Mites affect plum curculio (Coleoptera: Curculionidae) behavioural responses to attractive volatiles

Virginia Hock,[1] Gérald Chouinard, Éric Lucas, Daniel Cormier

Abstract—An infestation of *Histiostoma* Kramer sp. mites (Acari: Histiostomatidae) occurred in rearing colonies of the univoltine strain of plum curculio (PC), *Conotrachelus nenuphar* (Herbst) (Coleoptera: Curculionidae), established in 2010 in southern Québec, Canada. Tests conducted in a two-choice still-air vertical olfactometer with mite-infested and noninfested PC revealed that the number of beetles responding by walking towards normally attractive synthetic and natural odours was significantly lower for those infested with mites. Those mite-infested curculios that did discriminate between test odours and odour-free air all responded positively to the test volatiles, similar to the behaviour of noninfested insects. This indicates that mites affect PC ability to physically move towards attractive volatiles but not odour preference.

Résumé—Une infestation par les acariens phorétiques du genre *Histiostoma* Kramer (Acari: Histiostomatidae) s'est produite dans l'élevage de la souche univoltine du nord du charançon de la prune *Conotrachelus nenuphar* (Herbst) (Coleoptera: Curculionidae) établie en 2010 dans le sud du Québec, Canada. Des tests ont été effectués à l'aide d'un olfactomètre vertical à deux-voies, sans pression d'air. Les charançons infestés et non-infestés ont été testés et ont révélé un effet des acariens sur la réponse olfactométrique de cet insecte. Pour les odeurs synthétiques et naturelles attractives aux charançons, significativement moins de charançons infestés par des acariens ont répondu aux odeurs que de charançons non-infestés. Pour cette raison, l'attraction des charançons infestés par des acariens vers les sources des odeurs était difficile à établir. Cependant, tous les charançons infestés ayant répondu ont réagit positivement aux volatiles testés, d'une façon similaire aux insectes non-infestés. Ceci indique que les acariens affectent la capacité des charançons à répondre aux volatiles attractifs mais n'affectent pas leurs préférences.

Mites have been known to infest plum curculio (PC) *Conotrachelus nenuphar* (Herbst) (Coleoptera: Curculionidae) larvae and adults (Smith 1957; Broersma and Hays 1966; Amis and Snow 1985), and impair movement of this and other species of insects (Amis and Snow 1985; Smith 1988). We conducted olfactometric bioassays to determine if mite-infested PC adults behave differently than noninfested PC regarding response to volatiles.

Apples containing PC larvae were collected from the field in 2009 in Saint-Bruno-de-Montarville, Québec, Canada (45.55028°N, 73.31917°W) during June drop and placed in emergence cages. Emerging nonsexually mature adults were collected daily and immediately separated by sex (Thompson 1932) and transferred to overwintering cages (Le Blanc 1992) and placed outdoors under natural conditions throughout the winter. Overwintered adults were removed from their cages the following

Received 11 April 2012. Accepted 18 June 2012.

V. Hock,[1] Institut de recherche et de développement en agroenvironnement (IRDA), Laboratoire de Production Fruitière Intégrée 335, chemin des Vingt-Cinq Est Saint-Bruno-de-Montarville, Québec, J3V 0G7 Canada; and Université du Québec à Montréal (UQAM), Département des sciences biologiques, Laboratoire de lutte biologique, Case postale 8888, succursale Centre-ville, Montréal, Québec, H3C 3P8 Canada
G. Chouinard, D. Cormier, Institut de recherche et de développement en agroenvironnement (IRDA), Laboratoire de Production Fruitière Intégrée 335, chemin des Vingt-Cinq Est Saint-Bruno-de-Montarville, Québec, J3V 0G7 Canada
É. Lucas, Université du Québec à Montréal (UQAM), Département des sciences biologiques, Laboratoire de lutte biologique, Case postale 8888, succursale Centre-ville, Montréal, Québec, H3C 3P8 Canada

[1]Corresponding author (e-mail: virginia.hock@irda.qc.ca).
doi:10.4039/tce.2012.94

spring as soon as the soil thawed but while temperatures were still cool. This ensured that all emerging adults were of a similar age (since they emerged from pupation and adults were placed in overwintering cages at similar times). Several adults from each container were sampled to verify sex and sexual immaturity. Adults were then placed in 2 L containers with small apples (McIntosh cultivar, 2–3 cm in diameter) and water and kept at 27 °C, 70% relative humidity, 16L:8D (light:dark hours). A laboratory colony was established using some of these wild individuals as per Hoffmann *et al.* (2007). Both wild and laboratory-reared insects were used in experiments. In order to bring laboratory-reared PC to maturity we treated them with pyriproxyfen as described in Hoffmann *et al.* (2007). Previous observations over several generations (Hoffmann *et al.* 2007) reported no issues regarding PC treated with pyriproxyfen, nor were any observed differences between wild and laboratory-reared strains noted in our laboratory. In addition, an experiment conducted in our laboratory with pyriproxyfen-treated and untreated (wild) females regarding response to the odour of two mature males showed no difference in response to test versus control odours.

All PC used in experiments were sexually mature and all PC tested were female. Although the number of days elapsed since insect hatching may have differed between field-collected and laboratory-reared insects, insect age relative to sexual maturity was essentially the same. This is because wild individuals are still immature upon emergence from diapause requiring an average 13 days (Smith 1957; Smith and Salkeld 1964) to mature and lay eggs at 27 °C. Laboratory univoltine PCs emerging from diapause require treatment with pyriproxyfen to mature, and are able to lay eggs ~10 days after treatment (Hoffmann *et al.* 2007).

In our environmental chambers, wild and laboratory-reared individuals were found with mites and were quickly segregated to avoid infection of other wild and laboratory-reared PC colonies (uninfested PC). The source of infestation was most likely the apples used for maintaining wild and laboratory groups, as fruit flies were seen frequenting the apples and have previously been shown to carry phoretic mites with them (Amis and Snow 1985). In containers with infestations, all PC in the container were examined and found to have at least one or more mites present. Random samples of PC revealed an average mite infestation of ≈10–20 mites per PC. In laboratory-reared PC allowed to mate, the resulting offspring kept in the same environmental chambers and reared to adulthood were also found to be infested with mites. Infested PC used in our experiments carried about 10–20 mites each.

Plum essence (PE; Milne Fruit Products Inc., Prosser, Washington, United States of America) and the odour of two live males were previously shown to attract female PC in our laboratory and were used as test odours. PE is a synthetic mixture of plant essence, it is essentially a blend of concentrated plum juice that attracts PC (Coombs 2001, Whalon *et al.* 2006). Males have been previously shown to produce an aggregation pheromone termed grandisoic acid (Eller and Bartelt 1996), and two males have been shown to attract the greatest number of females in our laboratory. Either a 1.5 mL microcentrifuge tube (Sigma-Aldrich Canada Ltd., Oakville, Ontario, Canada) of PE containing a cotton wick for odour dispersion (Experiments 1 and 3), or two virgin males (Experiments 2 and 4), were put inside a 500 mL Mason jar (Bernardin Ltd., Richmond Hill, Ontario, Canada) and secured to the olfactometer with Parafilm®M (Sigma-Aldrich Canada Ltd.).

The olfactometer (Fig. 1) consisted of a large (105 mm inner diameter, 50 mm in height)

Fig. 1. Diagram of the olfactometer used in experiments consisting of a Pyrex® glass container with a Petri dish bottom and three main apical openings (left, right, centre). Lateral openings served as connectors to Mason jars containing either test or control odours (triangle in Mason jar represents PE odour); central opening served as the point of introduction for insect.

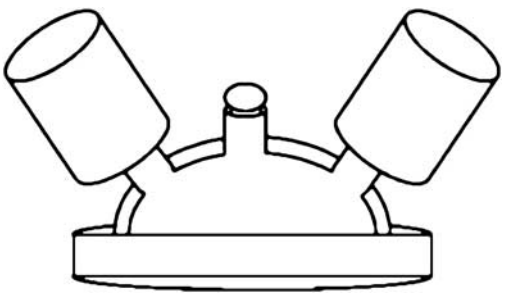


Table 1. Attraction of female PC to odour sources. (A) Proportion of responders (versus nonresponders) for noninfested and infested PC to PE and control odours. (B) PC responding to test odours (responders only).

Experiment	Mites treatment	Test odour	*n*	Proportion of females responding	±SE
(A) Responders versus nonresponders					
1A	No mites	PE	40	0.50	0.08
1B	Mites	PE	25	0.20	0.08
2A	No mites	2 males	18	0.78	0.10
2B	Mites	2 males	24	0.13	0.067
(B) Response to test versus control odours (responders only)					
3A	No mites	PE	20	0.75	0.09
3B	Mites	PE	5	1.00	0.00
4A	No mites	2 males	14	0.93	0.07
4B	Mites	2 males	3	1.00	0.00

SE = standard error.
n = number of single female choice tests run for 30 min during scotophase at 25 °C, 70% relative humidity.

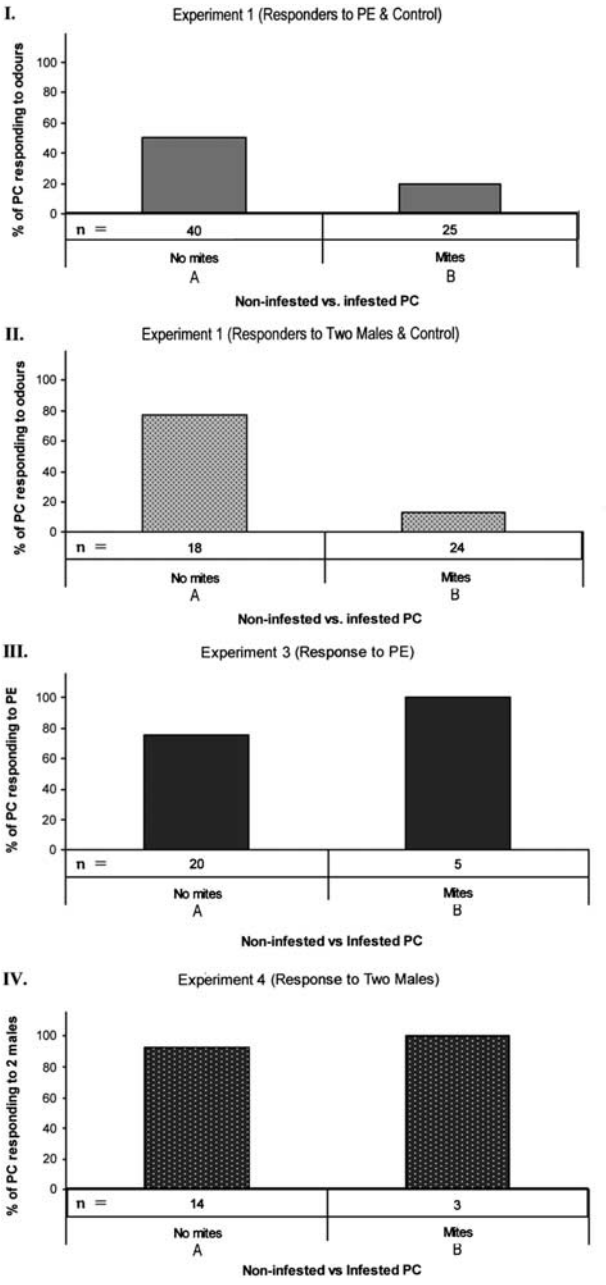
Pyrex® glass container (Corning Inc., Corning, New York, United States of America). Three main openings (24 mm inner diameter, 60 mm height) on top of the olfactometer served as a point of introduction for insects (central opening) into the arena and as connectors (left and right) to the odour jars, which were placed on top and secured to the olfactometer with Parafilm®. Each jar contained either the test odour or the control "odour" (air only). Standard mosquito mesh covered the openings to prevent females from escaping or entering the jars. A Pyrex® glass Petri dish (150 mm diameter, 20 mm height) served as the base.

Tests were conducted at 25 °C, 70% relative humidity during scotophase (Racette *et al.* 1991; Chouinard *et al.* 1993). A filter (LEE Filter, red primary no. 106®, Son-Art Production, Saint-Hyacinthe, Québec, Canada) covered the lights during experiments because PCs are not perturbed by red light (Prokopy *et al.* 1995). A single female PC was placed in the olfactometer for 30 minutes, after which its position was noted and the PC removed. PC inside or within 1 cm of tubes leading to test or control jars were considered "responders" (*i.e.*, PC having made an odour choice, either test or control); females in all other locations were considered "nonresponders" (no choice having been made). In addition to comparing PC odour choice (test versus control) among responders, the proportion of nonresponders was also compared with responders to gauge the effects of mites on PC ability to respond to odours. Odour zones were randomised after each replicate and the olfactometer was dismantled daily prior to each new experimental treatment, washed with Sparkleen™ (Fisherbrand, Pittsburgh, Pennsylvania, United States of America), and rinsed with acetone and hexane (Sigma-Aldrich Canada Ltd.).

SPSS statistical software (SPSS Inc. 2006, Chicago, Illinois, United States of America) was used for analysis. Comparisons between the number of "responder" PC choosing test versus control as well as the number of responding and nonresponding PC were done using a two-tailed χ^2-test. χ^2 statistics were used to test responders versus nonresponders and test versus control individually for noninfested and infested PC. This was to demonstrate the characteristics of a normal PC response to each odour (noninfested) in order to allow comparison against the response of infested PC (*i.e.*, if mites have no effect on PC, then the behaviour of infested PC should mimic that of noninfested PC regarding their response to each individual odour). Responses between experiments (PC with mites versus PC without mites for each of the two test odours) were compared using a Mann–Whitney *U*-test.

Infested PCs were sent to the Diagnostic Laboratory of the Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec (MAPAQ: Direction de la Phytoprotection, Québec, Québec, Canada) and identified as *Histiostoma* sp. (Acari: Histiostomatidae). The mites identified were phoretic deutonymphs that have an attachment organ with 10 sucker-like structures on the posterior venter of the body,

Fig. 2. (I and II) Total percentage of responders – females having made an odour choice (test and control), versus nonresponders – PC that did not make an odour choice (neither test nor control odours). (I) Using PE for testing noninfested and infested PC (Experiment 1A/B); (II) Using two males for testing noninfested and infested PC (Experiment 2A/B). (III and IV) Percent of PC choosing the test odour (based only on total number of PC having made an odour choice, excludes unresponsive PC – those that did not make a choice). (III) Using PE odour for noninfested and infested PC (Experiment 3); (IV) Using odour of two males for noninfested and infested PC (Experiment 4). Bars with an * denote significant differences between infested and noninfested PC. PC = plum curculio, test odour = odour tested other than control (*i.e.*, PE, two males), PE = plum essence, control odour = air, n = number of single female choice tests run for 30 min during scotophase at 25 °C, 70% relative humidity.



and were 0.15 mm in size with four pairs of frontwardly projecting legs ending in a single claw. The front-most pair of legs was longer than the other three pairs.

Response to odours (Table 1): no mites. There was no significant difference between proportion of responders versus nonresponders to odours in Experiment 1A (PE/control; $\chi^2 = 1.000$, df = 1, $P = 1.000$) but there was a significantly higher proportion of responders than nonresponders to odours in Experiment 2A (two males/control; $\chi^2 = 5.556$, df = 1, $P = 0.018$). Regarding odour preference, beetles responded significantly more to both PE ($\chi^2 = 5.000$, df = 1, $P = 0.025$) in Experiment 3A, and two males ($\chi^2 = 10.286$, df = 1, $P = 0.001$) versus the control in Experiment 4A. **Mites.** There were significantly more nonresponders than responders for both Experiment 1B (PE/control; $\chi^2 = 9.000$, df = 1, $P = 0.003$) and Experiment 2B (two males/control; $\chi^2 = 13.500$, df = 1, $P < 0.0001$) odours. All infested females chose PE (Experiment 3B) or two males versus the control (Experiment 4B). **No mites versus mites.** Noninfested females responded significantly more than infested females in both experiments (Experiment 1A/B [PE/control]: $Z = -2.400$, $P = 0.016$ [Fig. 2I]; Experiment 2A/B [two males/control]: $Z = -4.214$, $P < 0.0001$ [Fig. 2II]). There was no significant difference in odour choice between responding PC with or without mites regarding choice of test odours (Experiment 3A/B, PE: $Z = -1.225$, $P = 0.221$; Experiment 4A/B, two males: $Z = -0.463$, $P = 0.643$; Fig. 2III and IV, respectively).

The mites herein were phoretic deutonymphs of *Histiostoma* sp., and not the same mites previously identified as a phytoseiid species, *Garmania bulbicola* (Oudemans) (Acari: Phytoseiidae), that were reported to infest the PC laboratory colonies of Smith (1957). The species referred to by Smith (1957) as *G. bulbicola* is in fact *Proctolaelaps pygmaeus* (Müller) (Acari: Melicharidae) (Ehara 1964). To our knowledge, this is the first report of *Histiostoma* sp. present on PC. Histiostomatidae are associated with decomposition of organic material, with the deutonymph stage specialising in using arthropods for transport and dispersion (Scheucher 1957). The mites herein decreased PC ability to physically respond to odours, not their actual odour preference. Both PE and male PC odours were attractive to uninfested females, and all infested PC able to respond also chose the test odours (Fig. 2I and II). Thus, mites affect the ability of PC to actively respond and move towards odours but not PC odour preference itself. We noted that infested PC had problems climbing surfaces; the observed presence of mites on tarsi likely impaired PC movement as was noted by Amis and Snow (1985), where severe mite infestations hindered PC movement and metamorphosis. Mites can also inhibit insect flight and host-seeking behaviour (Elzinga and Broce 1988; Smith 1988). In our colonies heavy infestations (30+ mites) immobilised PC. Damage to host health is counter productive regarding mite dispersion but large quantities of mites may cause unavoidable damage. Generally, phoretic mites are thought to be commensals that disperse with no adverse effects to their hosts (Holte *et al.* 2001). Our study, like the observations made by Elzinga and Broce (1988), shows how the frontiers between phoretic (commensalistic) and parasitic (trophic negative) interactions is narrow and that the intensity of the phoretic load may determine the neutral or negative impact on host fitness.

Acknowledgements

The authors would like to thank Sylvie Bellerose, Franz Vanoosthyuse, Anne-Lyse Laroche, Marie-Pier Richard, Martin Duquette, and Robert Maheux for their assistance in the laboratory and field. They would also like to thank Tracey Leskey and Starker Wright of the United States Department of Agriculture for supplying the Plum Essence, and Mario Fréchette, Jean-Philippe Légaré, and Michèle Roy of the Laboratoire de Diagnostic en Phytoprotection for their help in identifying the mites. These studies were supported by a grant from the Programme de soutien à l'innovation en agroalimentaire de Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec (MAPAQ) (Québec, Canada).

References

Amis, A.A. and Snow, J.W. 1985. Conotrachelus nenuphar. *In*Handbook of insect rearing. *Edited by* P. Singh and R.F. Moore. Elsevier Science Publishers, Amsterdam, The Netherlands. pp. 227–235.

Broersma, D.B. and Hays, S.B. 1966. Improved methods for mass rearing plum curculio, *Conotrachelus nenuphar*. Journal of Economic Entomology, **59**: 235–236.

Chouinard, G., Hill, S.B., and Vincent, C. 1993. Spring behaviour of the plum curculio, (Coleoptera Curculionidae), within caged dwarf apple trees in spring. Journal of Insect Behavior, **5**: 385–394.

Coombs, A. 2001. Trap designs and attractants for monitoring plum curculio, *Conotrachelus nenuphar* (Herbst). M.Sc. thesis. Michigan State University, East Lansing, Michigan, United States of America.

Ehara, S. 1964. Some mites of the families Phytoseiidae and Blattisocidae from Japan (Acarina: Mesostigmata). Journal of the Faculty of Science, Hokkaido University, Series VI, Zoology, **15**: 378–394.

Eller, F.J. and Bartelt, R.J. 1996. Grandisoic acid a male produced aggregation pheromone from the plum curculio, *Conotrachelus nenuphar*. Journal of Natural Products, **59**: 451–453.

Elzinga, R.J. and Broce, A.B. 1988. Hypopi (Acari: Histiostomatidae) on house flies (Diptera: Muscidae): a case of detrimental phoresy. Journal of the Kansas Entomological Society, **61**: 208–213.

Hoffmann, E.J., Vanderjagt, J., and Whalon, M.E. 2007. Pyriproxyfen reproduction in prediapause northern strain plum curculio (*Conotrachelus nenuphar* Herbst). Pest Management Science, **63**: 835–840.

Holte, A.E., Houck, M.A., and Collie, N.L. 2001. Potential role of parasitism in the evolution of mutualism in astigmatid mites: *Hemisarcoptes cooremani* as a model. Experimental and Applied Acarology, **25**: 97–107.

Le Blanc, J.P.R. 1992. Trapping and monitoring techniques for plum curculio, *Conotrachelus nenuphar* (Herbst), (Coleoptera: Curculionidae) in a southwestern Quebec apple orchard. Ph.D. dissertation. McGill University, Québec, Canada.

Prokopy, R.J., Sylvia, J., Cooley, S., and Phelan, P.L. 1995. Bioassay approaches to assessing behavioral responses of plum curculio adults (Coleoptera: Curculionidae) to host fruit odor. Journal of Chemical Ecology, **21**: 1073–1084.

Racette, G., Chouinard, G., Hill, S.B., and Vincent, C. 1991. Activity of adult plum curculio (Coleoptera: Curculionidae) on apple trees in spring. Journal of Economic Entomology, **84**: 1827–1832.

Scheucher, R. 1957. Systematik und Ökologie der deutschen Anoetinen. Beiträge zur Systematik und Ökologie mitteleuropäischer Acarina, **1**: 233–384.

Smith, B.P. 1988. Host–parasite interaction and impact of larval water mites on insects. Annual Review of Entomology, **33**: 487–507.

Smith, E.H. 1957. A method for rearing plum curculio under laboratory conditions including some biological observations. Journal of Economic Entomology, **50**: 187–190.

Smith, E.H. and Salkeld, E.H. 1964. Ovary development and oviposition rates in the plum curculio, *Conotrachelus nenuphar* (Coleoptera: Curculionidae). Annals of the Entomological Society of America, **57**: 781–787.

Thompson, J.R. 1932. Sex differentiation of adults of *Conotrachelus nenuphar*. Journal of Economic Entomology, **25**: 807–810.

Whalon, M., Nortman, D., Wise, J., Gut, L., and Epstein, D. 2006. Plum curculio management and spray timing. Michigan State University Fruit Crop Advisory Team Newsletter, **21**: 1–3.