997; Lopez *et al.* 1997; Couturier *et al.* 2005), applying a similar assemblage of spontaneous or intuitive behaviours to the threat of water is likely to convey the largest fitness benefits.

Although flight is a dispersal method of choice for the colonisation of islands and remote territories, the importance of transport across water as an invasion mechanism is also well recognised (Weber 1950; Holzapfel and Harrell 1968). Transport across water tends to be associated to oceanic debris such as driftwood (Coulson *et al.* 2002) but floating without the assistance of debris (Hawes 2008) or in rafts (Mlot *et al.* 2011) also occurs frequently.

Based on the observations of Feytaud (1930), the adult Colorado potato beetle would be unable to face the threat of drowning directly (fight) by swimming away, back to the shore, and would therefore be limited to avoid the threat (fleeing, escaping) by avoiding crossing bodies of water or quiescence/death feigning to float and avoid drowning by postponing submergence. However, recent studies have provided increasing evidence that the inability of terrestrial insects to disperse in or across water may not be as absolute as was once presumed (Niehues *et al.* 1996; Heitler *et al.* 2005; Brouwers and Newton 2010a; Martay *et al.* 2014). Given the new evidence, this study was initiated with the objective of re-examining the response of adult Colorado potato beetles to water. Based on Colorado potato beetle literature, we hypothesised that the Colorado potato beetle response to the threat of water is limited to passive

escape by floating and that given its inability to fight its way out of the water environment, it would be advantageous for the Colorado potato beetle to have developed a behavioural avoidance of (crossing) water features when dispersing (*i.e.*, to consider water features as barriers). The hypothesis was tested through a series of laboratory trials re-examining the adult Colorado potato beetle avoidance of water crossings, survival at the surface of fresh water and salt water, survival at the surface of fresh water in the presence of driftwood, tolerance to submergence, and ability to swim. This study may be the first to investigate simultaneously floating and phoretic transport for a same species.

Materials and methods

Insects

Adult Colorado potato beetles were collected from a potato field receiving no insecticide treatment for some tests and from a colony (14–28-day-old adults) maintained on potato (*Solanum tuberosum* Linnaeus (Solanaceae); cultivar Kennebec) for other tests, all at the Potato Research Centre, Fredericton, New Brunswick, Canada. Male and female beetles were sexed according to Boiteau and Le Blanc (1992) when required. In some tests, beetles were marked individually using the necrotic spot method of Unruh and Chauvin (1993). Beetles were kept in ventilated containers with access to food until time of release in all tests.

Avoidance of water crossings

The impact of a body of water on the orientation of walking adult Colorado potato beetles was tested in the laboratory, at room temperature, using arenas 23 cm length × 23 cm width × 4 cm height made up of flat bottom Styrofoam containers. Treatments consisted of an uninterrupted flat arena (control), an arena interrupted by a 5 cm wide and 1 cm deep trench dug out across its centre and an arena interrupted by a trench filled with fresh water (distilled). The highly phototropic beetles released on one side of the trench (or equivalent marked area in the control) were drawn across the trench through a light gradient created by a light source set up across and parallel to the trench. Light intensity in the release, trench and

across trench areas of each container averaged 48, 52, and 65 lx, respectively (recorded using a Cooke Cal-Light 400 Calibrated Precision Light Meter (PCO-Tech (formerly Cooke Corporation), Romulus, Michigan, United States of America).

Six 15-day-old colony adult Colorado potato beetles were released on the floor of each container in the low light section closest to the observer. Each container was observed every five minutes over a period of 60 minutes to record the distribution of beetles in the release, trench, and across trench areas at that time. Whenever beetles were observed on or in the water, additional time was spent observing and noting the behaviour of beetles (floating, swimming, walking on bottom of trench, *etc.*). Beetles were not re-used in the tests. Six male and six female beetles were used alternatively in each test. Each test was repeated 15 times with males and 15 times with females.

Survival at the surface of fresh and salt water

The aim of this experiment was to determine if the survival period of floating beetles differed on fresh and salt water and was or not affected by water motion. Tests were carried out in open vials (16.5 × 6.5 cm with a 4 cm opening) filled with 2.5 cm of water placed on a laboratory bench or on an orbital shaker (VWR S-500 Model 980019 (VWR, Radnor, Pennsylvania, United States of America) – 3/4' circular orbit set to 25 rpm) to simulate water current. Six beetles (three males and three females) were introduced individually in each vial and there were two replicates of each treatment: flotation on still water (fresh and salt) and flotation on shaken water (fresh and salt). Salt water with a specific gravity 1.023 and salinity of 31.1 parts per thousand was prepared using commercially available Instant Ocean[®] Sea Salt (Blacksburg, Virginia, United States of America). Tests were carried out at 20 °C. Preliminary tests having established that adult Colorado potato beetle could only survive short periods of time floating at the surface of water, mortality was assessed after four days. The test was repeated three times with beetles collected from a field without insecticide sprays. Mortality was assessed by transferring inactive beetles to petri dishes lined with absorbent paper. Beetles that had not recovered after 24 hour were considered dead. Six beetles (three males and three females) were

introduced individually in each of two vials without water as control. No correction for mortality was required.

Survival and dispersal at the surface of fresh water in the presence of driftwood

Tests were carried out in plastic containers (34.3 × 21 × 12.1 cm (5.7 L)) filled with 18 mm of water and placed on a laboratory bench. Ten beetles were introduced in each of three replicate boxes. Ten beetles were also introduced in another box free of water serving as control and covered with netting to prevent escapes. Driftwood was simulated by the addition of a wood island (50 × 100 × 19 mm) inside each container. A metal weight in the middle of the island prevented it from floating and anchored it to the middle of the floor area of the box. Dental cotton rolls attached to the weight provided water to the beetles in the control box. The test was replicated twice over time using 29-day-old greenhouse colony beetles. Beetles were marked individually using the necrotic spot method of Unruh and Chauvin (1993).

The location of each beetle in each box (on the island/driftwood or in the water) and whether or not it was alive were recorded as often as possible throughout the day in test one and at least twice daily in test two. A fine camel-hair brush was used to touch immobile beetles and determine if they were alive or not. Beetles that reacted by moving their legs were considered alive and left at the same location. Beetles that did not react were removed and placed in a petri dish lined with absorbent paper. Beetles that resumed movement within a few hours were placed back into the box of origin and beetles that had still not resumed movement after 24 hours were considered dead. The first and second repetitions of the test were terminated shortly after 50% of the beetles had died. Room temperature throughout the test ranged between an average of 20 °C during the day and 21.5 °C at night.

Tolerance to submergence

The first test was carried out in an open transparent plastic container (34.3 × 21 × 12.1 cm (5.7 L)) filled in with 2 cm of fresh water, in the laboratory, at ~18 °C. A sheet of fibreglass window screening was adhered to the floor of the container to provide the rough surface required by

the Colorado potato beetle to hold onto the floor surface. Ten males and 10 females were pushed down underwater one by one until they grasped the bottom surface and observed visually. The duration of the stay underwater was recorded with a timer. The second test was carried out in 1-L beaker filled with water. Adult Colorado potato beetles were placed underwater in the beaker in groups of three in a small screen cage. The behaviour of the beetles was observed and the duration of the active period recorded. One group of beetles was kept underwater initially for two hours after all activity ceased and was then removed to a paper-lined petri dish where recovery was observed. The process was repeated for increasingly longer periods underwater with full recovery. Finally, 12 males and 12 females (in groups of three) were kept underwater for a period of 16.5 hours after all activity ceased and the per cent recovery recorded.

Ability to swim

The objective of this test was to determine if the dispersal of Colorado potato beetles floating at the surface of the water is only due to drift, as suggested in the literature, or also (partly) due to swimming. Tests were conducted in a rectangular white plastic arena (45 × 30.5 × 17.5 cm) filled in with 3.7 cm of water held at ~19 °C. A video camera (Panasonic WV-BP334-1/3' B&W CCD digital camera (Mississauga, Ontario, Canada) placed at 80 cm above the water surface was used to record the movement of the Colorado potato beetle introduced in the arena. A rubber stopper topped by wood (6.5 cm diameter) was placed at the centre of the arena in every other test to provide the experimental setup with an island accessible to test Colorado potato beetles. The top of the island was level with the surface of the water. Treatments consisted of (1) Colorado potato beetle dropped at the water surface of an arena with a wooden island at its centre and (2) without an island at the centre. Treatments were applied alternately and the tests carried out over a period of one month. The whole apparatus was placed inside a fine mesh cage (57 × 57 × 92 cm) that allowed the ambient light in but helped eliminate Colorado potato beetle drifting caused by the movement of the air in the room. A test was carried out to ensure that any remaining drifting was negligible. Six and nine dead Colorado potato

beetles were released in the same manner as the live Colorado potato beetles in arenas without and with islands, respectively, were found to drift a total distance of 23.9 and 7.9 cm ($df_{1,13} = 7.96$; $P > 0.014$) at a velocity of 0.061 and 0.065 mm/second ($df_{1,13} = 0.16$; $P > 0.700$). Because the sensitivity of the floating Colorado potato beetle to drifting is high, the cage did not entirely suppress the “abiotic” movement but reduced its impact substantially in each arena type. Because the total distance travelled or speed of travel represented such a small and variable proportion of the voluntary locomotion activity, data were not corrected for drifting before analysis (no data correction was considered necessary).

Tracks were recorded and analysed using (EthoVision[®] 3.1; Noldus Information Technology, Wageningen, The Netherlands) and SAS version 9.3 (SAS Institute 2002). The area of the island in treatment one and the corresponding area in treatment two were treated as a hidden zone. Comparisons of Colorado potato beetle behaviour between arenas with and without driftwood were made using the following parameters: distance moved (cm), velocity (cm/second), and number of 360° rotations (mean per Colorado potato beetle). Clockwise and anti-clockwise rotations were counted separately for each Colorado potato beetle.

Thirteen (six males and seven females) and 15 (nine males and six females) individuals were tested alternatively in arenas without and with island, respectively, over a period of 18 days. Each Colorado potato beetle was dropped from a pencil on the surface of the water in the lower left corner of the arena and the track recorded over a period of 120 minutes. The corresponding area was defined as a separate track recording zone for the Ethovision software. Turning angles and frequency at which the beetles reach the island or the island zone were used to determine the directionality of the travel path under both treatments.

Data analysis

In the test on the avoidance of water crossings, a 2 × 3 factorial analysis of variance (ANOVA) was carried out on the data to evaluate the variability of the mean number of Colorado potato beetles walking into the three zones of the arena in relation to treatment: no trench, trench, and trench filled with water and sex. The analysis was carried out using VassarStats (2015).

In the test on survival at the surface of fresh and salt water, the impact of shaken water on the percentage of potato beetles floating at the surface surviving in fresh or salt water was analysed after four days with a two-way ANOVA. An arcsin transformation was applied to the data but untransformed data are presented in the text and table. The survival of male and female beetles was compared in the combined data with a Student's *t*-test. The analysis was carried out using VassarStats (<http://www.vassarstats.net/>).

In the test on survival and dispersal at the surface of fresh water in the presence of an island/driftwood, the mean time to 50% mortality, the mean time spent on the release driftwood refuge before entering the water surface for the first time and the mean frequency of sightings off the refuge driftwood were compared for Colorado potato beetles in treatments with water and without water with a Student's *t*-test. The analysis was carried out using VassarStats (<http://www.vassarstats.net/>).

In the test on tolerance to submergence, the mean periods of activity for males and females were compared using a Student's *t*-test. The analysis was carried out using VassarStats (<http://www.vassarstats.net/>).

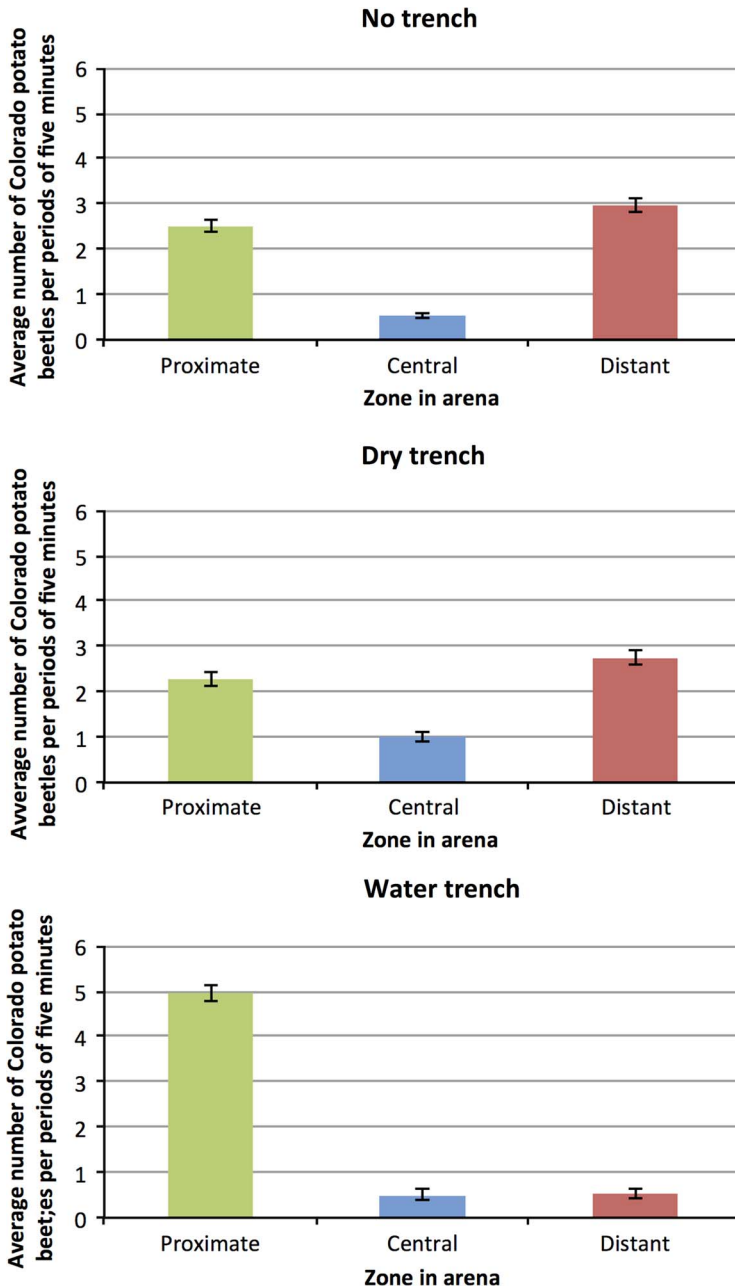
In the test on the ability to swim, the effect of the island on distance moved, velocity, and number of 360° rotations was tested using one-way ANOVA with post hoc Student's *t*-test. Data that did not meet the assumptions were log transformed. All tests were carried out using SAS (version 9.3). The effect of the island on turning angles and directionality of the Colorado potato beetle swim path was tested with circular statistics using (Oriana version 4.02 (Kovach Computing Services, Wales, United Kingdom)). The normal distribution and homogeneity of variance of the data was assessed for all tests whenever applicable.

Results

Avoidance of water crossings

The water filled trench interrupted the dispersal of the walking adult Colorado potato beetles across the phototropic gradient in the arena. The mean number of male and female Colorado potato beetles observed having crossed the trench filled with water (0.6 ± 0.11) (every five minutes over a one-hour period) was significantly lower than

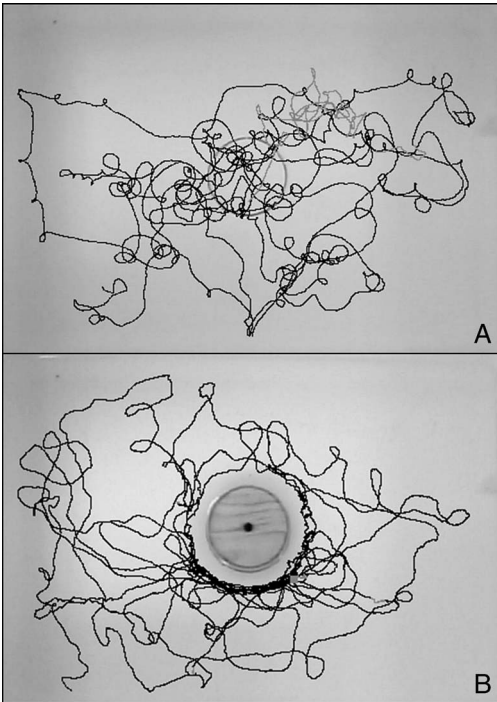
Fig. 1. Mean number of male and female Colorado potato beetles, released in the proximate zone of an arena, observed reaching the distal zone after crossing the central zone (water filled trench, dry trench, and no trench). The number of beetles was recorded every five minutes over one hour ($n = 30$). The dispersal took place across a phototropic gradient. Columns with different letters are significantly different according to Tukey's multiple comparison test ($P < 0.05$).



that observed across the dry trench (2.7 ± 0.16) or the no trench treatments (3.0 ± 0.15) ($F(2,84) = 94.03$; $P < 0.0001$) (Fig. 1). There was

no effect of sex ($F(1,84) = 3.59$; $P = 0.062$) and no interaction between sex and trench type ($F(2,84) = 2.91$; $P = 0.06$).

Fig. 2. Characteristic movement pattern of one adult Colorado potato beetle monitored for two hours swimming at the surface of a water-filled arena without (A) and with (B) a refuge island/driftwood in the middle. The dark point in the lower left corner indicates the location of the release.



Beetles moved up the light gradient across the arena within the first 5–10 minutes of each one-hour test in the dry and no trench treatments. Then, beetles moved back and forth throughout the arenas with close to half of the beetles released distributing themselves at each end of the light gradient (closer to the walls of the arena). In the water filled trench treatment, beetles walking up the light gradient either followed the edge of the body of water and turned back or turned back upon encountering the water. After one hour less than one beetle on average had crossed the water filled trench.

Survival at the surface of fresh and salt water

The percentage of beetles dead after four days floating at the water surface in the laboratory was significantly higher on salt (76.3 ± 5.86) than fresh (51.3 ± 6.92) water ($F(1,11) = 7.93$;

$P = 0.023$) but similar on calm (58.3 ± 5.66) and agitated (69.3 ± 10.02) water ($F(1,11) = 1.53$; $P = 0.250$). The average mortality of males (58.3 ± 2.41) did not differ significantly ($t = 2.92$; $P < 0.24$) from that of females (70.8 ± 12.73).

Survival and dispersal at the surface of fresh water in the presence of driftwood

Fifty per cent of Colorado potato beetles released on a driftwood refuge from surrounding fresh water survived over a period of 523 ± 20.8 hours whereas 50% of beetles released in a similar environment but without surrounding water survived 668 ± 17.1 hours ($df = 2$; $t = 4.303$; $P = 0.048$). Adult Colorado potato beetles spent significantly more time on the refuge surrounded by water before leaving it for the first time (235.5 ± 10.5 hours) than on the refuge without surrounding water (62 ± 13.0 hours) ($df = 2$; $t = 4.303$; $P = 0.009$). The frequency of sightings of individuals off the refuge was also higher in the treatment without water (50 ± 1.0) than with water (22 ± 1.0) but not significantly so ($df = 2$; $t = 6.314$; $P = 0.099$).

Tolerance to submergence

In the course of this study, a small number of beetles that fell or walked head first into shallow water were observed grasping the bottom of the water container and walking underwater (Fig. 3). Male and female beetles experimentally pushed to the bottom of an arena filled with water grasped the floor of the arena and voluntarily walked for 60 ± 12.8 and 56 ± 8.8 seconds ($df = 16$; $t = 2.119$; $P = 0.805$), respectively, before letting go and floating back to the surface. Male and female beetles held underwater in a screen enclosure kept walking on the walls of the enclosure for 344 ± 36.8 and 540 ± 75.3 seconds ($df = 23$; $t = 1.714$; $P = 0.029$), respectively, before slowing down, becoming uncoordinated and stopping all movement. All beetles pulled out of the water and placed in a petri dish looked dead, resting on their side with extended legs. However, after 10 minutes or less, beetles righted themselves, walked away slowly at first but before long behaved normally. All beetles kept submerged for increasing periods of time after they had become inactive survived submersion for up to 16.5 hours

after cessation of activity. Longer periods of submersion were not tested.

Ability to swim

Contrary to expectation, adult Colorado potato beetles demonstrated a great ability to swim (Fig. 2). They swam for considerable distances over the observation period of two hours (Table 1). There were, however, no significant differences in the total distance travelled ($F(3,24) = 0.45$; $P = 0.722$) in arenas with and without islands. The mean swimming distance was similar between arenas with and without island/driftwood ($F(1,24) = 0.08$; $P = 0.786$), between males and females ($F(1,24) = 0.12$; $P = 0.727$), and there were no interactions ($F(1,24) = 1.14$; $P = 0.297$). There were no significant differences in swimming velocity either ($F(3,24) = 1.17$; $P = 0.340$). The mean velocity was similar between arenas with and without driftwood ($F(1,24) = 2.15$; $P = 0.156$),

Fig. 3. Submerged adult Colorado potato beetle walking up a wooden rod underwater in a graduated cylinder.



males and females ($F(1,24) = 0.00$; $P = 0.963$), and there were no interactions ($F(1,24) = 1.37$; $P = 0.253$). There were no significant differences in the frequency of clockwise ($F(3,24) = 0.67$; $P = 0.579$) or counter-clockwise ($F(3,24) = 0.52$; $P = 0.674$) 360° rotations. The mean number of clockwise rotations was similar between arenas with and without driftwood ($F(1,24) = 0.06$; $P = 0.802$), males and females ($F(1,24) = 0.03$; $P = 0.846$), and there were no interactions ($F(1,24) = 1.91$; $P = 0.179$). The mean number of counter-clockwise rotations was similar between arenas with and without driftwood ($F(1,24) = 0.13$; $P = 0.718$), males and females ($F(1,24) = 1.05$; $P = 0.315$), and there were no interactions ($F(1,24) = 0.37$; $P = 0.551$).

The distribution of turning angles in arenas without a central platform (Rayleigh's $Z_{13} = 12.8$; $P < 0.001$) and with a central platform (Rayleigh's $Z_{15} = 14.6$; $P < 0.001$) were clearly non-uniform and concentrated around 18.0 ± 2.1 and $18.7 \pm 2.8^\circ$ in arenas without and with platforms, respectively.

Visual analysis of the tracks of individual beetles from release point to first encounter with the refuge indicated that a similar three out of 12 Colorado potato beetles and four out of 14 Colorado potato beetles possibly swam in the direction of the refuge zone or the refuge itself, respectively, whereas 3/12 and 6/14 did not reach or did not orient towards the respective areas. The remaining 6/12 and 4/14 did not swim in the direction of the refuge zone or the refuge itself, respectively, until towards the end of the track when it turned as if in response to the centre of the arena. Therefore, any orientation of the swimming beetles towards the centre of the arena did not appear to be in response to the presence of the refuge.

Table 1. Mean (\pm SE) locomotion parameters for male and female adult Colorado potato beetles swimming at the water surface in arenas with and without island/driftwood over a period of two hours.

| | | Mean total distance (cm) | Mean velocity (cm/s) | Mean clockwise rotations (#) | Mean counter-clockwise rotations (#) |
|-------|-----------|--------------------------|----------------------|------------------------------|--------------------------------------|
| Arena | Island | 742 \pm 177 | 0.14 \pm 0.02 | 3.9 \pm 1.36 | 3.7 \pm 1.20 |
| | No island | 815 \pm 191 | 0.19 \pm 0.02 | 4.4 \pm 1.09 | 4.3 \pm 1.25 |
| Sex | Female | 826 \pm 213 | 0.17 \pm 0.03 | 4.3 \pm 1.47 | 3.0 \pm 0.84 |
| | Male | 733 \pm 157 | 0.16 \pm 0.02 | 4.0 \pm 1.06 | 4.8 \pm 1.40 |
| | | NS | NS | NS | NS |

Note: NS indicates no significant differences between mean values for arena type or sex of beetles.

Discussion

Results confirm that the water landscape represents a threat for adult Colorado potato beetles but also provides new information on its survival strategy. Confronted by a threat, drowning in this case, the strategy of the Colorado potato beetle seems to give precedence to safety that dominates the behavioural response. Avoiding crossings of water features could be considered the Colorado potato beetle's adaptive behaviour to the presence of the possible threat in the agro-ecosystem. Observations suggest that the threat of drowning, as with the threat of predation, triggers a fight-or-flight response in the Colorado potato beetle. Essentially, by acting as a partial barrier to the dispersal of the walking Colorado potato beetles, bodies of water could be an important cause of local re-orientation and redistribution of dispersing populations and occasionally mid-distance to long-distance dispersal. However, in situations where water cannot be avoided, the unexpected ability of the Colorado potato beetle to swim over quantifiable distances and walk underwater provides it with the ability to fight off accidental falls or landings on the water. Its active response behaviours make it possible to reach floating refuges that can themselves be dispersed regionally or over large distances. The confirmed ability of the Colorado potato beetle to float for relatively long periods of time assisted by drifting would provide for passive escape. Its passive response behaviours make it possible for currents and wind to spread the beetle regionally. Although the passive long distance dispersal may occasionally be positive by expanding the range of the species, it could frequently be negative because it is likely to bring the Colorado potato beetle in inhospitable habitats (Boiteau and Heikkilä 2013).

Avoidance of water crossings

The adaptive avoidance of water crossings may be an extension of the navigation mechanism of the walking Colorado potato beetle among the physical elements found at the soil surface. In the terrestrial landscape, the walking Colorado potato beetle uses its antennae, legs, and eyes to examine the immediate landscape and maintain or alter its orientation (Pelletier and McLeod 1994; Pelletier and Caissie 2001; Boiteau *et al.* 2003). In the dry

trench treatment of our test arenas, Colorado potato beetles followed edges in search of a break where to resume their path failing which they walked down the vertical drop to cross the trench and continue their path in a manner relatively similar to that of beetles encountering a vertical wall and climbing it (Pelletier and Caissie 2001). In the water filled trench treatment, Colorado potato beetle behaviour along the water edge was initially the same as in the dry trench treatment but in the absence of a break in the obstacle, 80% more beetles turned away from their initial orientation in this treatment than in the dry trench treatment. This strong avoidance of water crossings supports earlier reports that bodies of water act as barriers to Colorado potato beetle dispersal. There was a trend for more male than female Colorado potato beetles reaching across the water filled trench but this is likely more a reflection of their higher level of walking activity (Boiteau *et al.* 2003) than of a greater ability to cross water. Most beetles crossing the water "barrier" did so after falling in the water as they inspected the interface trench/water, approached the water to drink or were accidentally pushed in by other beetles walking about. In spite of the efficacy of the avoidance behaviour, the frequency of involuntary falls in water followed by dispersal by flotation was measurable and therefore not negligible (Fig. 1). The relative importance of water avoidance as a barrier to Colorado potato beetle dispersal and therefore to the management of the pest in the agro-ecosystem remains unclear but it could be significant. For example, Niehues *et al.* (1996) found that a small stream was a barrier to genetic exchange between populations of *Carabus auronitens* Fabricius (Coleoptera: Carabidae), a woodland carabid tolerant to submergence.

Fight or flee response

For the beetles unable to avoid full contact with the water, their ability to fight or flee would determine their capacity to survive.

Survival at the surface of fresh and salt water

Our results confirmed that Colorado potato beetles can survive at the surface of fresh and salt water by floating passively and saving their energy for potential encounters with shorelines or

floating debris. Our results also demonstrated that Colorado potato beetles can survive by swimming their way from the water back to the shore or towards debris or rafts. This ability of the beetle to survive at the surface of water results in part, as with many insect species, from an average material density (the ratio of the insect mass over the volume of water displaced when submerged (Mlot *et al.* 2011)) similar to that of water. In the case of the Colorado potato beetle we calculated it at 1.05 ± 0.03 g/mL ($n = 9$), only slightly greater than the density of water. This near water density combined to the hydrophobic cuticle makes the beetle very buoyant. Supported by the water surface tension, these floating beetles can easily be dispersed by wind as observed by Boiteau and MacKinley (2015) in water-filled outdoor box plots. Adding soap to the water of some of our test bottles removed the water surface tension and beetles immediately sank just below the water surface.

Feytaud (1930), Dunn (1949), and Hurst (1975) suggested that the Colorado potato beetle could survive for 6–10 days at the water surface. Our tests, in the absence of debris on which to rest, produced similar periods of survival but demonstrated a shorter survival period on salt than fresh water. The probability of survival by swimming or passively fleeing using wave/wind driven dispersal is therefore slightly higher on fresh than salt water.

Adams *et al.* (2011) and Mlot *et al.* (2011) have shown that some insect species assemble into rafts to improve floatability and extend survival time on the water. Similar accumulations of Colorado potato beetle that could increase the probability of dispersal by waves and wind, perhaps quicker and over longer distances have been observed by fishermen at sea (Dunn 1949; Hurst 1975). These Colorado potato beetle rafts can be considerable, measuring as much as 245 cm in length and 180 cm in width consisting of large masses of adults piled on top of one another to a depth of 15–23 cm (Dunn 1949). The formation of rafts and the successful dispersal of the insects making up the rafts is likely much less probable for the Colorado potato beetle where rafting seems fortuitous than for species like *Solenopsis invicta* Buren (Hymenoptera: Formicidae) (Adams *et al.* 2011; Mlot *et al.* 2011) where rafting is adaptive (in response to the frequent flooding of the habitat of the ant). Those adaptive rafts can reach a stable

equilibrium within several minutes (Mlot *et al.* 2011) and can remain together for 12 hours to 12 days (Adams *et al.* 2011) whereas other rafts are ad hoc constructions where members are used interchangeably and randomly as flotation devices. The fire ant raft tends to self-heal with ants on the bottom moving to the top to preserve the average raft thickness when one ant changes position. Our random observations on the movement of potato beetles within a raft revealed no cooperative cycling of positions and frequent disintegration of the raft. These observations were made by dropping groups of 20 Colorado potato beetles on the water in plastic containers ($34.3 \times 21 \times 12.1$ cm) where they haphazardly formed rafts and separated as with many other invertebrates (*e.g.*, Hawes *et al.* 2008). Three days after formation, these Colorado potato beetle rafts remained loosely structured with a central patch with one or two out-layers. Contrary to the structured ant rafts (Mlot *et al.* 2011) only one or two beetles were attached to the rest of the group by a tarsal claw or otherwise with most of the beetles in the patch barely touching. The accumulation of frass underneath the beetle rafts at the bottom of the water containers suggested that the rafts remained in the same location over much of the period of observation. It is not clear if the stationary positioning resulted from beetle interaction as they tried to climb on each other to try and escape the water. Ants survive longer by cooperating and forming a raft (Mlot *et al.* 2011) whereas the Colorado potato beetle's loose assemblies seem the result of drifting and an adaptive behaviour to grasp and climb on encountered structures whether conspecifics or driftwood. It is not clear how these loose rafts could add substantially to the probability of Colorado potato beetle survival at the water surface.

Survival and dispersal at the surface of fresh water in the presence of island/driftwood

Our tests did demonstrate that solid debris such as driftwood could serve as refuges that would substantially extend Colorado potato beetle survival time on water from approximately four days to more than 22 days on average. The longer periods of survival in dry control containers than in those with water showed that the absence of food was not the key limiting factor to survival in

the water environment. Colorado potato beetles spent almost four times more time on the refuge before voluntarily or accidentally entering on the water surface confirming their reluctance to enter into water as shown in the trench test. This was also partially reflected in the almost two fold greater frequency of sightings off the refuge in the without water treatment than in the water treatment. It is not clear why this difference was not statistically significant except perhaps for the strong tendency of beetles in the control treatment to aggregate and remain around the cotton wicks, their source of drinking water.

The observed tendency for Colorado potato beetles that encounter floating refuges such as driftwood to avoid abandoning them combined to their capacity to survive without food is likely to play a substantial role in extending their stay on the water long enough for them to occasionally reach new shores (*e.g.*, Dunn 1949; Bartlett 1981). The proportion of beetles and the length of the survival periods in the laboratory trials are likely greater and longer than those outdoors. Many beetles have been observed throughout our laboratory trials falling into the water as they approached it to drink, sensed the boundaries of the driftwood, or pushed each other accidentally into the water. Outdoors, near real watercourses, falling off or abandoning driftwood could result in a shortened survival time as floating beetles are separated from their island by currents compared with the static environment of the laboratory tests. The temperature and light conditions during the laboratory trials were not conducive to beetle flight. Outdoors, Boiteau and MacKinley (2015) found that of more than 80% of Colorado potato beetles released on a platform in the middle of a water surface had abandoned it after 96 hours, with 64% flying off (rather than swimming off), demonstrating that flight can play an important role in improving their survival once drifting beetles have encountered driftwood or other floating debris.

Tolerance of submergence

The probability of Colorado potato beetle survival is further extended by their previously unreported ability to tolerate submergence and walk underwater. Because Colorado potato beetles tend to grasp objects and rough surfaces, they were able to resist the buoyancy force and remain underwater

at the bottom of rough-surface containers for one minute on average in our tests. Like with other terrestrial insects, this would correspond to situations where Colorado potato beetles may accidentally find themselves underwater in temporary ponds of flooded fields or after landing in a body of water and risk drowning (Pederson and Colmer 2012). This has been reported for a few other terrestrial insects. Heitler *et al.* (2005), for example, showed that desert locusts will actually stay submerged and walking for up to nine minutes.

In our tests, the submerged Colorado potato beetle had the same natural impulse that most insects have, above ground, to hold onto the surface of the structure on which they rest or to reach for the nearest object whether it is the bottom of a pond, a rock or a plant. In the case of the Colorado potato beetle above ground, adults hold onto plants with forces of up to 40 mN (Misener and Boiteau 1993). This impulse is not easily overridden. For example, a complex sequence of events that overrides the hold of the legs of the Colorado potato beetle on the ground surface is a prerequisite without which flight takeoff will not proceed (Boiteau 2002; Griff and Kane 2010). It may also be interrupted (broken) relatively quickly to escape predators by falling off plants to the ground. The same impulse to hold on the substrate was observed when one or more legs of a Colorado potato beetle falling head first into the water were observed to try and grasp the bottom of the arena so the insect could interrupt its fall through water, establish a hold on the substrate and resume walking. Even with aquatic insects, buoyancy is high and insects float on the surface unless they cling to objects underwater (Ditschek-Kuru *et al.* 2012; Seymour and Matthews 2013). The loss of traction rather than an active release of the grasping behaviour could have explained some of the observations. This would be particularly frequent on plastic or glass surfaces that provided little or no hold in comparison to the screening material. The grasping reflex was not overridden by a depletion of the store of oxygen in the tracheal system of the beetles as males and females continued to walk underwater for additional 3.8 and 7.6 minutes, respectively, on average, when forced to remain underwater. It is possible that the energetic cost of holding onto the floor of the arena against the buoyancy forces had become physiologically unacceptable.

The eventual development of uncoordinated movements followed by immobilisation may have been more indicative of some level of oxygen depletion. It becomes difficult and perhaps impossible for insects with closed spiracles to keep oxygen and carbon dioxide levels in balance unless they are in a resting state (Kovac *et al.* 2007). Although the immobile submerged potato beetles displayed many of the apparent characteristics of dead beetles (extended legs, lowered pronotum and head, abdomen depressed in the elytral cavity, *etc.*), the full recovery of all individuals maintained underwater over 16 hours does suggest that they were at rest. It is likely that the spiracles remained closed preventing the beetles from drowning. It is interesting that the retraction of the abdomen into the elytral cavity occurred at the same time as the beetles were suddenly becoming immobile and remained so until the beetles were taken out of the water. A few minutes after the beetles were out of the water, the abdomen could be clearly seen to slowly come back to its original size with the edges of the elytra not quite covering the abdomen. The behaviour of potato beetles maintained underwater for extended periods of time was in fact remarkably similar to that of fruit flies exposed by Krishnan *et al.* (1997) to anoxia for periods of 5–240 minutes, which lost coordination and became motionless after 1–2 minutes in anoxia but recovered fully even after up to four hours in a complete nitrogen atmosphere. The Colorado potato beetle is probably a good example of the capacity that some insects have to live for hours or days without breathing by closing their spiracles and curbing their activity (Krishnan *et al.* 1997). The ability of terrestrial insects to survive submergence varies considerably among insects. If some ants can survive up to eight days (Fielde 1904), rice thrips (Thysanoptera) rarely survived for more than three days (Thankachan and Nadarajan 2005). Obviously the capacity to recover from prolonged submersion is limited, probably by the eventual need to re-open the spiracles in response to unacceptably high levels of carbon dioxide. This capacity, tested here for up to 16 hours, is limited and likely less than the four days Colorado potato beetles survived at the surface of fresh water in another test. Other strategies used by insects to survive underwater are the use of oxygen from trapped air bubbles (Pederson and Colmer 2012)

and plastron respiration (Hebets and Chapman 2000). These were not investigated with the Colorado potato beetle but the period of time spent walking underwater voluntarily by a Colorado potato beetle was so short that its time underwater is more likely to be limited by the volume of oxygen already in its system than by trapped air.

Other insect species, such as adult desert locusts (*Schistocerca gregaria* Forsskål; Orthoptera: Acrididae) will submerge themselves completely underwater, and walk along the bottom (Heitler *et al.* 2005). Some species of wetland carabids are submersion tolerant (Rothenbacher and Schaefer 2006), float and can survive for over a week trapped in water without air pockets (Martay *et al.* 2014). We can only speculate on the likelihood of Colorado potato beetle walking underwater in the agro-ecosystem. In our tests, beetles voluntarily engaged into an “underwater” walk only twice after falling into the water head first. However, beetles that were brought underwater while holding on the end of a pencil frequently grasped without hesitation the nearby floor of the arenas and walked across or to the edge of the water filled trench. These observations suggest that beetles that for some reason penetrate below the surface tension of a body of water or are swept at the edge of a body of water would in nature actually perform underwater short walks.

The ability of Colorado potato beetles to walk underwater and stop breathing for periods of at least up to 16 hours underwater is likely to play an important role in their survival when trapped and unable to reach the surface. This extra time would allow a beetle surfacing under driftwood, for example, to walk underneath the log underwater until it reaches the above water sections. In an insect raft it would provide the insects with an increased probability of fighting its way back to the surface of the raft when pushed underneath the assemblage of insects. The sudden arrest of this period of walking activity underwater would provide an extended period of passive survival during which they may be dislodged from underneath a structure trapping them or float to the surface where they can be blown by the wind to the shore. The significantly longer period of active dispersal underwater for females than males could provide them with a slight survival advantage over males. If this extended to a longer period of inactive survival underwater, this might provide

an even more significant advantage to females reaching shorelines. As an invasive species, the foci of colonisation depend more on the successful colonisation by females than by males because of the high proportion of females already fertilised which could lay eggs and initiate a foci.

Ability to swim

Early observations of Colorado potato beetles floating on water (*e.g.*, Feytaud 1930) indicated that beetles had their legs folded back against the ventral surface and were therefore not in a position to swim. In our observations beetles were generally floating with their legs extended. Beetles finding themselves floating on the water in our tests did seem ineffective in their effort to move across the water surface and escape but even in the absence of morphological structures adapted to motion in water, did move forward slightly between bouts of passive floating. Our test has shown that the terrestrial Colorado potato beetle does have the ability to swim at the surface of water but at low speed (0.14–0.19 cm/second) compared with the semi-aquatic curculionid beetles (*e.g.*, 1.38 cm/second for *Ludovix. fasciatus* (Gyllenhal) (Coleoptera: Curculionidae) and 1.53 cm/second for *Lissorhoptrus oryzophilus* Kuschel (Coleoptera: Curculionidae); de Sousa *et al.* 2007) or the whirligig beetles (Coleoptera: Gyrinidae) that reach 23.3 cm/second (Xu *et al.* 2012). With these observations, the Colorado potato beetle joins other terrestrial insects such as desert locusts and wood crickets, which have been recently shown to readily swim across small watercourses (Heitler *et al.* 2005; Brouwers and Newton 2010b). The looping behaviour of the swimming Colorado potato beetle is likely to serve, as has been suggested for insects on land, to increase the chances of finding hosts or refuges as they float or swim. The observed looping behaviour has been interpreted in the context of systematic search strategies (Turchin 1998; Bengtsson *et al.* 2004) for insects that find themselves without food or in an unsuitable environment. The similar proportion of left and right looping observed here is similar to that obtained by Bengtsson *et al.* (2004) (average total number of loops 15.2 ± 1.02 /hour) with *Protaphorura armata* (Tullberg) (Collembola: Onychiuridae) in dry petri dishes. Although the unfavourable environments studied have usually been terrestrial,

our results show that it might be part of a survival strategy on water. The frequency of loops was similar in tests with and without islands, as one would expect as long as beetles continue to search. Although wind and waves are likely to disperse floating Colorado potato beetles at speeds greater than their swimming speed on calm water, the ability to swim could be critical near shorelines or debris to increase the probability of climbing ashore or on refuges. The swimming tracks in tests with and without island displayed a level of directionality but there was no evidence that Colorado potato beetles used their ability to swim to orient towards refuges.

No attempt was made to analyse the swimming method of the Colorado potato beetle but the water surface tension limited their ability to flex and provide propulsion by trapping the extended legs of floating Colorado potato beetle. Occasionally, beetles were observed floating sideways on the water, with one or more legs deeper in the water, below the surface tension. These “unbalanced” beetles tended to disperse over relatively long distance propelled by one, two, or three legs. The side of the beetles on top of the water surface seemed to be sliding on the surface tension propelled by the one or more legs submerged. Because of the odd positioning of the body the path travelled was often nonlinear and of variable length.

In our tests in containers floating beetles swam as much towards the driftwood refuge as away from it. In an experimental container this means that the beetles floating away will inevitably have to turn around when they have reached the walls of the arena whereas in an open lake for example, they may continue to swim away for the original island until they find another island or the shore or die. This means that many of these beetles will die after four or five days of drifting as we observed in the shaker test. Therefore the survival in our island test is likely to overestimate the survival time and the survival frequency.

Role of the water landscape in Colorado potato beetle integrated pest management

A prerequisite to the use of the agro-ecosystem landscape in managing the dispersal of the Colorado potato beetles between fields or across farms as well as between countries is the identification of landscape barriers. Historically (Feytaud 1930; Dunn 1949; Kaczmarek 1955), ponds,

lakes, rivers, and oceans have been considered barriers to local spread of walking Colorado potato beetles with the recognition that occasional survival of floating beetles contributed to long distance expansion of the range of the beetle. Our results with the Colorado potato beetle extend the observations with other insect species that the dispersal limits imposed by waterways are less than once presumed (Niehues *et al.* 1996; Martay *et al.* 2014).

Hawes *et al.* (2008) reminded their readers that Charles Darwin had highlighted the importance of relatively rare events such as drifting and rafting in determining the dispersal of animals. The role of this marine pleuston (Peck 1994) drifting may have been underestimated because often presumed unpredictable (Gillespie *et al.* 2012). However, these infrequent events with low probability of success do occur and must be taken onto consideration in developing plans for the management of invasive species (Trewick 2001) such as the Colorado potato beetle. Given the expected increase in the frequency of flooding brought about by global climate change (Pullin 1999), a better understanding of the impact of water on the movement of the adult Colorado potato beetle can only lead to better management practices against this endemic and invasive insect pest (Boiteau and Heikkilä 2013). The ability of the Colorado potato beetle to swim, tolerate submergence and float on water all help increase the probability of surviving the threat of drowning and transform a water barrier into a non-barrier. Changing temperature above water that may at times force beetles to land on their surface (Hurst 1975) may also make it possible for them to fly off floating debris (Boiteau and MacKinley 2015). It is not clear how far above water the Colorado potato beetle can fly but the absence of successful Colorado potato beetle mass flight over the English Channel provides us with a maximum upper limit of 30 km (Hurst 1975). Given the ability of the Colorado potato beetle to survive, like many other land arthropods (Coulson *et al.* 2002), at the water surface for periods of three weeks or more, they could cross short distances such as the English Channel but might not cross longer distances without intermediate stopovers on islands or driftwood (Gillespie *et al.* 2012). Given that Colorado potato beetles control their dispersal over water only when flying or swimming, wind

and currents are likely to be responsible for much of its orientation. With sufficient information on local dominant wind current direction, it should soon be possible to predict the most likely direction of Colorado potato beetle drifters (see Gillespie *et al.* 2012).

The relatively high temperature at which this study was carried out could be considered representative of summer conditions in ponds and lakes but we could only speculate as to whether the cooler ocean water temperature would increase or decrease the ability of the Colorado potato beetle to survive on the water. With an activity threshold estimated at 15 °C (Boiteau *et al.* 2003) the Colorado potato beetle is likely to retain its ability to swim or survive submerged in most oceans in summer except in more northern oceans where water temperature is frequently at or below 8 °C.

Practically, potato field submergence has been proposed by Niem *et al.* (2013) for the control of some soil borne diseases (Niem *et al.* 2013) and could possibly be integrated to the management of the Colorado potato beetle. Thankachan and Nadarajan (2005) proposed sustained submergence as a non-insecticidal approach to manage rice thrips. The ability of the Colorado potato beetle to tolerate submergence, walk underwater and readily float to the edges of ponds and lakes by surface wind suggests that the method is unlikely to bring significant changes in the abundance of Colorado potato beetle populations. However, the control method should not be rejected until the tolerance of diapausing (overwintering) Colorado potato beetles to submergence has been determined as submergence of potato fields would be most likely to occur naturally or artificially in early spring or late fall.

Even with relatively short survival rates or survival time periods, floating, rafting, and especially phoretic transport can be significant modes of dispersal. The probability of survival will increase dramatically within short distances or with the presence of driftwood or other structures.

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