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COVER: *Aeshna interrupta* Walker (Odonata: Aeshnidae)

Aeshna interrupta (Variable Darner) is one of the most common large dragonflies in British Columbia. It is a boreal species, ranging across North America from Newfoundland to Alaska and south in the western mountains to California and New Mexico. It lives in marshes and peatlands and is the typical *Aeshna* of grassland ponds and lakes in the Interior. On the Coast it is one of the predominant dragonflies in peat bogs.

The scientific name "*interrupta*" refers to the shape of the stripes on the sides of the thorax. In eastern North America and on the Pacific coast, these are "interrupted", that is, each is broken into two spots. On the Great Plains and in the BC Interior, the stripes are unbroken but thinner than in any other species. The common name "variable" describes these stripes. The stripes and spots of the male are blue; those of the female are blue or, more commonly, yellow.

Most dragonflies spend the majority of their lives in the aquatic larval (nymphal) stage. After about 10 to 14 moults, depending on the species and environmental conditions, the fully grown larva metamorphoses into an adult inside its last larval skin, then crawls out of the water. Now exposed to air, the dragonfly begins its final moult – the top of the thorax splits open and the adult squeezes out. It pumps blood into its wings and abdomen, which expand slowly, and gradually the body hardens. After an hour or two the dragonfly can fly, but only weakly at first. It leaves the empty larval skin, the exuvia, clinging to the support.

Photograph details:

Male *Aeshna interrupta* photographed during emergence at a grassland pond near Riske Creek, Chilcotin region, BC, 15 June 1978. Pentax Spotmatic II with 50 mm/1.4 Macro Takumar lens, handheld and with available light. Kodachrome 64 film. Robert A. Cannings.

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Melacoryphus admirabilis (Uhler) (Hemiptera: Lygaeidae) new to Canada, with additional Canadian provincial records for other Heteroptera

G.G.E. SCUDDER¹

ABSTRACT

The lygaeid *Melacoryphus admirabilis* (Uhler) is recorded from Saskatchewan and new to Canada. New provincial records are given for 16 other species of Heteroptera, belonging to the families Alydidae, Artheneidae, Cymidae, Geocoridae, Lygaeidae, Miridae, Oxycarenidae, Rhyparochromidae and Tingidae.

INTRODUCTION

Further research on collections of Canadian Heteroptera has resulted in the discovery of another species new to Canada. In addition, new provincial records have been established for 16 other species. Some of these significantly change the known distribution of species in Canada.

Data cited are those on specimen labels. The order follows Maw et al. (2000). Collection and Museum abbreviations used in the text are as follows:

CNC: Canadian National Collection of Insects, Agriculture and Agri-Food Canada,

Ottawa, ON (R.G. Foottit).

DBUC: Department of Biological Sciences, University of Calgary, Calgary, AB (J.E. Swann).

LC: D.J. Larson Private collection, Maple Creek, SK.

LM: Lyman Entomological Museum, Macdonald College, McGill University, Ste. Anne-de-Bellevue, QC (T. Wheeler).

UCCB: Department of Biology, University College of Cape Breton, Sydney, NS (D. McCorquodale).

NEW RECORDS

Family MIRIDAE

Phytocoris eureka Bliven

In Canada, previously only reported from British Columbia (Stonedahl 1988; Maw et al. 2000), but widely distributed in the western United States (Stonedahl 1988).

New provincial record. AB: 1♀, Kananaskis, U. of C. Field Station, 51° 0'49"N 114°12'01"W, 11-19.viii.2004 (L. Wooldridge) [DBUC].

Family TINGIDAE

Corythucha distincta Osborn & Drake

In Canada, previously only reported from British Columbia (Parshley 1919; Downes 1925, 1927; MacNay 1952; Maw et al. 2000), but in the United States re-

corded south to California and in South Dakota (Froeschner 1988b).

New provincial record. AB: 1♂, Fish Creek Provincial Park, 50°54.406'N 114° 01.260'W, sweep of field near ranch house, 17.viii.2009 (J.E. Swann & G. Hull) [DBUC]; 1♂, Fish Creek Provincial Park, 50°54.594'N 114°01.698'W, sweep, 17.viii.2009 (J.E. Swann & G. Hull) [DBUC]; 1♂, Waterton Lakes National Pk., Cardston Entrance, Malaise, 13.viii.1989 (R. Longair) [DBUC].

Corythucha salicata Gibson

In Canada, reported from British Columbia, Manitoba, Northwest Territories, Ontario and Saskatchewan (Maw et al.

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2000). This species is confined to the Western Cordilleran region in the United States (Froeschner 1988b).

New provincial record. AB: 17♂ 6♀, Fish Creek Provincial Park, 50°54.594'N 114°01.698'W, sweep, 17.viii.2009 (J.E. Swann & G. Hull) [DBUC]; 2♂ Fish Creek Provincial Park, 50°55.717'N 114°07.307'W, sweep, goldenrod, 19.viii.2009 (G. Hull) [DBUC].

Family ALYDIDAE

Protenor belfragei Haglund

In Canada, previously reported from Saskatchewan east to Prince Edward Island (Maw et al. 2000), and widely distributed in the United States (Froeschner 1988a).

New provincial record. NS: 1♂, Cape Breton Co., Sydney, UCCB, 1.xi.1999 (M.I. Kerr) [UCCB]; 1♀, *id.*, 1.ix.1999 (B.H.W. MacIntosh) [UCCB].

Family ARTHENEIDAE

Chilacis typhae (Perris)

An alien species in Canada previously reported from British Columbia and Ontario (Scudder 2000; Maw et al. 2000; Scudder and Foottit 2006), as well as New Brunswick, Nova Scotia, Prince Edward Island (Wheeler 2002) and Quebec (Roch 2008). In the United States it is recorded from both the east (Wheeler and Fetter 1987) and the west (Wheeler and Stoops 1999). Wheeler (2002) also added 21 new U.S. state records for *C. typhae*.

New provincial record. AB: 1♀, Calgary, U. of Calgary, 11.vi.2009 (Tim Loh) [DBUC]. SK: 1♀, Battle Creek, near Merry Flats, 30.ix.2006 (D. Larson) [LC]; 2♂, Larson Ranch, Hwy. 21, 16 km S. Maple Creek, 24.ix.2008 (D. Larson) [CNC; LC]; 2♂ 1♀, *id.*, 2.viii.2009 (D. Larson) [CNC; LC].

Family CYMIDAE

Cymus coriacipennis (Stål)

In Canada, previously only reported from British Columbia (Scudder 1961; Maw et al. 2000). It is a Western Cordilleran species in the United States (Hamid 1975; Ashlock and Slater 1988).

New provincial record. SK: 1♂, Maple Ck., Hwy. 21, 16 km S, 8.vii.2003 (D. Larson) [LC]; 1♂, *id.*, 8.vi.2004 (D. Larson)

[CNC].

Family GEOCORIDAE

Geocoris atricolor Montandon

In Canada, previously only reported from British Columbia (Parshley 1919; Walley 1934; Maw et al. 2000) and Alberta (Walley 1934, Strickland 1953; Maw et al. 2000). A Western Cordilleran species in the United States (Ashlock and Slater 1988).

New provincial record. SK: 1♂, Cypress Hills Pk., Center Block, Highland Trail, 2.x.2008 (D. Larson) [LC]; 1♀, Larson Ranch, Hwy. 21, 16 km S. Maple Creek, 12.vii.2002 (D. Larson) [LC]; 1♂, *id.*, 17.ix.2008 (D. Larson) [LC]; 2♂, 1♀, *id.*, 24.ix.2008 (D. Larson) [CNC; LC]; 1♀, Maple Creek, 6 km N, 9 km E, sandy road allowance, 12.ix.2008 (D. Larson) [LC].

Geocoris howardi Montandon

In Canada, previously reported from Alberta, British Columbia, Northwest Territories and Yukon (Maw et al. 2000). Also known from Alaska, the species is distributed across boreal North America (Radio and Sweet 1982; Ashlock and Slater 1988).

New provincial records. MB: 1♀, Carberry, 29.vii.1953 (Brooks-Kelton) [CNC]; 1♀, Churchill, 31.vii.1937 (W.J. Brown) [CNC]; 1♂ 3♀, *id.*, 10.viii.1937 (W.J. Brown) [CNC]; 1♀, *id.*, 12.vii.1952 (J.G. Chillcott) [CNC]; 2♂ 1♀ 1 immature, Churchill, 4 km W, Akudik marsh, 58°44'47"N 94°06'47"W, gen'l. coll., 16.vii.2006 (Boreal & Arctic Entomol) [UM]; 1 immature, Churchill, 6 km E No. Stud. Ctr., 58°46'14"N 93°54'46"W, krumholz tundra, gen'l. coll. (39), 11.viii.2006 (Boreal & Arctic Entomol) [UM]; 1 immature, Churchill, 12 km W, Launch Rd., 58°45'18"N 93°59'04"W, bluffs + fen near A-frame, gen'l. coll. (84), 17.viii.2006 (Boreal & Arctic Entomol) [UM]; 1 immature, Churchill, 15 km S, Stud. Ctr., 58°37'00"N 93°49'15"W, gen'l. coll. near pond, Sample 16, 9.viii.2006 (Boreal & Arctic Entomol) [UM]; 1♂ 2♀ 1 immature, Churchill, 15 km S No. Stud. Ctr., 58°37'00"N 93°49'15"W, burned area-boreal for., gen'l. coll. (62), 14.viii.2006 (Bor. & Arc. Entomol.) [UM]; 1♀, Turtle Mt., 28.vii.1953 (Brooks-Kelton) [CNC]. NS: 1♀, S. Berwick,

22.viii.1963 (V.R. Vickery) [LM]. ON: 1♂ 3♀, Black Hawk, 3.viii.1960 (Kelton & Whitney) [CNC]; 1♀, Eagle River, 11.viii.1960 (Kelton & Whitney) [CNC]; 1♂, Hastings Co., 29.viii.1954 (J.F. Brimley) [CNC]; 3♀, Kapuskasing, 18.vii.1961 (G. Brumpton) [CNC]; 5♂, Little Current, 9.vii.1961 (G. Brumpton) [CNC]; 1♂, Nestorville, 24.vi.1965 (K.P. Butler) [LM]; 2♀, One Sided Lake, 1.viii.1960 (Kelton & Whitney) [CNC]; 3♀, *id.*, 2.viii.1960 (Kelton & Whitney) [CNC]; 1♀, Sioux Narrows, 6.viii.1960 (Kelton & Whitney) [CNC]. QC: 2♂ 1♀, Kazubazua, 18.viii.1931 (G.S. Walley) [CNC]; 1♀, *id.*, 25.vii.1933 (G.S. Walley) [CNC]; 1♂, Laniel, 21.viii.1932 (W.J. Brown) [CNC]. SK: 1♂ 1♀, Christopher Lake, 13.vii.1959 (A. & J. Brooks) [CNC]; 2♀, *id.*, 15.vii.1959 (A. & J. Brooks) [CNC]; 1♂, Cypress Hills, E. Block, Ambrose Place, 25 km SE Maple Creek, 10.vii.2006 (D. Larson) [LC]; 2♂ 1♀, Cypress Hills Pk., Center Block, Highland Trail, 2.x.2008 (D. Larson) [LC]; 1♀, Saskatoon, 22.vii.1949 (L. Konotopetz) [CNC]; 1♀, Torch River, 23.viii.1950 (L.A. Konotopetz) [CNC].

Geocoris pallens Stål

In Canada, previously only reported from Alberta, British Columbia (Forbes 1900; Torre-Bueno 1946; Slater 1964; Ashlock and Slater 1988; Maw et al. 2000). *G. pallens* has been collected from most of the western United States, and has a range extending eastward to Indiana, Illinois, Missouri and Arkansas (Readio and Sweet 1982), but also occurs from Mexico to Central America and in Hawaii (Ashlock and Slater 1988).

New provincial record. SK: 1♂, Larson Rch., 16 km S Maple Creek, Hwy. 21, 5.viii.2002 (D. Larson) [CNC].

Geocoris uliginosus (Say)

In Canada, reported from Newfoundland (Lindberg 1958), Ontario (Walley 1934) and Quebec (Walley 1934; Moore 1944, 1950; Béique and Robert 1963; Larochelle 1984; Roch 2008). *G. uliginosus* var. *speculator* Montandon was synonymized with *G. uliginosus* by Readio and Sweet (1982).

Early records for British Columbia

(Downes 1927), repeated by Walley (1934) and noted by Lindberg (1958), are incorrect. Downes (1927) reported the species from Merritt on August 11, 1923 by R. Hopping, and also at Victoria. I have been unable to locate the Merritt material taken by Hopping on that date, but other specimens now in the CNC collected in 1923 by R. Hopping have been found to be *G. bullatus* (Say). Also, specimens in the CNC from Victoria, 5.ix.1923 (K.F. Auden) also prove to be *G. bullatus*.

In North America, in general *G. uliginosus* has a range that extends from the Gulf Coast north to southern Canada, and from the east coast west to the foothills of the Rocky Mountains in Colorado (Readio and Sweet 1982; Ashlock and Slater 1988). Also known from Cuba (Alayo 1973) and the West Indies (Baranowski and Slater 2005).

New provincial record. NB: 1♂, Fundy Nat. Pk., 8.viii.1954 (J.F. Brimley) [CNC].

Family LYGAEIDAE

Subfamily LYGAEINAE

Melacoryphus admirabilis (Uhler)

This species and genus is keyed by Slater (1992) and is macropterous with a black membrane with a narrow white margin, and with clavus and corium black for most part, with costal margin and apical third of corium red. The species is widely distributed in the United States and occurs in Mexico (Ashlock & Slater 1988).

New Canadian record. SK: 1♀, Grasslands Natl. Pk., Tp. 1 & 2, Rge. 6 & 7, W3, EBGE sweep samples, 29-30.vii.2008 [CNC].

Subfamily ORSILLINAE

Nysius angustatus Uhler

Widely distributed across Canada, and previously reported from the Northwest Territories east to New Brunswick (Maw et al. 2000), and known from Mexico and most of the United States (Ashlock and Slater 1988).

New provincial record. NS: 2♂ 2♀, Coldbrook, 22.viii.1963 (V.R. Vickery) [LM].

Nysius niger Baker

In Canada, previously reported from

Yukon and the Northwest Territories to Newfoundland (Maw et al. 2000), but not previously recorded from Nova Scotia and Prince Edward Island. Recorded from most of the United States, Bermuda, Mexico to Central America, and the West Indies (Ashlock and Slater 1988), although not noted from the latter by Baranowski and Slater (2005).

New provincial record. NS: 3♀, Kentville, 15-17.vii.1966 (L.A. Kelton) [CNC].

Nysius tenellus Baker

In Canada, previously only reported from British Columbia (Barber 1947; Maw et al. 2000). However, the species is recorded from Florida, most of the western United States, Mexico, Central America and the West Indies (Barber 1947; Ashlock and Slater 1988; Baranowski and Slater 2005).

New provincial record. SK: 1♂, Jones Peak, 9 km W, Eastend, 49°30'N 108°57'W, 14.viii.2005 (Larson) [CNC].

Family OXYCARENIDAE

Crophius bohemani (Stål)

In Canada, previously only reported from British Columbia (Downes 1927; Walley 1934; Barber 1938; Maw et al. 2000). The record for the "North West Territories" (Gibson 1911) is obviously an error, and was not included by Barber (1938). In the United States *C. bohemani* is a Western Cordilleran species.

New provincial record. SK: 1♂1♀, Cypress Hills Park, Center Bloc, Highland Trail, 25.ix.2008 (D. Larson) [CNC; LC]; 1♀, Cypress Hills, Center Block, War Lodge Coulee, 29.ix.2008 (D. Larson) [LC].

Family RHYPAROCHROMIDAE

Subfamily RHYPAROCHROMINAE

Tribe DRYMINI

Eremocoris ferus (Say)

In Canada, previously reported from British Columbia, Nova Scotia, Ontario and Quebec (Maw et al. 2000). Sweet (1977) elevated *Eremocoris borealis* (Dallas) from synonymy with *E. ferus* and stated that this species has a Carolinian and Austroriparian Zone distribution extending from the Gulf of Mexico in the eastern United States north

to lowland locations in New England, with the northern records being from Massachusetts, New Hampshire, Connecticut and southern New York. He also noted that specimens from Illinois, Iowa and Indiana were all referable to *E. ferus*, and appeared to mark the northern limit of the distribution of the species in the Midwest. Furthermore, Sweet (1977) stated that he had not seen *E. ferus* specimens from west of the 100° meridian, although relictual populations in Texas might indicate that the species extends west of this meridian.

Over the past few years I have studied collections of *Eremocoris* from the west, and have specimens that I consider to be *E. ferus* from not only British Columbia and Saskatchewan, but also from Arizona, California, Idaho, Montana, Oregon, Utah and Washington state.

New provincial record. SK: 1♀, Cypress Hills Pk., C. Block, Ski Lodge, 25.vi.2004 (D. Larson) [CNC]; 1♀, Larson Ranch, Hwy. 21, 16 km S. Maple Creek, 16.v.2009 (D. Larson) [LC].

Tribe MEGALONOTINI

Megalonotus sabulicola (Thomson)

An alien species, in Canada previously reported from British Columbia (Scudder 1960, 1961; Asquith and Lattin 1991), Ontario (Maw et al. 2000) and Quebec (Scudder and Foottit 2006). Asquith and Lattin (1991) discussed the occurrence of this species in the Pacific Northwest, with records in the United States shown for California, Idaho, Oregon, Utah and Washington. Wheeler (1989) also discussed the occurrence of *M. sabulicola* in the eastern United States, with new records for Delaware, New Jersey, Pennsylvania, Rhode Island, Virginia and West Virginia, and additional localities in Maryland and New York. I have also collected *M. sabulicola* in Montana (Scudder 2010). Wheeler (1989) found that in the mid-Atlantic region of the United States, this bug feeds mainly on the fallen seeds of the spotted knapweed, *Centaurea biebersteinii* DC (= *C. maculosa* auct. non Lam).

New provincial record. SK: 1♂, Larson Ranch, Hwy. 21, 16 km S Maple Creek,

10.v.2008 (D. Larson) [LC]; 4♂ 2♀, *id.*, flooded grass, 12.vi.208 [CNC; LC].

Tribe MYODOCHINI

Neopamera albocincta (Barber)

To date in Canada, only reported from Ontario (Scudder 1985; Maw et al. 2000). Recorded from most of the eastern United States to Texas in the south, as well as the West Indies, and Mexico to South America (Ashlock and Slater 1988).

New provincial record. QC: 1♀, Riv. Du Sud Co., Iberville, CH322, 31.vii.1975 (N. Dorion) [CNC]; 1♂, *id.*, CH500, 26.viii.1974 (N. Dorion) [CNC]; 1♀, *id.*, CH503, 26.viii.1975 (N. Dorion) [CNC].

Sisamnes claviger (Uhler)

So far only recorded from British Columbia in Canada (Scudder 1985, 1992, 1993, 1994; Maw et al. 2000), but widely distributed in the United States (Ashlock and Slater 1988).

New provincial record. SK: 2♀, Cypress Hills, Center Block, War Lodge Coulee, 29.ix.2008 (D. Larson) [CNC; LC]; 2♀, Cypress Hills Pk., Center Block, Highland Trail, 14.iv.2009 (D. Larson) [CNC; LC]; 3♀, Larson Ranch, Hwy. 21, 16 km S Maple Creek, 10.v.2008 (D. Larson) [CNC; LC]; 1♂ 1♀, *id.*, 24.ix.2008 (D. Larson) [LC]; 1♂, Sand Hills, 7 km W Piapot, 4.vii.2009 (D. Larson) [LC].

Tribe STYGNOCORINI

Stygnocoris rusticus (Fallén)

In Canada, this alien is recorded from British Columbia east to Newfoundland (Maw et al. 2000), but until now there have been no records for either Manitoba or Saskatchewan.

Asquith and Lattin (1991) mapped the restricted distribution in the Pacific Northwest, with records shown for Oregon and Washington. Wheeler (1983) reviewed the more extensive distribution in the eastern United States.

New provincial record. SK: 1♂, Cypress Hills, Center Block, War Lodge Coulee, 29.ix.2009 (D. Larson) [LC]; 1♂, Cypress Hills Park, Center Block, Highland Trail, 25.ix.2008, (D. Larson) [CNC]; 1♂, Frenchman R. Valley, Cypress L., 20.viii.2009, (D. Larson) [LC]; 1♂, Larson Ranch, Hwy. 21, 16 km S Maple Creek, 23.ix.2008, (D. Larson) [LC]; 2♂ 1♀, *id.*, 24.ix.2008 (D. Larson) [CNC; LC].

Tribe UDEOCORINI

Neosuris castanea (Baker)

In Canada, to date reported only from British Columbia (Scudder 1993, 1994; Maw et al. 2000). Recorded from the Western Cordilleran states, to the south and Mexico (Ashlock and Slater 1988).

New provincial record. SK: 1♀, Maple Ck., Hwy. 21, 16 km S, 30.vii.2003 (D. Larson) [LC]; 1♂, Old-Man-on-his-Back Ridge, 49°11'N 109°16'W, 5.ix.2009 (D. Larson) [CNC].

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Taxonomic changes in *Dicraneura* Hardy, *Colladonus* Ball and *Macrosteles* Fieber (Homoptera-Auchenorrhyncha) in the Montane Cordilleran Ecozone

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ABSTRACT

A neotype is proposed for *Typhlocyba carneola* Stål, 1858 (= *Dikraneura carneola* var. *shoshone* DeLong & Caldwell, 1937, syn.nov.), and *Dikraneura sitkana* Ball & DeLong is elevated to specific rank for the taxon previously known as *Dikraneura carneola*. Five other species are described from the Montane Cordilleran Ecozone: *Colladonus keltoni* Hamilton, *Colladonus okanaganus* Hamilton, *Macrosteles frigidus* Kwon, *Macrosteles similis* Kwon and *Macrosteles vulgaris* Kwon.

INTRODUCTION

Eleven apparently undescribed species were discovered during the preparation of a faunal synopsis of the Montane Cordillera Ecozone (MCE) of British Columbia. Six of these species belong to well studied genera. *Colladonus* Ball was revised by Nielson (1957) for the species north of Mexico and 6 species have been added subsequently (Nielson 1962, Hamilton and Langor 1987); this contribution adds two more. *Dikraneura* Hardy was revised by Knight (1968) and a former synonym is elevated to specific status following designation of a neotype for the species with which it has been confused. *Macrosteles* Fieber is a large genus discussed by Kwon (1988, unpublished); his dissection techniques and five of his new species from the Atlantic Maritime Ecozone will appear elsewhere (Hamilton and Kwon 2010) and three others, occurring in the MCE, are formally described below.

Four other species belong to the typical subgenus *Empoasca* Walsh, which is a taxon with more than 600 species; it is in need of revision before any additional species are described. A fifth species is known only from a single female. Since it belongs to the Delphacid genus *Delphacodes* Fie-

ber, in which only males can be recognized with certainty, no formal description can be presented.

Specimens and notes examined in this study are deposited in the following institutions:

AMNH: American Museum of Natural History, New York.

CNC: Canadian National Collection of Insects, Arachnids and Nematodes, Ottawa, ON

GL: Grassland Leafhopper survey; field notes from H.H. Ross in CNC.

MLBM: Monte L. Bean Life Science Museum, Brigham Young University, Provo, UT

NCSU: North Carolina State University, Raleigh

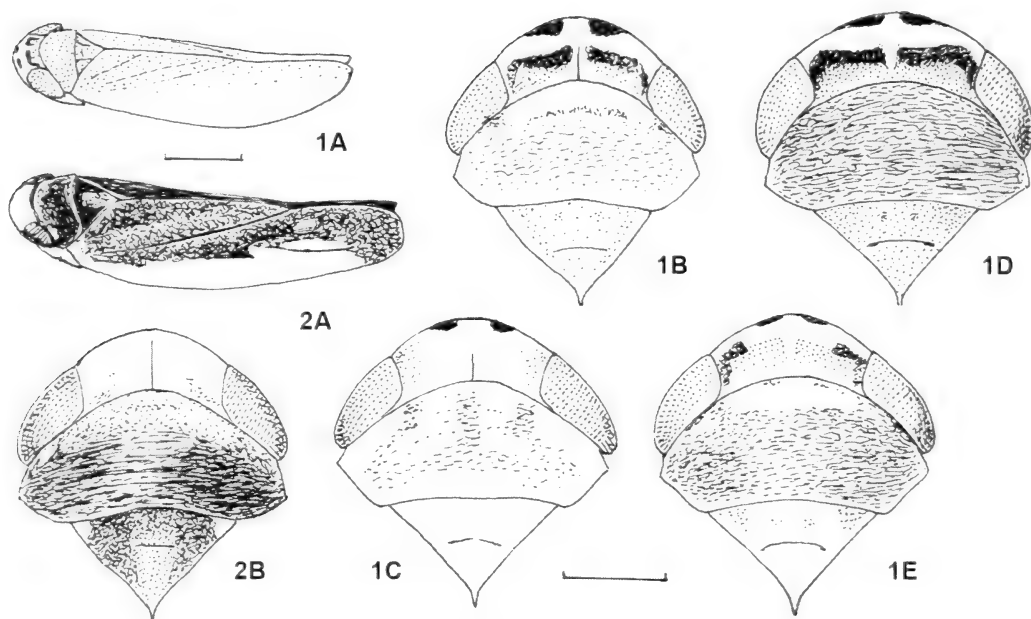
OrSU: Oregon State University, Corvallis

***Colladonus keltoni* Hamilton, sp.n.**

Diagnosis. Crown slightly less than half as long as wide, apically rounded (Fig. 1B-E); colour uniform brown, paler on tegmina, unmarked except for crown of head: apex with 2 black spots and usually also a mustache-shaped mark between eyes; tegmina hyaline with dark hind wing veins visible (Fig. 1A). Male genitalic characters

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Figures 1-2. *Colladonus* spp. 1, *C. keltoni*; 2, *C. okanaganus*: A, habitus, dorsolateral aspect; B-C, dorsum of male; D-E, dorsum of female. Habitus to larger scale (1 mm), dorsum to smaller scale (0.5 mm).

as in *C. ponderosus* Ball (Nielson 1957, figs. 4A, C): male pygofer produced on caudoventral margin and bearing a short spine at tip; style tips curved outwards, but with gonopore at midlength of shaft, and terminal processes extending at least half length of shaft, as in *C. tahotus* Ball (Nielson 1957, fig. 47B). Length: male, 4.5-4.9 mm; female, 4.8-5.2 mm.

Types. Holotype male, **B.C.**- Cranbrook, 23 July 1959 (L.A. Kelton). Paratypes: 3 females, same data as holotype; 3 females, Yahk, 22 July 1959 (L.A. Kelton), lodgepole [pine]; 1 female, Penticton, 27 June 1974 (M.W. Nielson); 1 male, Okanagan Mission, 18 June-2 July 1971, sticky board on *Prunus emarginata*; 1 male, same data, 1-16 July 1971; 1 male, Bear Creek (Okanagan Valley), 8 Aug. 1970 (K.G.A. Hamilton); 1 female, Okanagan Falls, 4 July 1976 (K.G.A. Hamilton); 1 female, Little Fort, 3 July 1976 (K.G.A. Hamilton); 2 males, Elko, 14 Aug. 1985 (K.G.A. Hamilton); **MT**- 1 male, 5 km E Grantsdale, 3 June 1992 (K.G.A. Hamilton). All types (7 males, 8 females) No. 21835 in CNC.

Remarks. All females and the darkest

males may be distinguished by the head markings. This is one of three species of *Colladonus* that are associated with pines, all of which have strongly produced male pygofers. The combination of genitalic characters distinguishes males from the other two pine species, *C. ponderosus* and *C. tahotus*.

***Colladonus okanaganus* Hamilton, sp.n.**

Diagnosis. Crown slightly less than half as long as wide, apically rounded (Figs. 2B); head and venter yellow, strongly contrasting with blackish brown notum and tegmina; costa and small spots on preapical cells hyaline (Fig. 2A). Male genitalic characters as in *C. flavocapitatus* (Van Duzee): pygofer spine arising from midlength of truncate pygofer tip, styles tips long and curved outwards, and terminal processes of aedeagus extending to midlength of shaft (Nielson 1957, figs. 45 A-C), but with process of pygofer slightly longer, as long as terminal processes of aedeagus, and gonopore basad of midlength of shaft (as in Nielson 1957, fig. 19B). Length: male, 4.6-5.0 (holotype); female unknown.

Types. Holotype male, **B.C.**- Okanagan Mission site 12 (Okanagan Valley), 30 July

1974 (J.E.H.). Paratypes: 1 male, Armstrong, 15-29 July 1971, sticky board on *Prunus emarginata*; 1 male, Penticton, site 1 SB, 18 July 1974 (J.E.H.). All types No. 21835 in CNC.

Remarks. The boldly contrasting colour is similar only to that of an undescribed species of *Colladonus* from the west coast of Vancouver Island.

***Dikraneura carneola* (Stål)**

Typhlocyba carneola Stål, 1858: 196 (Sitka Island, Alaska).

Dikraneura carneola var. *shoshone* DeLong & Caldwell, 1937: 27, **syn.nov.** (Idaho).

Diagnosis. This species is closely allied to a sister species, formerly considered as a "variety" *sitkana* (name elevated to specific rank, below). The latter differs in minor details of male genitalia and has a more southerly range (Figs. 3-4), overlapping that of *D. carneola* in southern B.C. to Idaho.

Remarks. This taxon was described from unusually pinkish specimens. Ball and DeLong (1925) considered the species to be very widespread in western North America, with variable colour. Specimens from Utah were especially yellower and were named as "var. *sitkana*," possibly as geographic variants, but more probably these are ecophenotypes. Later, DeLong & Caldwell (1937) figured the male genitalia for the first time. They considered the genitalic characters to be variable and associated the widespread "*D. carneola*" with a different aedeagal type than that of "var. *shoshone*" from Idaho. Knight (1968) concluded that these two aedeagal types represent separate species. Lack of material from Alaska prompted him to retain the name "*D. carneola*" for the widespread species. However, 78 specimens were later taken close to Sitka at Haines (1 nymph, 12 males, 16 females, GL 1142) and Potter (14 males, 35 females, GL 1127). Five males dissected from each series are referable to *D. shoshone*. A female from much farther northwest on coastal Alaska (King Salmon) is probably conspecific. Three males from inland sites in northwestern Canada (Banff, AB; Atlin and Chicotin, BC) indicate that

the coastal populations have a montane connection to the southern BC and ID populations. The two names are therefore synonyms and the southern "variety" is indeed a valid species.

Types. Stål's types have been sought, but have not been found (Knight 1968). Neotype of *carneola*, here designated: male, AK- Haines, 5 August 1968 (Ross, Ross & Miller) GL 1142.

***Dikraneura sitkana* (Ball & DeLong), stat.nov.**

Typhlocyba carneola var. *sitkana* Ball & DeLong, 1925: 330.

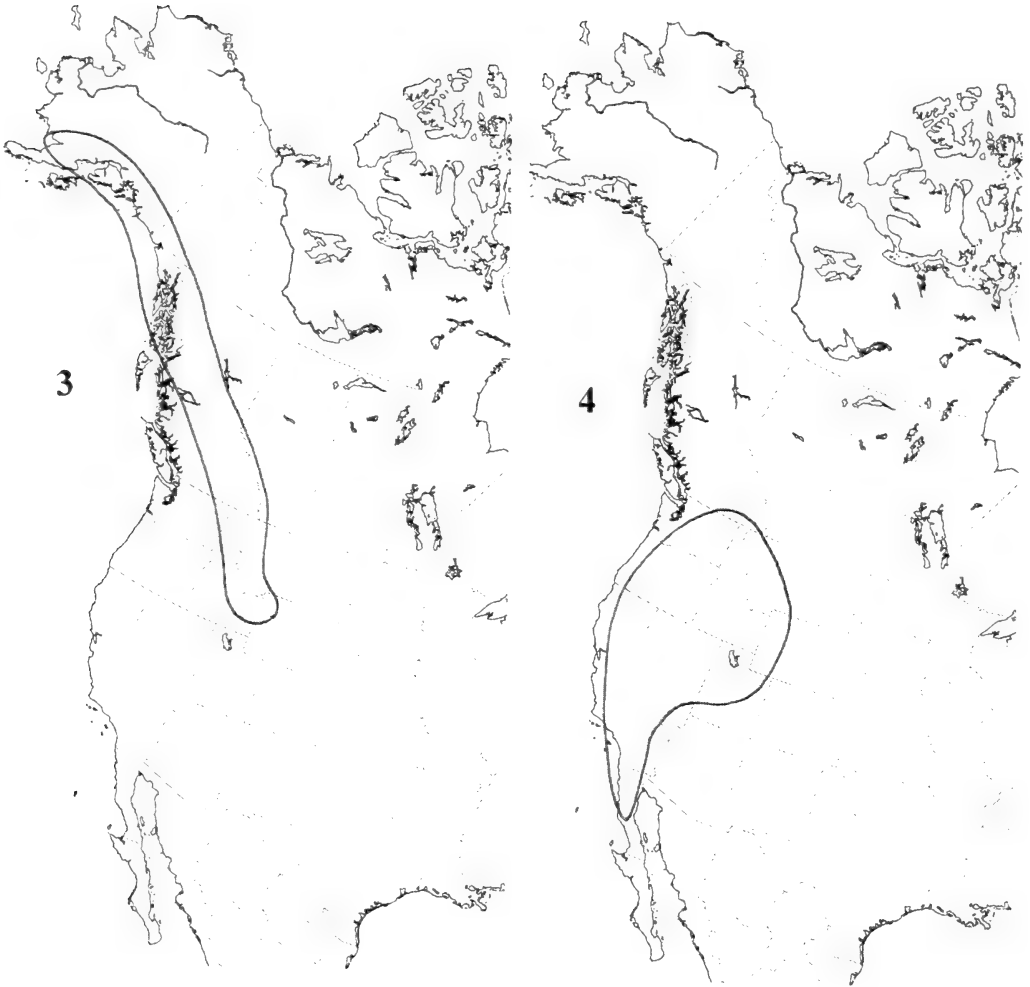
Typhlocyba carneola: Knight, 1968: (misidentification).

Diagnosis. Characters and distribution as in Knight (1968) for *D. carneola* [nec Stål], but the single female of "*carneola*" from Edmonton, AB cannot be positively identified. This female probably belongs to the common prairie-inhabiting *D. variata* Hardy, which sometimes has pinkish females.

Material examined: 52 males, 66 females from B.C.- Baldy Mtn. 7000-7550' [2300-2500 m ASL], 13 Aug. 1970 (K.G.A. Hamilton); 48 males, 48 females, MT- Bozeman, 9 Sept 1971 (H.H. Ross) [GL] 1271. All types No. 21835 in CNC. Ten males from each of these series were dissected to verify that there is no admixture of other species.

***Macrosteles frigidus* Kwon, sp.nov.**

Diagnosis. Yellow to yellowish green, often with faint smoky tint on tegmina (Fig. 5A-B). Crown with anterior margin rounded in male, more or less pointed in female; anterior black spots isolated, subequal in size to posterior spots, which are always prominent and isolated; median and lateral spots absent; frons without any prominent dark streaks (Fig. 5C-E). Tegmina more or less mottled with large, discoid pale areas. Male abdomen with 1st acrotergite only as broad as long, 2nd acrotergite with "neck" shorter than half of acrotergite width; 1st tergal apodeme reaching posteriorly to two-thirds of tergite length (Fig. 5F); 1st sternal apodemes with posterior lobes slightly longer (1.1-1.2 ×)



Figures 3-4. Ranges of *Dikraneura* spp. 3, *D. carneola*; 4, *D. sitkana*.

than wide (Fig. 5G, J-L); 2nd sternal apodemes with posterior lobes slightly exceeding twice basal width (Fig. 5N-Q); apophysis processes narrowly produced (Fig. 5M). Aedeagal shaft in posterior aspect (Fig. 5W-Z) with apical processes reflexed basally, crossed, extending nearly to middle of shaft, and subapex narrowed, bearing paired lateral teeth slightly above gonopore; in lateral aspect (Fig. 5S-V) more or less convex subapically on dorsal side, spiculate ventrobasally; subgenital plates (Fig. 5R) each with mesal margin about as long as basal margin. Length: male, 2.9-3.2 mm; female, 3.2-3.4 mm.

Types. Holotype male, **B.C.**- Quesnel, 31 Aug. 1948 (G.J. Spencer). Paratypes: 5 females, same data as holotype; 2 males, 4

females, Minnie Lake, 27 July 1925 (H.G. Crawford); 3 males, 4 females, Stanley, 3 Aug. 1949 (R. Stace-Smith); 2 males, 1 female, 7 mi S of Nelson, 6 Aug. 1969 (P. Oman); **Alta.**- 3 males, 4 females, Wainwright, 27 July 1957 (A.R. & J.E. Brooks); 1 male, High Prairie, 17 July 1961 (A.R. Brooks); 1 male, Grande Prairie, 26 July 1961 (A.R. Brooks); **Sask.**- 1 male, Torch R., 20 July 1950 (L.A. Konotopetz); 1 male, 2 females, Prince Albert, 23 July 1959 (A.R. & J.E. Brooks); 1 male, Saskatoon, 1 Aug. 1960 (A.R. Brooks); **Man.**- 2 males, 2 females, Virden, 9 July 1953 (Brooks & Kelton); **Y.T.**- 2 males, Snag, 24 July 1948 (Mason & Hughes); **AK.**- 3 males, 1 female, Big Delta, 13 July 1951 (J.R. McGillis). Holotype and 41 paratypes

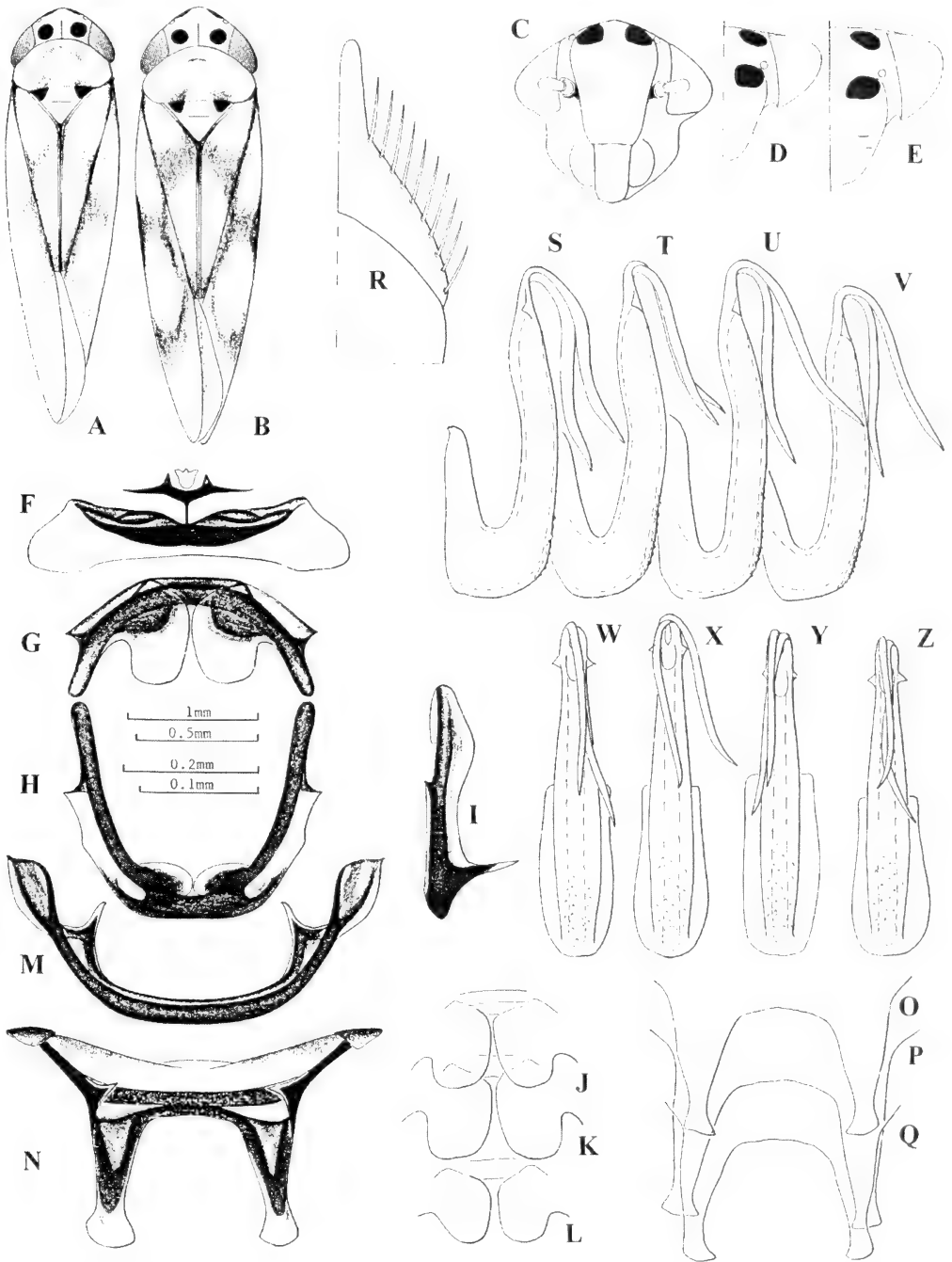


Figure 5. *Macrosteles frigidus*, based on specimens from B.C., except variants (from Yukon, Alta. and AK respectively): A, habitus of male, dorsal aspect; B, same, of female; C, male head in facial aspect; D, same, in anterior aspect; E, same, of female; F, male abdominal tergites 1-2 in dorsal aspect; G, male abdominal sternite 1 in dorso-anterior aspect; H, same, in anterior aspect; I, same, in lateral aspect; J-L, same, variation of outlines of sternal lobes; M, male abdominal sternite 2 in anterior aspect; N, same, in dorsal aspect; O-Q, same, variation of outlines of sternal lobes; R, male subgenital plate, ventral aspect; S-V, variation in aedeagus, lateral aspect; W-Z, same, posterior aspect. Habitus (A-B) to largest scale; face (C-E) to second largest scale; abdominal plates (F-R) to second smallest scale; aedeagus (S-Z) to smallest scale.

No. 22874 in CNC; 3 paratypes in OrSU and 1 paratype in NCSU.

Additional specimens, excluded from the type series, were examined from Texas (36 specimens), Alberta and Saskatchewan (3 specimens). The Texan morph has somewhat shorter head and apodemes, while the others have more strongly pointed heads, especially in the female.

Remarks. This species is similar to *M. galeae* Hamilton (in Hamilton and Langor 1987), but is readily distinguished by the very bold coronal spots between the eyes. From all other species in the genus with crossed aedeagal processes it may be distinguished by its convex subapex of the aedeagus in lateral aspect.

***Macrosteles similis* Kwon, sp.nov.**

Diagnosis. Relatively elongate; yellow to yellowish green, often with very faint smoky tint on fore wings (Fig. 6A-B). Crown somewhat rounded in male, more or less pointed in female; black anterior spots often fused together with lateral spots to form a transverse band along coronal margin, and with median and lateral spots often confluent; frons with prominent, dark transverse bands on either side of median stripe (Fig. 6C-E). Tegmina with dark stripe along claval suture. Male abdomen with 1st acrotergite broad, 2nd acrotergite nearly transverse, triangularly produced ventrally, "neck" slender, less than half as long as width of acrotergite; 2nd tergal apodeme often reaching to middle of tergite (Fig. 6F); 1st sternal apodemes with posterior lobes usually as long as wide (Fig. 6G, J-L), slightly inclined in lateral aspect (Fig. 6I); 2nd sternal apodemes with posterior lobes about twice as long as basal width (Fig. 6N-Q); apophysis processes narrowly produced (Fig. 6M). Aedeagal shaft smooth ventrally, with serrate lateral flanges lying along anterior edge of shaft in lateral aspect, in posterior aspect (Fig. 6V-X) narrowly developed, apical processes slender, convergent, or crossed apically in posterior aspect, in lateral aspect (Fig. 6S-U) turned anteriorly, then hooked dorsad; subgenital plates each with mesal margin longer than basal margin (Fig. 6R). Length: male, 3.6-4.0 mm; fe-

male, 4.0-4.4 mm.

Types. Holotype male, **AK-** Big Delta, 13 July 1951 (J.R. McGillis). Paratypes: 1 female, same data as holotype; 1 male, 1 female, same locality, 30 June-26 July 1951 (W. Mason); 1 male, Fairbanks, 4 Aug. 1951 (H.C. Severin); 2 males, Circle Hot Spgs., 4 Aug. 1951 (H.C. Severin); **B.C.-** 3 males, Atlin 2200' [700m], 29-30 July 1955 (B.A. Gibbard); 1 male, 10 mi S of Revelstoke, 22 Aug. 1978 (K.G.A. Hamilton) on *Juncus* spp.; 1 male, Orchard Pt. bog, Brooks Pen., Vancouver Is., 4 Aug. 1981 (R.A. & S.G. Cannings); **N.W.T.-** 2 males, 4 females, Rocknest Lake 65°39'N 114°20'W, 26 Aug. 1966 (G.E. Shewell); **Qué.-** 5 males, Natashquan, 7 Aug. 1929 (W.J. Brown); **CO-** 1 male, 2 mi S of Gould, 9000' [2400m], 13 Aug. 1968 (P. Oman); **UT-** 16 males and 38 females, Uinta Mts. 10,000' [3000m], Uintah Co., 21 Aug. 1983 (M.W. Nielson) on *Carex* sp. Holotype and 22 paratypes No. 22876 in CNC; 54 paratypes in MLBM; 1 paratype in OrSU.

Remarks. Similar to *M. fieberi* (Edwards), but differing in the much shorter posterior lobes on the male 2nd sternal apodemes, and by the serrate lateral flanges of the aedeagus lying along the anterior edge of the shaft instead of along the sides (Beirne 1952, figs. 91-92). This aedeagal character appears to be variable in European populations of *M. fieberi*, but not in North American populations.

***Macrosteles vulgaris* Kwon, sp.nov.**

Diagnosis. Yellow to yellowish green, frequently with smoky markings on body and tegmina (Fig. 7A-C). Crown broad, slightly more than twice as wide as long, often rounded in male, more or less pointed in female; spot pattern confluent or isolated, as in *M. quadrilineatus* (Forbes). Frons with black transverse bands usually confluent (Fig. 7D-G). Tegmina unmarked or faintly crossbanded. Male abdomen with 1st acrotergite very broad, 2nd acrotergite nearly transverse, triangularly produced ventrally, "neck" slender and elongate, only slightly shorter than acrotergite; 2nd tergal apodeme reaching middle of tergite (Fig.

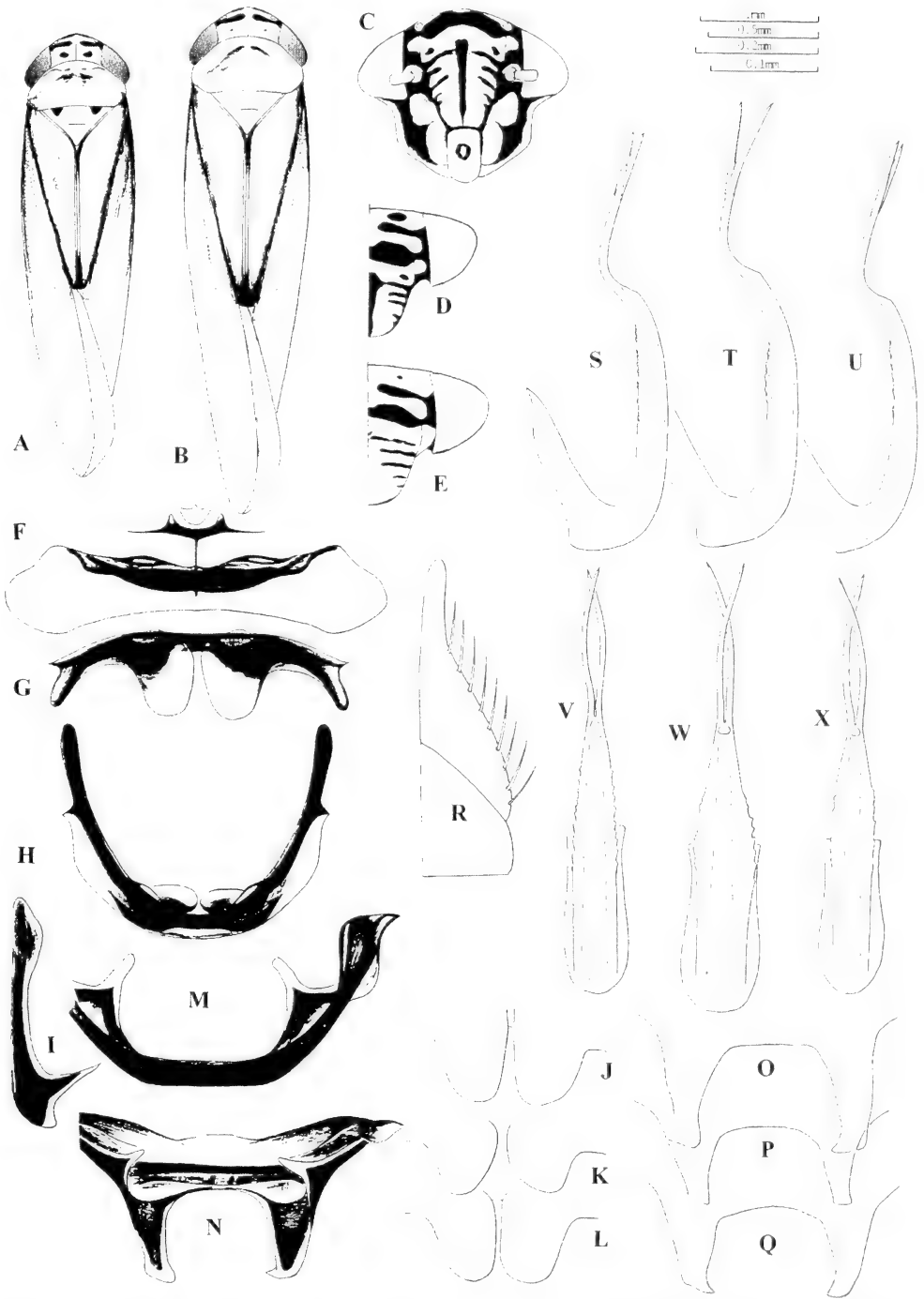


Figure 6. *Macrosteles similis*, based on males from Qué. and female from N.W.T.: A, habitus of male, dorsal aspect; B, same, of female; C, male head in facial aspect; D, same, in anterior aspect; E, same, of female; F, male abdominal tergites 1-2 in dorsal aspect; G, male abdominal sternite 1 in dorso-anterior aspect; H, same, in anterior aspect; I, same, in lateral aspect; J-L, same, variation of outlines of sternal lobes; M, male abdominal sternite 2 in anterior aspect; N, same, in dorsal aspect; O-Q, same, variation of outlines of sternal lobes; R, male subgenital plate, ventral aspect; S-U, variation in aedeagus, lateral aspect; V-X, same, posterior aspect. Habitus (A-B) to largest scale; face (C-E) to second largest scale; abdominal plates (F-R) to second smallest scale; aedeagus (S-X) to smallest scale.

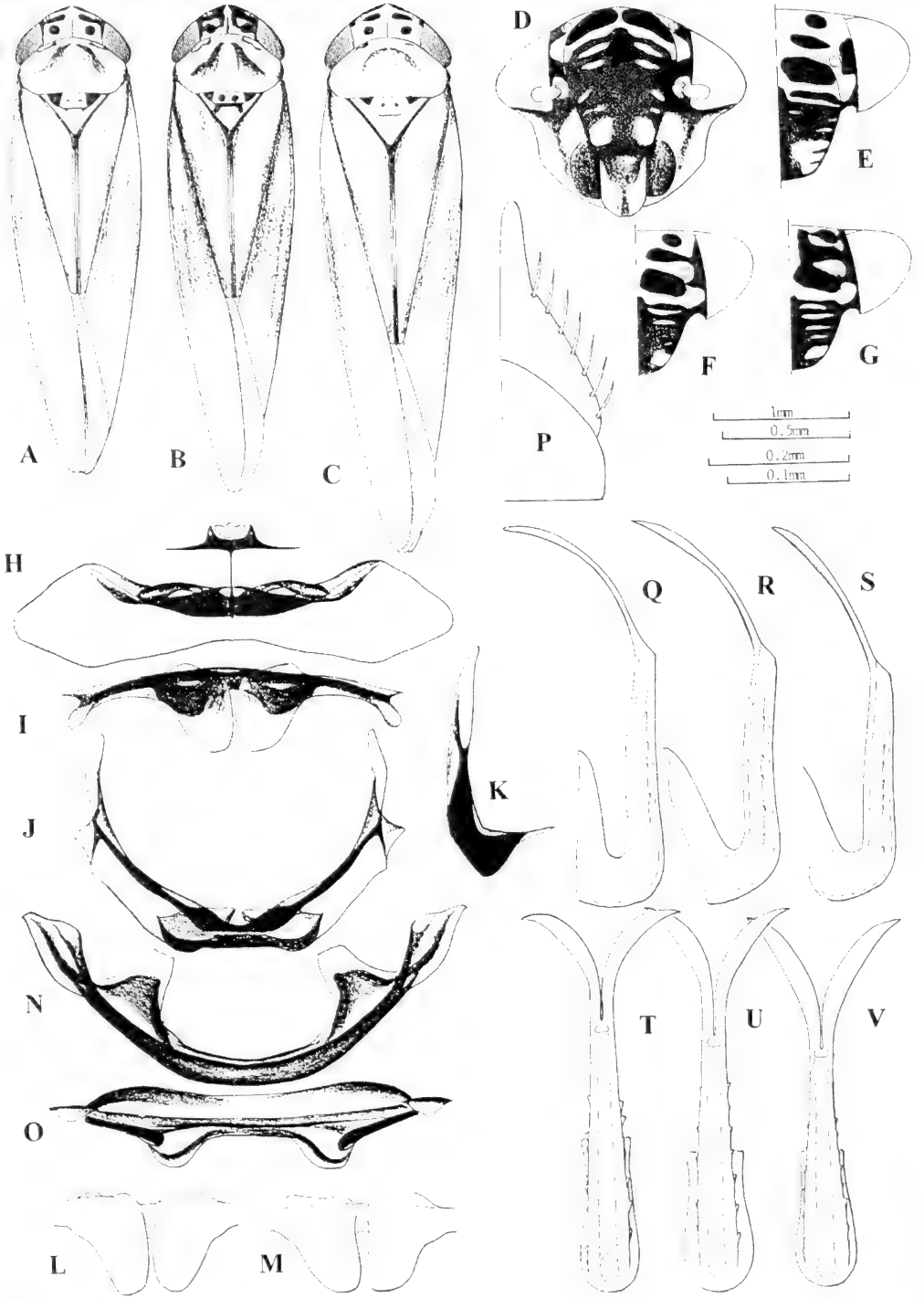


Figure 7. *Macrosteles vulgaris*, based on specimens from B.C.: A, B, habitus of male, dorsal aspect; C, same, of female; D, male head in facial aspect; E, same, of female in anterior aspect; F, G, same, of male; H, male abdominal tergites 1-2 in dorsal aspect; I, male abdominal sternite 1 in dorso-anterior aspect; J, same, in anterior aspect; K, same, in lateral aspect; L, M, same, variation of outlines of sternal lobes; N, male abdominal sternite 2 in anterior aspect; O, same, in dorsal aspect; P, male subgenital plate, ventral aspect; Q-S, variation in aedeagus, lateral aspect; T-V, same, posterior aspect. Habitus (A-C) to largest scale; face (D-G) to second largest scale; abdominal plates (H-P) to second smallest scale; aedeagus (Q-V) to smallest scale.

7H); 1st sternal apodemes with posterior lobes about as long as basal width (Fig. 7I, L-M); apophyses abruptly bent at middle in anterior aspect (Fig. 7J), dorsal aspect with lower part narrower than median part between posterior lobes; 2nd sternal apodemes apically truncate (Fig. 7N) with posterior lobes reduced, triangular (Fig. 7O). Aedeagus (Fig. 7Q-V) as in *M. quadrilineatus*; subgenital plates each with mesal margin longer than basal margin (Fig. 7P). Length: male, 3.2-3.7 mm; female, 3.5-4.2 mm.

Types. Holotype male, **B.C.**- Cowichan Lake, 6 June 1955 (R. Coyles). Paratypes: 65 males, 30 females, same data as holotype; 8 males, Kootenay Bay, 23 June-29 Aug. 1948-49 (D.B. Waddell); 1 male, Soda Creek, 21 July 1950 (G.J. Spencer); 1 male, 3 females, Malahat, 20 Sept. 1950 (W. Downes); 22 males, 11 females, Oliver 2500' [800m], 2 July 1953 (J.R. McGillis); 1 male, Diamond Head Trail 3200' [1000m], Squamish, 7 Aug. 1953 (G.J. Spencer); 1 male, 6 females, Duncan, 9 June 1955 (R. Coyles); 1 male, 5 females, Miracle Is. Park, 11 June 1955 (R. Coyles); 3 males, 3 females, same locality, 29 May 1959 (R. Madge & R.E. Leech); 1 male, Spectacle Lake, Oliver, 10 June 1959 (L.A. Kelton); 11 males, 14 females, Terrace, 15 July-3 Aug. 1960 (W.R. Richards); 1 male, Shames, 18 mi SW of Terrace, 17 July 1960 (C.H. Mann); 8 males, 11 females, 5 mi E of Sidney, 23 Aug. 1971 (J. Sawbridge); **Man.**- 1 male, Shoal Lk., 28 June 1976 (K.G.A. Hamilton) on *Distichlis stricta*; **Sask.**- 1 male, Pipestone Creek, 7 June 1958 (A.R. Brooks); **CA**- 3 males, Truckee, Nevada Co., 29 Aug. 1967 (L. Kelton); 1 male, 1 female, 38 mi SE Mt. Shasta, 10 July 1972 (P. Oman); 1 male, 4 females, 18 mi W of Susanville 5400' [1530m], 10 July 1972 (P. Oman); 1 male, 7.6 mi N of Bridgeport, 19 June 1982 (P. Oman); 1 male, 2 females, Squaw Valley, Placer Co., 5 Oct. 1983 (D.G. Denning); **ID**- 1 male, Paris, 8 July 1920 (F 4741); **MT**- 1 male, 24 females, Bozeman, 9 Sept. 1971 (H.H. Ross); **OR**- 6 males, 5 females, Corvallis, 14 Aug. 1928 (O.A.

Hills); 1 female, same data, but 4 July 1927; 1 male, Lostine, 12 Aug. 1929 (O.A. Hills); 10 males, Gresham, 8 July 1949 (R. Rosenstiel) on strawberry; 1 male, Astoria, 13 June 1951 (E.A. Dickason); 1 male, Oregon City, 28 Aug. 1962 (Koontz) on potato; 6 males, 3 females, Forest Grove, 2 July 1965 (F.P. Larson) black light trap; 3 males, Woodburn at Pudding R., 26 July 1965 (F.P. Larson) black light trap; 3 males, same data, but 11 Sept. 1966; 1 male, Hillsboro, 28 July 1965 (F.P. Larson) black light trap; 2 males, Troutdale, 18 July 1966 (F.P. Larson) black light trap; 2 males, 2 females, Canby, 29 Aug. 1966 (F.P. Larson) black light trap in corn; 3 males, Lily Lake 13 mi E of French Glen 7200' [2000m], 10 July 1968 (P. Oman); 3 males, MacDonald Forest at Corvallis, 17 July 1968 (P. Oman); 2 males, 6 females, Seal Rock, 31 Aug. 1968 (P. Oman); 8 males, 13 females, same data, but 1 May 1970; 4 males, 5 females, same data, but 1-27 May; 1 male, 2 mi NW of Banks, 18 June 1969 (P. Oman); 1 male, 25 mi SE of Joseph, 10 Aug. 1969 (P. Oman); 7 males, 5 females, Tou Velle Park, Jackson Co., 2 May 1970 (P. Oman); 1 male, 3 females, Agate Desert, Jackson Co., 2 May 1970 (P. Oman); 1 male, 2 females, same data, but 19 May 1971; 1 male, 2 females, Joseph, 6 June 1970 (P. Oman); 1 male, 10 mi ESE of Ruch, 14 May 1971 (P. Oman); 10 males, 11 females, 19 mi W of Klamath Falls, 24 June 1971 (P. Oman); 1 male, 20 mi E of Seneca, 14 Aug. 1971 (P. Oman); 3 males, 1 female, 12 mi W of Silver Lake 5000' [1500m], Lake Co., 12 July 1978 (P. Oman); 11 males, 4 females, Johnson Meadow, Klamath Co., 17 July 1979 (P. Oman); 1 male, 2 females, north edge of Big Lake 4650' [1350m], Linn Co., 1 Oct. 1979 (P. Oman); 2 males, 2 females, Saunders Lake 2 mi N of Hauser, 8 Oct. 1979 (P. Oman); **WA**- 2 mi S of Humptulips, 22 Aug. 1971 (Viraktamath). Holotype and 240 paratypes No. 22877 in CNC; 190 paratypes in OrSU, 13 paratypes in MLBM, 3 in University of Kentucky, Lexington and 1 in AMNH.

Additional records, based on unassoci-

ated females and therefore excluded from the type series, are: **CA**- Fort Ord., 2 mi E of Gasquet; **OR**- 2 mi E of Carlton, Merlin, Mt. Vernon, Odell, Sams Valley (N of Medford), Skookum Meadow (Klamath Co.).

Remarks. Distinguished from *M. quad-*

rilineatus and related species by the angulate apophyses of the male 2nd sternal apodeme, and by the unusual length of the 2nd acrotergite "neck." This species appears to replace *M. quadrilineatus* as the most common temperate-zone *Macrosteles* west of the Rocky Mountains.

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First records of the banded elm bark beetle, *Scolytus schevyrewi* Semenov (Coleoptera: Curculionidae: Scolytinae), in British Columbia

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ABSTRACT

The banded elm bark beetle, *Scolytus schevyrewi* Semenov was detected for the first time in British Columbia near Kelowna during 2010. Fifty-eight *S. schevyrewi* were captured in an experiment that targeted the European elm bark beetle, *Scolytus multistriatus* (Marsham). It was a test of the efficacy of a new trap design relative to the multiple funnel trap currently used in surveillance programs for invasive bark- and wood-boring Coleoptera. Data on the seasonal occurrence of the banded elm bark beetle are presented.

Key Words: banded elm bark beetle, *Scolytus schevyrewi*, forest pest, invasive species

INTRODUCTION

The banded elm bark beetle, *Scolytus schevyrewi* Semenov (Coleoptera: Curculionidae: Scolytinae), is an invasive bark beetle native to central and eastern Asia (Negrón et al. 2005; CABI/EPPO 2009; Lee et al. 2009). It was first reported from North America in Colorado and Utah in 2003 (Negrón et al. 2005) and was soon found to be more widely distributed (Negrón et al. 2005). In the U.S.A., *S. schevyrewi* is now reported from 28 states including all states west of the Mississippi River (except Arkansas, Iowa, Louisiana and North Dakota) as well as from Connecticut, Delaware, Illinois, Indiana, Maryland, Michigan, Minnesota, Missouri, New Jersey, Ohio, Pennsylvania and Virginia, (Lee et al. 2009; NAPIS 2010). Specimens in reference collections indicate that *S. schevyrewi* was present in the U.S.A. (Colorado) as early as 1994 (Lee et al. 2009). In Canada, banded elm bark beetle was first detected in Alberta

in 2006 (Langor et al. 2009) and has subsequently been reported from locations in Saskatchewan, Manitoba and Ontario (CABI/EPPO 2009). While species of elms (*Ulmus*) are the only reported hosts for *S. schevyrewi* in North America and are its primary hosts in Asia, it has also been recorded to attack *Caragana*, *Elaeagnus*, *Malus*, *Prunus*, *Pyrus* and *Salix* across its native central and eastern Asian range (Wood and Bright 1992; Bright and Skidmore 1997, 2002; Negrón et al. 2005).

S. schevyrewi is of immediate concern as a potential vector of Dutch elm disease (DED), caused by the fungal pathogens *Ophiostoma himal-ulmi* Brasier & M.D. Mehrota, *Ophiostoma novo-ulmi* Brasier and *Ophiostoma ulmi* (Buisman) Nannf. (Harrington et al. 2001). Jacobi et al. (2007) isolated DED from adult *S. schevyrewi* emerging from infected *Ulmus americana* L. at levels similar to those from co-

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emergent European elm bark beetle, *Scolytus multistriatus* (Marsham), the primary vector of DED in North America. Concurrently, Koski and Jacobi (2007) demonstrated that DED was transmitted to feeding wounds during maturation feeding by *S. schevyrewi* adults artificially inoculated with the disease. The efficiency of *S. schevyrewi* as a disease vector remains unknown as neither its efficiency as a DED

vector (Lee et al. 2009) nor field transmission of DED from infected to uninfected elms have been determined (Negrón et al. 2005). However, the evidence suggests that the risk of *S. schevyrewi* serving as an additional vector of DED is very high. We report the first records for *S. schevyrewi* in British Columbia (BC) and document its seasonal occurrence.

MATERIALS AND METHODS

S. schevyrewi was detected in traps near Kelowna, BC during ongoing trials to test the efficacy of bottle traps relative to 12-unit multiple funnel traps (ConTech Inc., Delta, BC). Each bottle trap was constructed from an inverted clear 2-liter pop bottle with half of the circumference of the side wall removed. The threaded portion of the bottle's neck was inserted into a 2.54 cm diameter hole drilled in the lid of a 16 oz white plastic cosmetic jar (Industrial Plastics, Victoria, BC). The trap and the lures were hung from the pivoting triangular loops of picture frame hangers riveted (2.7 cm below the base) to the outside and inside walls of the bottle, respectively.

Ten replicates of a bottle trap paired with a funnel trap were established on a berm on the west margin of the Glenmore Landfill (49.9556°, -119.4235°). The landfill is situated in the Okanagan Very Dry Hot Ponderosa Pine Variant (PPxh1) of the Ponderosa Pine biogeoclimatic zone (Hope et al. 1991); ridges dominated by Ponderosa pine (*Pinus ponderosa* P. & C. Lawson) are present 70 m west of and 800 m east of the berm. The berm is landscaped with both ornamental and native trees including Colorado blue spruce (*Picea pungens* Engelm.), corkscrew willow (*Salix* sp.), Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco), Lombardy poplar (*Populus nigra* L. cv. 'Italica'), London plane (*Platanus x acerifolia* (Air.) Willd.), maple (*Acer* spp.), mountain ash (*Sorbus* sp.), Ponderosa pine, Rus-

sian olive (*Elaeagnus angustifolia* L.), Scots pine (*Pinus sylvestris* L.), and sumac (*Rhus* sp.).

All traps were baited with a proprietary release system releasing multistriatin, 4-methyl-3-heptanol and alpha-cubebene at sub-milligram rates per day at 20 °C (ConTech Inc., Delta, B.C.) and half of the replicates were also baited with a half-size ultra-high release ethanol lure (270 mg/day at 20 °C, ConTech Inc., Delta, B.C.). The primary lure for the experiment was selected to target *S. multistriatus*, which is widely distributed in the study area (van Sickle and Fiddick 1982). Traps within a pair were separated by 4-5 m and pairs were separated by at least 30 m. Collecting cups contained 125 ml of propylene glycol to retain any captured insects. Traps were deployed on 13 April 2010 and serviced approximately every two weeks through 5 August 2010 when all lures were replaced. The screening aid of LaBonte et al. (2003) was used to separate *S. schevyrewi* from other species of *Scolytus* present in the samples. While the experiment is still ongoing and identifications of all insects captured are not complete, all *S. multistriatus* and *S. schevyrewi* recovered to 28 September 2010 have been determined. Because *S. schevyrewi* is new to the fauna of British Columbia, we feel it is important to document its occurrence in the province prior to the completion of the study.

RESULTS

In total 27, 551 *S. multistriatus* and 58 *S. schevyrewi* were captured between 13 Apr. and 28 Sep. 2010. Collection dates [number of males and females] for *S. schevyrewi* are: 12-28.v.2010 [1♂, 4♀]; 28.v-11.vi.2010 [3♂]; 11-28.vi.2010 [15♂, 4♀]; 28.vi-15.vii.2010 [11♂, 10♀]; 15-28.vii.2010 [2♂, 4♀]; 5-20.viii.2010 [1♂]; and 2-28.ix.2010 [1♂, 2♀]. No *S. schevyrewi* were recovered from the 13-29.iv.2010, 29.iv-12.v.2010, 28.vii-5.viii.2010 and the 20.viii-2.ix.2010 sample periods. In contrast, *S. multistriatus* was

recovered throughout the complete sampling period. Voucher specimens of *S. schevyrewi* have been deposited in the Canadian National Collection (CNC), Ottawa, ON; the Royal British Columbia Museum (RBCM), Victoria, B.C., Natural Resources Canada, Canadian Forest Service, Pacific Forestry Centre (PFCA), Victoria, BC; and the Spencer Entomological Collection (UBCZ), Beaty Biodiversity Museum, University of British Columbia, Vancouver, BC.

DISCUSSION

The high numbers of *S. multistriatus* and the detection of *S. schevyrewi* at the Kelowna landfill are surprising as no *Ulmus* are planted on the site. Sixteen elms were located around a parking lot 400 m to the north and scattered mature trees were also noted on a rural property 725 m south of the trap line. Deciduous hosts growing along the berm at the landfill and dead and dying limbs of the elms to the north of the landfill were examined by LMH, EJ and MN on 5 August 2010 for signs of attack by bark beetles. None of the hosts exhibited signs of attack, thus the source of the *S. multistriatus* and *S. schevyrewi* populations remains unknown. The traps were also well removed from two other potential sources of

the *Scolytus* spp., yard waste and solid wood packaging. The collection site for urban yard waste is 600-800 m to the north-east, while that for wood waste is 600-700 m to the east.

Scolytus schevyrewi has replaced *S. multistriatus* as the predominant bark beetle attacking elms in Colorado, Utah and New Mexico (Lee et al 2009) and has been implicated as the causal agent of Siberian elm, *Ulmus pumila* L., mortality in Colorado (Negrón et al 2005). Siberian elm is widely planted in the arid interior of BC and has naturalized in the Okanagan, Similkameen and Kettle valleys (Brayshaw 1996), and may be impacted by *S. schevyrewi* populations.

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DNA barcoding identifies the first North American records of the Eurasian moth, *Eupithecia pusillata* (Lepidoptera: Geometridae)

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ABSTRACT

The first North American records of the juniper pug moth, *Eupithecia pusillata* (Denis & Schiffermüller, 1775) (Lepidoptera: Geometridae), brought to our attention using DNA barcoding, are presented. Documentation and collection localities suggest it was introduced, established, and likely has persisted, at least in the Greater Vancouver area of British Columbia since the mid-1970s. We discuss the integration of DNA barcoding into routine biosurveillance and forest insect surveys to prevent such delay in recognition of non-indigenous species—in this case, 34 years.

Key Words: *Eupithecia pusillata*, *Eupithecia interruptofasciata*, *Eupithecia niphadophilata*, juniper pug moth, *Juniperus*, non-indigenous species, invasive species, DNA barcoding

INTRODUCTION

DNA barcoding of biological specimens has demonstrated repeatedly its utility as a molecular diagnostic technique that merits integration into biosurveillance programs. In contrast to other molecular tools commonly employed for species identification of intercepted organisms, DNA barcoding is a generic and standardized approach that meets international standards of data quality and transparency (Floyd et al. 2010). Several studies have demonstrated the efficacy of this technique for detecting non-indigenous species and determining native provenance, for example in leeches (Siddall

and Budinoff 2005), agromyzid leafminers (Scheffer et al. 2006), tephritid fruit flies (Armstrong and Ball 2005; Barr 2009), siricid wasps (Wilson and Schiff 2010), true bugs (Nadel et al. 2010), and numerous taxa of moths (Ball and Armstrong 2006; Simonsen et al. 2008; Humble et al. 2009; deWaard et al. 2009; Gilligan and Epstein 2009; Armstrong 2010). Here we report the first North American records of the juniper pug moth, *Eupithecia pusillata* (Denis & Schiffermüller, 1775) revealed by DNA barcoding.

MATERIALS AND METHODS

While compiling a DNA barcode library for the Geometridae of British Columbia

(deWaard et al., submitted), the cytochrome *c* oxidase subunit I (COI) sequences de-

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rived from two *Eupithecia* specimens were found to be divergent from known native *Eupithecia*. The two sequences were compared to a reference barcode database of Lepidoptera barcodes using the identification engine (BOLD-ID) of the Barcode of Life Data Systems (BOLD) (Ratnasingham and Hebert 2007), and tentatively identified as *Eupithecia pusillata*, a Eurasian species not known to occur in North America. The reference barcode database for Geometridae used by BOLD-ID is continually validated by specialists to ensure accurate identifications, and is particularly well parameterized due to a global campaign to barcode the nearly 23,000 species of the family (see <http://www.lepbarcoding.org/geometridae/index.php>). The nine sequences with identical and near-identical matches from Europe were obtained from Axel Hausmann (Zoological State Collection, Munich, Germany) and Marko Mutanen (University of Oulu, Oulu, Finland) and combined with related North American specimens (*sensu* Bolte 1990). A neighbour-joining tree was constructed on BOLD using the Kimura-2-parameter distance method (Fig. 1).

To pursue confirmation of the identity of the specimens, the two putative *E. pusillata* specimens obtained from the RBCM (Royal British Columbia Museum, Victoria, BC) and PFC (Arthropod reference collection, Pacific Forestry Centre (PFC),

Natural Resources Canada, Canadian Forest Service, Pacific Forestry Centre, Victoria, BC), were dissected to examine the genitalia following the methods given by Lafontaine (2004). Images of genitalia were taken using a Leica M205C microscope equipped with a Leica DFC490 camera kit and Leica LAS Montage system that assembles multiple images in successive planes of focus into a single image with a large depth of field. The specimens were verified by comparison of the structure of genitalia with specimens held in the CNC (Canadian National Collection of Insects, Arachnids and Nematodes, Ottawa, ON), and figures of *E. pusillata* in Skou (1986) and Mironov (2003). Related species in the *E. niphado-philata* Dyar, 1904 group (Bolte 1990) were ruled out by genitalic comparison to specimens in the CNC, as were other North American species.

Historical data associated with the specimens were compiled from specimen labels and Forest Insect and Disease Survey (FIDS) records (Van Sickle et al. 2001). The single specimen from PFC, collected by FIDS, is uniquely identified by a registration number (e.g. 76-9-0019-01) that links the specimen to a FIDS sampling form, completed at the time of sample collection, as well as a rearing record documenting the status of laboratory rearings. These records are held on file at PFC.

RESULTS

Specimens examined: 1♂ – *label data* (handwritten information in italics, individual lines separated by comma, multiple labels separated by ‘|’):

No. 76-9-0019-01, Date 19 vii, F.I.[D.] S.1976 | *c. juniper*, Port. Coquitlam BC Ac. No. PFC, 2007-0271.

The specimen was initially identified as *Eupithecia unicolor* (Hulst). The FIDS records document that this specimen was one of two adults reared from five larvae and five pupae (10 individuals in total) collected by the B.C. Forest Service on Mt. Burke, Port Coquitlam (UTM 10 53 546 [49.3, -122.7], Elevation 900 ft), on 15 May

1976. The host recorded was common juniper (*Juniperus communis* L.); Remarks & Symptoms state “Attacking several ornamentals with moderate damage”. The date recorded on the specimen label is the date of adult eclosion. While the Rearing Record indicates a second adult eclosed on 8.vii.76 and was subsequently spread, the specimen could not be found in the PFC reference collection.

1♀ – *label data*:

BC, N. Vancouver, 5 AUG 1986, C.S. Guppy | ROYAL BRITISH, COLUMBIA MUSEUM, ENT991-12573 |.

This specimen was identified as

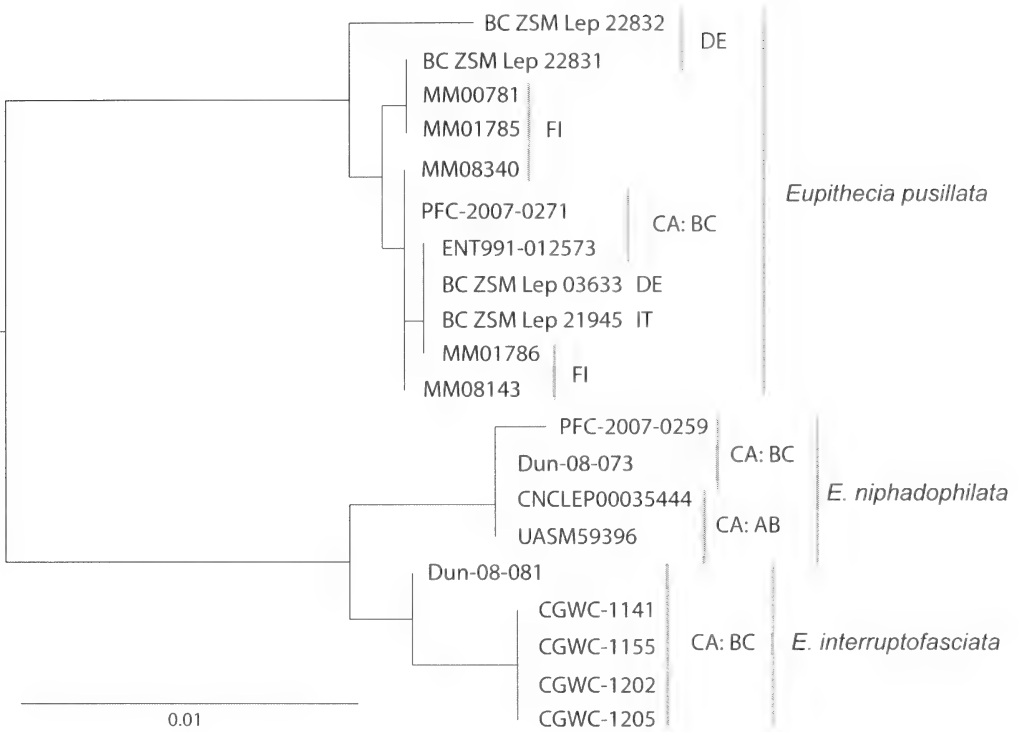


Figure 1. Neighbour-joining tree of *Eupithecia pusillata* and two closely related species, *E. niphadophilata* and *E. interruptofasciata*. Tree was reconstructed with the barcode fragment of the cytochrome oxidase I (COI) gene. Sequences shaded in grey are from two individuals collected in Vancouver, Canada. Abbreviations: DE – Germany, FI – Finland, IT – Italy, CA – Canada, BC – British Columbia, AB – Alberta.

Eupithecia sp. in the collection before tentative assignment to *Eupithecia intricata taylorata* Swett by JRD.

Diagnosis: *Eupithecia pusillata* is most similar to *E. niphadophilata* and particularly *E. interruptofasciata*, but a number of *Eupithecia* species are superficially very similar and identification should be based on examination of genitalia. Compared to *E. interruptofasciata*, which is structurally most similar, the male 8th sternite apical prongs are narrower, more blunt and the apical cleft is shallower; the base of the sternite is also narrower overall with a shallower medial invagination. The basal half of the male vesica is armed with one spine, not two as in *E. interruptofasciata*. In the female genitalia, the large spines on the left side of the ductus bursae do not extend beyond the mid-point of the ductus, but extend beyond the midpoint in both *E. interruptofasciata* and *E. niphadophilata*.

Description: A small moth with a wingspan of 16–22 mm (Mironov 2003) (Figs. 2a, 2e). Forewing narrow, mostly shades of light brown with black transverse lines and oblong discal spot. Hindwing pale grey-brown with weakly marked transverse lines and variable discal spot. Abdomen pale grayish brown with narrow black lateral stripes. Male genitalia (Fig. 2d) composed of broad valva with small ventral process, heavily sclerotized sacculus, vesica with three horn-like cornuti, simple aedeagus (Fig. 2c) and elongated 8th sternite with two narrow apical processes (Fig. 2b). Female genitalia composed of elongate and sclerotized bursa copulatrix (Fig. 2h) with small spines at base and larger spines at margin. Ovipositor is simple with long setae (Fig. 2f). Terminal segment of pupal case is stout with prominent lateral lobes and cremaster bearing four pairs of hook-like setae (Figs. 2h, 2i).

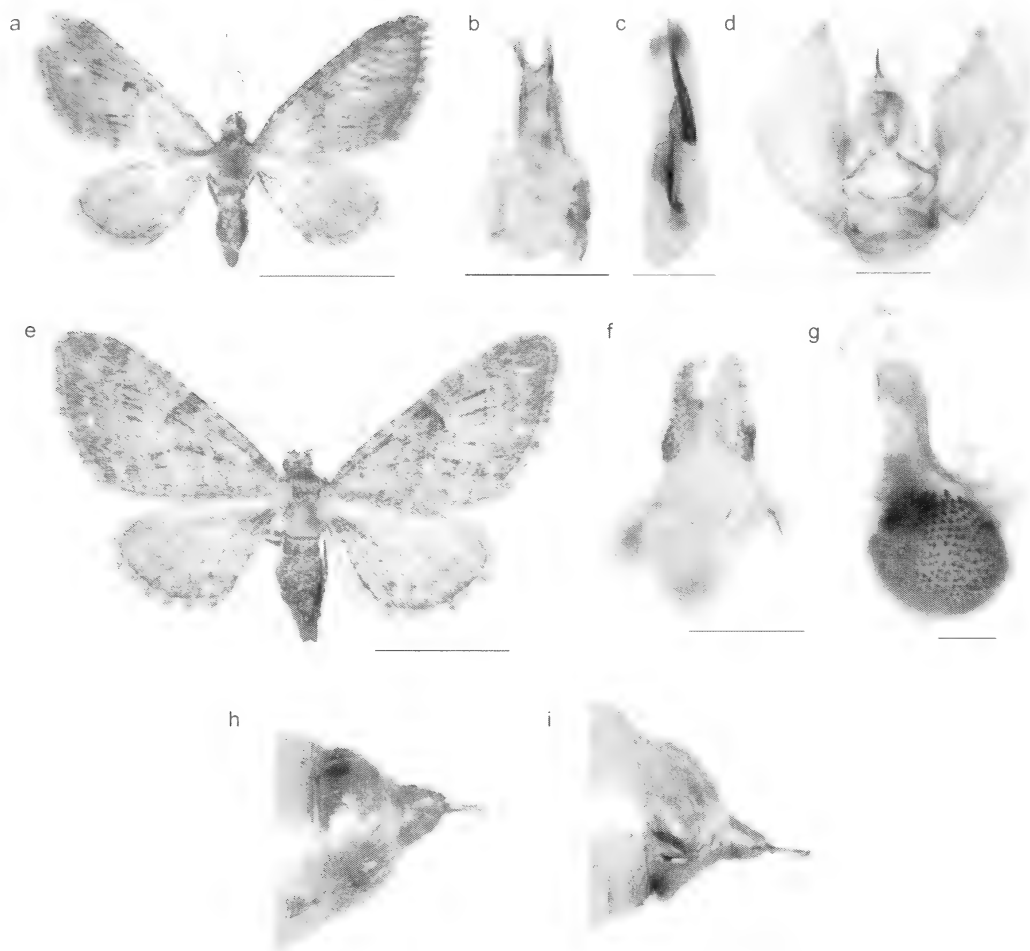


Figure 2. Morphology of *Eupithecia pusillata*. a) male, dorsal view, b) male, 8th sternite, c) male, aedeagus, d) male, genital capsule e) female, dorsal view, f) female, ovipositor, g) female, bursa copulatrix, h) pupa, terminal segment, dorsal view, i) pupa, terminal segment, lateral view. Scale bars: a, e = 5 mm; b–d, f–i = 0.5 mm. A colour version of this figure is available from Dr. Lee Humble.

Distribution and Habitat: In its native European range, the nominate subspecies is widely distributed from southern Europe, its range extends to the Mediterranean from eastern Spain to mainland Greece and Romania, then extends north and west across northern Ukraine into western Russia. With the exception of Corsica, it has not been recorded from the islands of the Mediterranean. To the north it is present in the British Isles, through central Europe, north to northern Scandinavia, and into western Russia across the southern Kola Peninsula (Skou 1986; Mironov 2003; Karsholt & van Nieukerken 2010). A disjunct population of

E. pusillata is present in the Caucasus Mountains (Mironov 2003). In Asia, its range extends across Russia from Sakhalin through Siberia, the Altai and Caucasus regions (Skou 1986). The subspecies *E. pusillata scoriata* Staudinger, 1857 has been recorded only from Iceland and south-western Greenland (Mironov 2003). Mironov et al. (2008) recently described a third subspecies, *E. pusillata kashmirica* Mironov and Ratzel from the Himalayas. In natural settings, *E. pusillata* can be found in heaths, forest edges, rocky cliffs, and similar habitats where the primary host grows. In urban areas, it can be common in gar-

dens. It is known from sea level up to approximately 2,500 m elevation in the Sierra Nevada (Spain) and the Alps (Switzerland) (Weigt 1993; Mironov 2003).

Life History and Notes: The following data are based on European populations, and it is expected that flight times, voltinism and larval hosts will be similar in North America, should extant populations be discovered. Univoltine, with larval stage from late April to mid-June and adult flight period from mid-July to late September (Skou 1986; Mironov 2003). As its common name implies, the primary host of *E. pusillata* is common juniper, *Juniperus communis* L. (Cupressaceae) (Skou 1986), of which it feeds on young needles and flowers. It is

generally regarded as monophagous (Mironov 2003), although it has also been recorded feeding on Douglas-fir, *Pseudotsuga menziesii* (Mirb.) Franco (Pinaceae) in France (Roques et al. 2006), where this North American tree is cultivated. The host of the subspecies *scoriata* and *kashmirica* is not known, but is presumed to also be *Juniperus*. *Eupithecia pusillata* overwinters in the egg stage and pupates in a loose web in the ground (Skou 1986). It is attacked by a variety of ichneumonid and braconid species listed in Mironov (2003). It is not known if other native or ornamental species of *Juniperus* are suitable hosts in British Columbia.

DISCUSSION

Eupithecia Curtis is a large genus with 1529 described species and subspecies (Scoble 1999; Scoble & Hausmann 2007), and about 160 species in North America (Powell and Opler 2009). The North American species were revised by McDunnough (1949), and the Canadian fauna was revised by Bolte (1990). *Eupithecia pusillata* is part of the *niphadophilata* species group, which includes two Nearctic and one Palearctic species (Bolte 1990), all feeding primarily on junipers (Skou 1986; Bolte 1990).

Although we currently have only two specimens of *Eupithecia pusillata* from North America, we can extract a great deal of information from the associated data documentation. First of all, the collections were made in urbanized Vancouver, BC, suggesting the species was introduced. The lack of records, particularly from inland BC (which is well-surveyed for macro-Lepidoptera), the Yukon Territory and Alaska, lead us to conclude that the species is not naturally Holarctic like some *Eupithecia* (see Skou 1986, Bolte 1990). Furthermore, the six *Eupithecia* species considered Holarctic all show at least 1% COI sequence divergence (data not shown) indicative of separation in the Pleistocene. The absence of additional records also suggests that there has not been substantial

spread beyond the point of introduction. Secondly, the locality of the first collection (Mt. Burke), the number of individuals recorded (ten), and the damage observations in the FIDS record, all indicate that there was an established *E. pusillata* population in BC in 1976 (but note this is the only FIDS record of a *Eupithecia* on juniper from greater Vancouver). And lastly, the 1986 collection from North Vancouver suggests that the population has persisted, or it did so for at least a decade. Subsequent surveys, initially in the Vancouver area, are required to determine the contemporary status of this species.

The excellent documentation of FIDS that enabled inferences about the status of *E. pusillata* is unfortunately a relict of the past; the program ceased in 1996 after almost 50 years of operation due to budgetary cut-backs (Van Sickle et al. 2001). Programs such as this, based on surveying or inventorying diversity, are simultaneously a) a tremendous resource for managers, foresters and scientists, and b) reliant on tremendous resources themselves particularly in terms of highly qualified personnel (e.g. Marshall et al. 1994). The present case illustrates the value of these long-term, well-documented biological surveys, but these programs are often hindered by the

necessity to rear immatures to allow the diagnosis of species. Just as DNA barcoding makes an invaluable tool for biosurveillance (Floyd et al. 2010), it could likewise assist any regional or national biomonitoring program of similar scope to FIDS. Barcoding could not only identify immature stages (Ahrens et al. 2007) making rearing nonobligatory, it could also identify the plant meal of gut contents (Miller et al. 2007), identify parasitoids (Rougerie et al. in press), and trace complex food webs (Sheppard et al. 2004; Smith et al. in press). Decreasing costs and increasing capabilities of sequencing (e.g. Shokralla et al. 2010)

are certain to make species diagnosis in this form time- and cost-effective. Furthermore, most years of the FIDS program predated electronic databases, so it would also be better served by modern and online relational databases such as BOLD (Ratnasingham and Hebert 2007). With DNA barcoding in place, a resource similar to FIDS could once again be realized, and without having to expend substantial resources as a cost. It would also, without question, speed the time of non-indigenous species detection—from years (34 in the case of *E. pusillata*) to days.

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The Fireflies (Coleoptera: Lampyridae) of British Columbia, with special emphasis on the light-flashing species and their distribution, status and biology

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ABSTRACT

In British Columbia the family Lampyridae (fireflies) is poorly known. Ten species in five genera are recorded but adults of only two species produce flashed bioluminescent signals. Before 1997, museum collections that we examined held specimens of only one flashing species from three BC localities, despite recent and widespread reports of flashing fireflies in the province. A solicitation of specimens and sight records from entomologists and naturalists resulted in the discovery of 14 additional collection localities for the two species. Sight records are summarized but are not recorded in detail. As far as is known, *Photuris pennsylvanica* (DeGeer) (*sensu lato*), is restricted in BC to the southern Rocky Mountain Trench (East Kootenay region). *Photinus obscurellus* LeConte, herein recorded in BC for the first time, is widespread in the northeast, central and southern Interior of the province. This paper briefly summarizes the BC lampyrid fauna, examining the status, distribution and biology of the two flashing species in more detail.

Key Words: Lampyridae, fireflies, flashing species, *Photuris*, *Photinus*, British Columbia, distribution, identification

INTRODUCTION

Beetles of the family Lampyridae (fireflies) are poorly known in British Columbia (BC). McNamara (1991) recorded nine species in four genera, but adults of only one of these species produce flashing signals. This paper briefly summarizes the BC lampyrid fauna but examines, in more detail, the status of the two flashing species, one in each of the genera *Photuris* LeConte and *Photinus* Laporte, now known to occur in BC. One of these species is recorded in the province for the first time.

Adults of nocturnal bioluminescent species use their flashed signals in courtship. Characteristics of these signals, such as flash number, flash duration and the inter-

val between flashes, are important in species recognition but, in some cases, such as in *Photuris*, are still not completely reliable for identification (Lloyd and Branham in press, Branham and Greenfield 1996, J.E. Lloyd pers. comm.). These beetles are chemically defended and, when handled or attacked, often exude defensive compounds from the body, especially the elytra (Eisner *et al.* 1978). As larvae, many lampyrid species are soil dwelling predators that eat insects, snails, worms and other invertebrates, while others are arboreal or aquatic and feed on snails (LaBella and Lloyd 1991). *Photinus* larvae are subterranean, seldom observed, and may specialize on eating

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³ Died 13 May 2008. We dedicate this paper to the memory of Bob McVickar, whose enthusiasm for the project got it off the ground.

earthworms; *Photuris* larvae are surface dwelling, omnivorous predators and scavengers of dead insects and fallen berries (Buschman 1984, Lloyd 2002). All lamyrid larvae have a luminous organ on abdominal segment 8 (Lloyd 2002); light production warns predators that these larvae are distasteful (Sivinski 1981, De Cock and Matthysen 2003).

Adults of the best known BC fireflies are diurnal, non-flashing species. These taxa use pheromonal communication for pair formation (Lloyd 2002, Branham and Wenzel 2003). *Ellychnia* Blanchard is the most diverse genus in BC with five species: *E. corrusca* (Linnaeus) (widespread), *E. facula* LeConte (Okanagan, central coast), *E. greeni* Fender (southern Interior), *E. hatchi* Fender (widespread on coast), and *E. lacustris* LeConte (Terrace). All but *E. corrusca*, which is transcontinental, are restricted in Canada to BC (McNamara 1991). *Phausis nigra* Hopping (southern Interior) is unknown outside BC but *Phausis rhombica* Fender (southern Interior) is also known from Alberta (McNamara 1991), Washington and Oregon (Fender 1961) and Montana (M.A. Branham pers. obs.) -- both are Cordilleran species. *Pyrogyga nigricans* (Say) (southern Interior) ranges from BC east to the Atlantic Ocean (McNamara 1991).

Before this study, collections of flashing

lamyrid adults in BC were rare. The only literature records for BC are a reference to *Photuris pennsylvanica* (DeGeer) in south-eastern BC (Fender 1961) and a subsequent inclusion of the species in the BC fauna by McNamara (1991). These references evidently refer to collections in 1928 and 1958 (in RBCM and UBC collections, respectively [see Specimens Examined]). Sightings of flashing fireflies, beginning in 1996 in the central and south-central Interior of the province, where no such species had been collected before, stimulated two of us, Cannings and McVickar, to solicit specimens and sight records of flashing beetles from the BC naturalist and entomological communities by word of mouth and through various newsletters (Cannings 1999).

In 2010, an additional extensive compilation of observations made by naturalists, ranchers and others in the East Kootenay region was organized by the Columbia Wetlands Stewardship Partners (Jamieson 2010). This study, motivated by plans to promote fireflies as iconic wetland inhabitants and as a focus for conservation and wetland education, improved our knowledge of the distribution of *Photuris* in the area. No specimens were collected. The survey covered the East Kootenay Trench from the US border to Donald, (north of Golden), a distance of about 345 kilometres.

RESULTS AND DISCUSSION

Specimens examined

Specimens received by the RBCM were identified by Marc Branham and James Lloyd and are listed below along with those examined from museum collections (see also Fig. 1).

Museum collection abbreviations: CNC – Canadian National Collection of Insects, Arachnids and Nematodes, Ottawa, ON; PFC -- Pacific Forestry Centre, Victoria, BC; RBCM -- Royal British Columbia Museum, Victoria, BC; UBC -- Spencer Entomological Collection, Beaty Biodiversity Museum, University of BC, Vancouver, BC. There are no BC specimens of *Photuris*

or *Photinus* in the E.H. Strickland Entomological Museum, University of Alberta, Edmonton AB, the Oregon State Arthropod collection, Oregon State University, Corvallis, OR or the California Academy of Sciences, San Francisco, CA, the museum that contains much of the Ralph Hopping and Hugh Leech beetle collections (including considerable BC material) and thus the US collection most likely to house significant numbers of BC beetles.

Photuris pennsylvanica (DeGeer 1774)

CANADA, BC, Fort Steele, 16.vi.1958, R.J. Andrews (8♂, UBC); Fort Steele, 25.vi.1959, Forest Insect Survey (41♂



Figure 1. Distribution of *Photuris pennsylvanica* (■) and *Photinus obscurellus* (●) in British Columbia as represented by specimens.

caught in flight, UBC; 4♂ caught in flight, RBCM); Fort Steele, CP railway tracks, south yard switch, 30.vi.1998, Greg Ross (3♂, RBCM); Haha Creek, between Haha Creek Rd. and Haha Creek, 11.vii.1998, Greg Ross (2♂, RBCM); Ta Ta Lake, 18.vi.1958, E.K. White (3♂, UBC); Windermere, 27.vi.1928, W.B. Anderson (1♂, RBCM).

Photinus obscurellus LeConte 1852

CANADA, BC, Bednesti Lake (53°51'11"N x 123°22'07"W), 30.vi.2007. R.V. Rea (1♂, RBCM); Bonaparte Lake, 17 km W at Moose Lake (51°18'14"N x 120°55'44"W), 15.vii.1997, Joe Cortese (1♀, PFC, with photos, Fig. 2). Fort St.

John, 20 km SW (56° 06'37"N x 121°04'59"W), 4.vii.2009, Mark Phinney (6♂, 1♀, RBCM); Fort St. John, 20 km SW (56° 06'47"N x 121°05'19"W), 4.vii.2009, Mark Phinney (3♂, RBCM); Horsefly, swamp 6 km S of Bells Lake, 3.vii.2002, Marcus Charles (1♂, RBCM); 100 Mile House, Horse Lake, Fawn Creek Rd., 10.vii.2002, Pat Griffin (4♂, RBCM); Prince George, Ness Lake, 9690 Anne Rd., 10.vii.1999, Marie Pearson (1♂, RBCM); Quesnel, Beryl Rd. (52° 57'22"N x 122°26'34"W), 10.viii.2010, Clint Tibideau (1♂, RBCM); Quesnel, Cottonwood House (53°05'20"N x 122°12'45"W), 13.vi.2005, John Massier (1♀, RBCM); Sheridan Lake,

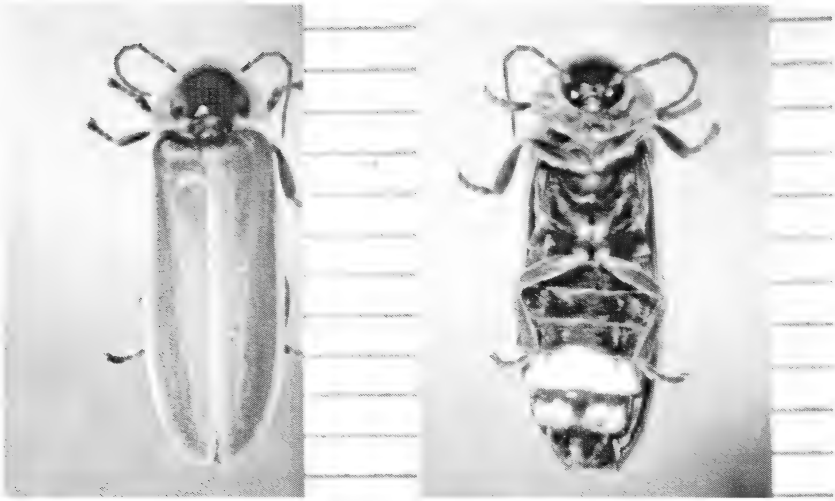


Figure 2. *Photinus obscurellus* (♀): left, dorsal view; right, ventral view. Scale lines are 1 mm apart. Specimen photographed at Pacific Forestry Centre by Bob Duncan. Collected by Joe Cortese at Moose Lake, 17 km west of Bonaparte Lake (51°18'14"N x 120°55'44"W) on 15 July 1997 (Males look similar but have light-producing organs covering the 5th and 6th visible ventral segments (true abdominal segments VI and VII).

vii.2003, Frank McFadden (4♂, RBCM); Shuswap Lake, Eagle Bay, Herman Lake, N end (50°55'08"N x 119°09'45"W), 24.vi.1997, Dawn Kellie (3♂, RBCM); same locality and date, Dennis St. John (4♂, RBCM); same locality and date, R.H. McVickar (2♂, RBCM; 1♂, CNC); same locality, 10.vii.1996, R.H. McVickar (2♂, RBCM); Skmana Lake, 7.vii.1998, R.H. McVickar (1♂, RBCM).

Sight records

In addition to the collections and associated observations listed above, numerous sightings of flashing fireflies were reported by 40 respondents across the province. Sixteen people participated in the 2010 survey in the East Kootenays; observations included direct sightings as well as hearsay, past and present. Some recent sightings included exact coordinates and dates or even photographs; others were less precise. Unless an observer is experienced, sightings of flashing fireflies are difficult to assign to species, and most of the sight records gathered were not verified by voucher specimens. For these reasons and because, for the most part, collected specimens fall within the known distributions of the two flashing species in BC (Fig. 1), sight records are not listed in detail. They do, how-

ever, support the notion that these beetles are not rare and that their populations, especially those of *Photinus*, are widespread. We have reports from two areas outside the generally known ranges of *Photuris* and *Photinus* in BC. There are three from the West Kootenay region (two in the Nelson area, one near Trout Lake) (Jakob Dulisse, pers. comm.). Nelson is not far from the Cranbrook populations of *Photuris*, but the two areas are separated by extensive mountainous terrain. Trout Lake is closer to the Shuswap region where *Photinus* is found. Three other unsubstantiated reports come from Vancouver Island (Campbell River, Parksville, Sidney) (R.A. Cannings, unpubl. data) but there are no others from the BC coast and no specimens have been seen or reported from west of the Coast Mountains.

Photuris pennsylvanica

Before the 2010 survey, sightings were reported from nine localities in the East Kootenay region (some the same as specimen localities), all presumably for *Photuris*. Dates range from 1966 to 2008 and localities range from Canal Flats south to Haha Creek and the Bull River Fish Hatchery near Wardner. Photographs of specimens (no vouchers collected) accompany the record documented by Barb Houston at

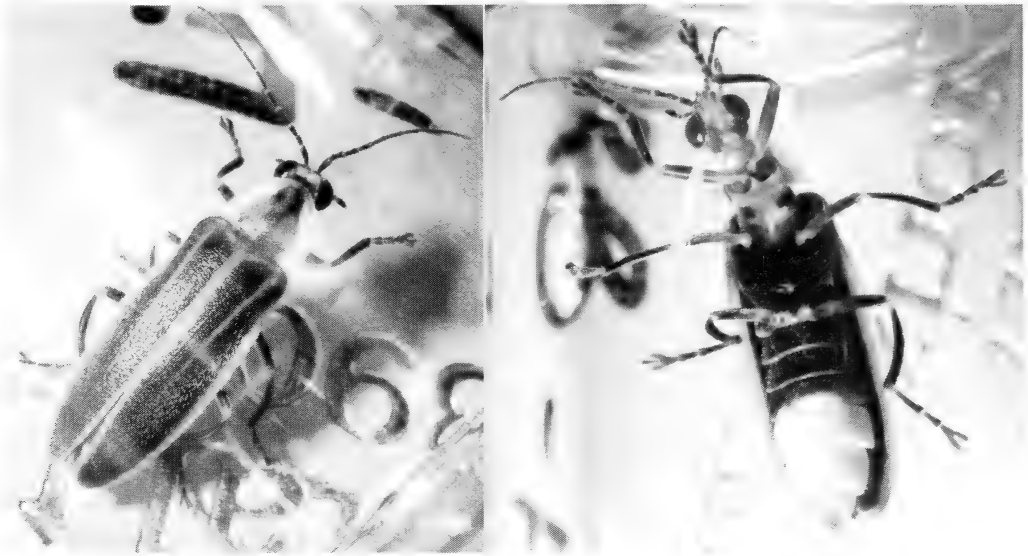


Figure 3. *Photuris pennsylvanica* (♂): left, dorsal view; right, ventral view. Live male specimen photographed in vial by Barb Houston at Bummers Flats, north of Cranbrook (49°39'20"N x 115°40'29"W) on 19 June 2008.

Bummers Flats (49°39'20"N x 115°40'29"W) on 19.vi.2008 (Fig. 3). In the 2010 information survey, 16 additional localities were reported, ranging from Brisco in the north to Newgate near the US border in the south. Most of these observations were actually made in earlier years but seven were of fireflies seen in 2010. However, only three of these records represent sites not recorded before 2010 (Jamieson 2010).

Photinus obscurellus

About 20 of the sight records reported came from within the known range of *Photinus obscurellus*; they are assumed to be of this species. Even as long ago as the 1920s, anecdotal records from localities as far apart as Enderby, Prince George and the Kiskatinaw River in the Peace River region embraced the present known latitudinal range of the species. Other general areas represented are Prince George north to McLeod Lake, Quesnel and the Shuswap region. The most westerly sightings range from Vanderhoof in the north, to Nazko Lakes Provincial Park (Pynn 1996) on the Chilcotin Plateau, to the Carpenter Lake area in the south.

The identification of *Photuris* and *Photinus* in BC

Adults of the two known species of flashing fireflies in BC can be distinguished from the other lampyrids in the province by the presence of pale, light-producing organs on the underside of the abdomen (Figs. 2, 3). They completely cover the 5th and 6th visible ventral segments in males but form a more restricted band in females.

These species can be separated from each other by the structure of the fore and mid tarsal claws -- in *Photuris* these claws are bifid (one of the two claws is "split") while those of *Photinus* are simple (Lloyd 2002). In *Photuris*, the legs are long and slender (Fig. 3); in *Photinus* they are shorter and more flattened (Fig. 2).

With few exceptions, North American *Photuris* cannot be accurately identified to species using either morphological or flash pattern characters. Although the morphology and scarce behavioural observations that are available for *Photuris* specimens collected in BC are consistent with characteristics of *Photuris pennsylvanica* (J.E. Lloyd, pers. comm.), this identification should not be considered more than a convenient and useful referent and working designation. Nevertheless, we are using the name here because it is already in use for the BC records (Fender 1961, McNamara

1991) of *Photuris*.

An understanding of the complex taxonomic history of *Photinus obscurellus* is useful for those attempting to identify Nearctic *Photinus* specimens. LeConte (1852) described the species but in his synopsis (LeConte 1881) he considered it a synonym of *Photinus ardens* LeConte. Lloyd (1966), describing the behaviour of *Photinus ardens* (p. 47-49), is actually referring to *Photinus obscurellus*; at the time he was following LeConte (1881) and Green (1956). But his field studies later revealed that *Photinus obscurellus* deserved formal recognition, and in 1969 he described the flashing behavior of the true *Photinus ardens* and restored *Photinus obscurellus* to species status (Lloyd 1969). Because *Photinus obscurellus* was treated as a synonym of *Photinus ardens* in Green's (1956) widely used identification key, specimens of *Photinus obscurellus* in museum collections are almost always misidentified as *Photinus ardens*.

Behaviour and habitat

Photuris pennsylvanica

We have little information on details of the behaviour of *Photuris pennsylvanica* in southeastern BC. Six collection records in the East Kootenay region range from 16 June to 11 July; sight records extend this period only one day earlier. At Haha Creek on 11 July 1998 Greg Ross collected two specimens and noted that individuals irregularly produced a 1-second flash every 8 or 9 seconds.

Jamieson (2010) notes that in the East Kootenays, the species inhabits pothole wetlands on the benches of the main valley (Hahas, Ta Ta, Butts and Cub Lakes) as well as the wetlands along the major rivers -- the Kootenay (Fort Steele, Bammers Flats, and so on) and the Columbia (Canal Flats, Luxor Creek, Brisco areas). These habitats are typically associated with springs or small creeks that flow year-round. The springs sometimes emerge in the bottom of wetlands or ponds.

Photinus obscurellus

In BC, 14 collection records range from 13 June to 10 August; the latter date is a

month later than any others. At Herman Lake near Shuswap Lake, McVickar recorded flashing in May 1996 and it continued for six weeks. In 1997 the onset was later but continued into July and was not finished by 8 July. The site is a cattail (*Typha latifolia* Linnaeus) marsh bordering a small lake about 250 m long; the fireflies mostly flash in and above the cattails but are active up to 250 m inland from the marsh. The first flashes appear when the last light is fading but the full performance commences during complete darkness, about midnight in June. Before the onset of the full flashing display there appears to be a warm-up period, which begins before the onset of full darkness. Females emit as many as 7 or 8 flashes in quick succession. Males settle into a pattern of two or three flashes produced while flying in a straight line followed by another flash given on a curved flight path. The most common pattern is: flash, flash, curving flash. Females settle into a pattern of single flashes given at considerable intervals.

On warm nights between 1 and 21 July 2002 near Horse Lake in the Cariboo, about 15 insects at a time flew and flashed. Specimens were collected on 10 July about 22:15 PDT, just after dark (Pat Griffin, pers. comm.). Marie Pearson (pers. comm.) observed at least 100 fireflies (and collected one) flashing at 23:30 PDT near Ness Lake northwest of Prince George on 10 July 1999. The habitat consisted of a small, spring-fed marsh, flooded in spring but only moist in summer; typical ground cover is moss, willows (*Salix* spp.), Buckbean (*Menyanthes trifoliata* Linnaeus) and Bog laurel (*Kalmia microphylla* (Hook.) Heller). In 1997, *Photinus obscurellus* individuals were active between mid-July and 5 August; the Pearsons have seen them there since 1983.

In the Peace River region near Fort St. John, Mark Phinney collected specimens in sedge meadow wetlands within a forested landscape from 00:30 to 01:15 MDT on 4 July 2009. At one site about 25 were flashing, not flying, but perched on the tops of willows and sedges. The signals usually

consisted of two long flashes, each about 2 seconds in duration, separated by about 1 second. The time between these sessions was variable and seemed affected by the flashes of neighbours.

Distribution and status

Photuris pennsylvanica

Species of *Photuris* range from Canada to Argentina, with 22 known species in North America and 28 new species descriptions in preparation (J.E. Lloyd, pers. comm.). They range mostly in the eastern United States, west to Colorado and southwest Texas (Lloyd 2002).

As mentioned above, no detailed statement can be made about the geographical distribution of *Photuris pennsylvanica*, as the majority of "determined" specimens in collections are questionably identified. The six BC collection records from the Kootenay and Columbia valleys range from Windermere in the north to Haha Creek in the south (Fig. 1). Sight records extend this almost linear distribution in the Rocky Mountain Trench from Brisco in the north to Newgate in the south. In general, according to the anecdotal information gathered from residents in the region, populations probably have declined in the past several decades. Specimen and sight records come from 25 localities between 2001 and 2010, while at 10 additional sites, beetles have not been seen since they were reported between 1950 and 2000 (Jamieson 2010).

Because the genus needs revision based on behavioural, morphological and molecular data and because the flash patterns of the BC species have not been studied, the specific identity of the BC population remains provisional at this time.

Photinus obscurellus

Species of *Photinus* range from Canada to Argentina, with 34 described species in North America and an additional 13 known but undescribed (J.E. Lloyd, pers. comm.). The genus is widely distributed on the continent but there are only scattered populations west of Texas and Kansas (Lloyd 2002).

Photinus obscurellus ranges from Newfoundland, Nova Scotia, New Brunswick

and Maine through southern Quebec and Ontario west to North and South Dakota, Manitoba and Saskatchewan (Lloyd 1969, unpublished CNC data) with an outlying population in BC. We are unaware of any collection records from Alberta, Washington, Idaho or Montana. In BC the species appears restricted to the Peace River region and the central Interior from about Mackenzie south to the Shuswap Lake area (Fig. 1). A sight record of this species extends the range west to the central Chilcotin.

The dates of all BC collections of *Photinus* range from 1996 to 2010, the result of the present study aided by the BC naturalist community. Why no specimens were collected before this time is a mystery because many entomologists have collected insects in central BC over the last century. Cannings has never seen a flashing firefly in BC despite having a strong interest in insects in the province for almost 50 years; until the present project was begun, he had never heard of any reports from the central Interior. Why did Ralph Hopping, Hugh Leech or James Grant, all avid, professional coleopterists working at various times between about 1920 and 1980 within the southern part of the present range of *Photinus obscurellus*, apparently never collect any? On the other hand, as reported above, the population of *Photuris* in the East Kootenays was known from collections as far back as 1928.

A lack of collections might be attributed in part to a lack of communication between residents and entomologists interested in these beetles. When directly asked in this study, naturalists and ranchers responded with numerous memories of flashing fireflies as far back as the 1920s in the Shuswap, Prince George and Peace River regions, but professional entomologists may not have heard such accounts.

Although a reason for the significant separation of *Photinus* records between northern Saskatchewan and northeastern BC (approximately 800 km) might be lack of collecting in this geographical gap, this hiatus and the historical lack of specimens

from BC have suggested to some that *Photinus obscurellus* might be recently introduced to BC from the East. However, the widespread distribution of the species in BC and the newly reported sight records in the province from many areas over many decades indicate that this is unlikely. It is possible that the beetle arrived in BC via the railways and was somehow helped in its spread by various railway lines. Even today, most of the sightings and collection records occur within 30 km of a railway. Similarly, in the East Kootenay region of southeastern BC, *Photuris pennsylvanica* apparently inhabits wetlands of the Rocky Mountain Trench, about 220 km from north to south, with all records close to railway tracks. Members of this genus exhibit a very patchy distribution in western North

America; this or other species of *Photuris* also occur in Montana, just southeast of the BC population (M. Ivie, pers. comm.). The apparent association between these firefly distributions and railway lines is interesting and raises several points for consideration. Wetland habitats are commonly situated in valleys where many railroads are located. In addition, the presence of a railroad berm and the additional weight it applies to the surrounding soil might be in part responsible for the creation of new wetland habitat (M. Ivie, pers. comm.).

We hope this paper stimulates future systematic, ecological and behavioural research on these firefly populations and that more targeted studies are able to evaluate hypotheses concerning these interesting distribution patterns.

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Dominant bacteria associated with broods of mountain pine beetle, *Dendroctonus ponderosae* (Coleoptera: Curculionidae, Scolytinae)

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ABSTRACT

Mountain pine beetle (MPB) is the most damaging insect of mature pine forests in western North America. The current outbreak in British Columbia is the largest ever recorded. During a survey of beetle occurrence, well-established infestations were sampled in central B.C. and found to possess larval mortality. Bacteria or other microbes were among the potential causes of the mortality. Bacteria were isolated from living larvae and adults, as well as larval and adult beetle cadavers found in bark samples. Bacteria were identified by fatty acid methyl ester (FAME) analysis, which indicated 32 species of bacteria present in the MPB larvae. The predominant bacteria (*Serratia liquefaciens*, *S. plymuthica*) were detected in about a third of all sampled larvae, regardless of mortality. *Rahnella aquatilis* was found in 11% of all larvae examined and was usually (93%) associated with larval mortality. Interactions between two bluestaining fungal symbionts of the MPB (*Grossmannia clavigera*, *Ophiostoma . montium*) and two of the isolated bacteria (*S. liquefaciens* and *R. aquatilis*) were assessed. *S. liquefaciens* and *R. aquatilis* both inhibited the growth of beetle-associated bluestain fungi by 72%. The bluestain fungi did not impede bacterial growth, and both bacteria grew on autoclaved bluestain mycelium. Combinations of the two bacterial species formed aggregates on practical-grade (crab) chitin, but there was no aggregation in pure cultures or on the autoclaved mycelium of *G. clavigera* or *O. montium*. These results indicated that the two bacteria may be capable of aggregation within the insects, and this may have implications for their combined effects in the beetle. The role of *S. liquefaciens* and *R. aquatilis* in MPB biology requires further investigation.

Key Words: bark beetle, disease, larva, pathosystem

INTRODUCTION

As associates, antagonists, and pathogens, microbes play important roles in the life cycle of bark beetles (Barras and Perry, 1975), including the Mountain Pine Beetle (MPB), *Dendroctonus ponderosae* Hopkins. MPB has been responsible for billions of board feet of timber losses (Anonymous 2005, Bellows *et al.* 1998); a current outbreak has spread over a vast area of British Columbia (BC), killing at its peak ca. 1.41×10^8 m³ of merchantable pine; during 2008 the outbreak was still killing 3.6-4.3

$\times 10^6$ m³ of merchantable pine (Walton, 2009). The potential for microbial populations to constrain the beetle is therefore of potential interest. In most of BC, MPB has a 1-year life cycle, where incipient or epidemic populations attack trees *en masse*. Young female adults emerge from host trees during late summer, and initiate the attack on new host trees by burrowing into the phloem and tunnelling vertically to form egg galleries. Hatched larvae overwinter and feed in the phloem. Pathogenic

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bluestain fungi associated with the beetles spread from the galleries; eventually, trees succumb to this combined attack. (Safranyik and Carroll, 2006) During the spring of 2003, a routine survey of larval and adult MPB in central BC detected the presence of large numbers of dead larvae. The larvae were in lodgepole pine (*Pinus contorta* Dougl. ex Loud.) trees containing well-established broods. Although the patchy occurrence of very cold temperatures in the region was suspected as one possible cause for the mortality, the atypically darkened and distended hindguts of the larvae suggested that microbial factors should not be ruled-out.

Bluestain fungi are microbes that have a close relationship with the MPB life cycle; they also figure prominently in interactions with other beetle-associated microbes. MPB possesses a specialized mycangium that carries the inoculum of bluestain fungi from tree to tree (Whitney and Farris 1970). The main fungi associated with MPB are *Grossmannia clavigera* (Robinson & Davidson) Zipfel, deBeer & Wingf. and *Ophiostoma montium* (Rumbold) von Arx, and their relative abundances in populations varies (e.g., Kim et al. 2005, Six and Bentz 2007, Bleiker and Six 2007, 2009). As larval broods develop, the fungi colonize the phloem and sapwood (Whitney 1971, Bleiker and Six 2009). Mining larvae may ingest phloem colonized by hyphae and teneral adults consume spores lining pupal

chambers if present prior to emergence (Whitney 1971, Bleiker and Six 2007, 2009). These fungi are reported to have mutualistic and antagonistic relationships with bacteria and yeasts found in the beetle galleries (Adams *et al.* 2008).

Natural enemies of *Dendroctonus* spp. are diverse (Bellows *et al.* 1998, Bushing 1965, Dahlsten 1982, Moore 1971, 1972a, 1972b). They play a role in holding MPB populations in check during their endemic phase (Moeck and Safranyik 1984, Hofstetter *et al.* 2006). Some natural enemies of MPB have been studied as potential biological control agents, for example the insect pathogen *Beauveria bassiana* (Balsamo) Vuillemin (Hunt *et al.* 1984). Various options have been suggested for augmenting natural biological control effects, including the inundative release of microbes that may be antagonistic to the bluestain fungi (Safranyik *et al.* 2002). The objective of this research was to determine the dominant bacteria present in living and dead larvae and adults of MPB and to characterize interactions between the two principal bluestain fungi associated with MPB and the dominant bacteria associated with larvae. Our hypothesis was that different bacteria would predominate in living vs. dead insects, and that bacteria predominating in dead insects would be inhibitory to microbial associates of the living beetles, i.e. bluestain fungi and predominant bacteria.

MATERIALS AND METHODS

Isolation of Bacteria and Fungi from Beetles. During the first week of March, 2003, Rectangular 20x30-cm slabs of sapwood with the bark and phloem intact were cut with a chainsaw from five randomly-selected beetle-attacked lodgepole pine trees at each of ten sites (Table 1, Fig. 1). The slabs were taken at points 1.3 m from the ground at a randomly selected aspect (north, south, east or west). The geographic position of each site was recorded with a GPS unit, and site moisture (humid, mesic, or xeric) and the percentage of currently

attacked trees in the surrounding stand were visually estimated. The slabs were stored at 20°C on a laboratory bench for several days, thus ensuring activity of living larvae. In the laboratory, the bark was peeled from the sapwood with a knife and MPB adults (parental) or larvae were excised with forceps and surface-sterilized according to the method of Winder and Watson (1994). Insect mortality was assessed in each slab as the percentage of dead larvae and adults versus the total number of insects present. Larvae were considered to be dead when

Table 1.

Descriptions of sites in British Columbia where mountain pine beetle larvae were collected in March, 2003.

| Site (no.) | Location | Elevation (m) | Moisture (class) | Infested trees (%) | Terrain (type) |
|------------|------------|---------------|------------------|--------------------|----------------|
| 1 | Agodak L. | 1132 | Hydric | 50 | Flat |
| 2 | Quesnel B. | 1189 | Hydric | 60 | Flat |
| 3 | 5100 Rd. 1 | 1007 | Hydric | 60 | Northern slope |
| 4 | 5100 Rd. 2 | 1040 | Mesic | 70 | Southern slope |
| 5 | Baker C 1 | 1009 | Mesic | 80 | Western slope |
| 6 | Baker C 2 | 976 | Mesic | 60 | Western slope |
| 7 | 6600 Rd. 1 | 979 | Mesic | 60 | Western slope |
| 8 | 6600 Rd. 2 | 854 | Mesic | 50 | Eastern slope |
| 9 | Redstone 1 | 900 | Xeric | 10 | Flat |
| 10 | Redstone 2 | 859 | Xeric | 50 | Flat |

they appeared to have abnormal, distended hindguts and they failed to move upon firm, repeated probing with a dissecting needle. Adults were considered to be dead when they were overcome with yeasts or fungal hyphae and non-reactive to probing. Zero to three live larvae, zero to three dead larvae, and one to two adults (living or dead) were selected from each slab, using the number available to a maximum of three. The larvae and beetles were placed on nutrient agar (NA) in Petri plates; NA was composed of 8 gL⁻¹ Difco Bacto® dehydrated nutrient broth and 20 gL⁻¹ agar (Sigma). The insects were tamped downward with enough force to partially embed them in the medium. The plates were sealed with paraffin film and incubated at 20±2°C for 7d to allow microbial colonies to form. A colony possessing predominant morphology (colour, size, and growth pattern) was selected from each insect sample and aseptically streaked on NA plates for selection of pure cultures. The plates were sealed with paraffin film and incubated at 20±2°C for 1-2 weeks prior to identification. One pure colony possessing predominant morphology was selected per insect, for subsequent culturing according to the same method.

Identification of Bacteria. Bacteria were identified by fatty acid methyl ester (FAME) analysis using the Sherlock[®] Mi-

crobial Identification System (MIDI Inc., Newark, DE, USA). Pure colonies were transferred by quadrant-streaking onto plates of BBL[®] Trypticase Soy Broth agar (TSBA) and incubated for 24h at 28±2°C. This was repeated once again prior to analysis. Bacterial biomass was harvested from the third quadrant of the final cultures, and fatty acids were saponified and methylated according to the protocols provided by MIDI Inc. for the Sherlock[®] system. The resultant fatty acid methyl esters were extracted into 1:1 (volume-to-volume) hexane: methyl tertiary butyl ether (MTBE) and analyzed with a gas chromatograph (5890A Series II with HP-Ultra 2 column, Hewlett-Packard Co., Mississauga, Ont., Canada) using the TSBA40 method for aerobic bacteria, also provided by MIDI Inc. for the Sherlock[®] system. Extracted *Stenotrophomonas maltophilia* (Hugh) Palleroni & Bradbury (ATCC 13637) and a hexane:MTBE reagent blank were used as positive and negative controls, respectively. Bacterial isolates were identified from comparisons made against reference strain data (Anonymous, 2002) in the MIDI TSBA40 database, version 4.10 (MIDI Inc., Newark, DE, USA). Using the protocol and standards published by Weyant et al. (1996), a similarity index (SI) ≥ 0.500 was considered an acceptable identification to the level



Figure 1. Sites sampled for mountain pine beetles, with numbered locations corresponding to Table 1.

of species. SI values ≥ 0.300 were considered an acceptable identification to the level of genus, while lower similarity index values were considered inconclusive. When comparisons resulted in multiple species or genera exceeding these identity thresholds, data corresponding to the highest SI value

were used for the identification. Dendrogram cluster analysis based on unweighted pair-matching of fatty acid profiles (Sherlock[®] analysis software, MIDI Inc., Newark, DE, USA) was used to explore relatedness among unidentified isolates.

Bacterial inhibition of Bluestain

Fungi. A nested experimental design was used to assess interactions between the three most dominant bacteria: *Serratia liquefaciens* (Grimes & Hennerty) Bascomb *et al.*, *Serratia plymuthica* (Lehmann and Neumann) Breed *et al.*, and *Rahnella aquatilis* IZARD *et al.*, and two bluestain fungi commonly associated with *D. ponderosae*, *G. clavigera* and *O. montium* (Solheim and Krokene 1998). Fungal isolates were obtained from C. Breuil (University of British Columbia, Vancouver, BC) as cultures growing in Petri Dishes containing malt extract agar. Small pieces of these cultures were aseptically transferred to Petri dishes containing 3:7 (v:v) malt extract agar:NA. This substrate was optimal for simultaneous fungal and bacterial growth in preliminary trials combining different ratios of the two agar media (data not shown). To account for the variable effects of cultural moisture on microbial growth, Petri dishes were treated as experimental units, and the difference in fungal growth in the presence or absence of bacteria was evaluated in each dish. Half of each dish was inoculated with a 'lawn' of either *S. liquefaciens*, *S. plymuthica*, or *R. aquatilis*. In each dish, a 5 mm-diam. agar plug colonized by either *G. clavigera* or *O. montium* was placed on the bacterial side, and another was placed on the bacteria-free side. In each plate, the bacterial inoculum was taken from one of five randomly selected isolates from each of the three species of bacteria collected from the beetles. Each isolate originated from a different beetle. Each combination of fungus and bacterial isolate was replicated five times. The plates were incubated for 7 d at 20°C. The radial growth of fungal colonies was measured after incubation and percentage of growth inhibition was calculated for each plate by dividing the maximal radius of the fungal colony on the bacterial side by the maximal radius of colony in the bacteria-free area, and multiplying by 100.

Interactions between bacteria and chitin-containing substrates. A second completely randomized experiment was used to assess the interactive effect of bac-

teria and chitin-containing substrates on bacterial growth and aggregation, because it is possible for bacterial consortia to affect insect health (Hentzer and Givskov 2003) or ice nucleation (Pierson *et al.* 1998) and therefore cold tolerance. A sterile transfer loop was used to aseptically transfer bacteria from cultures of *R. aquatilis* and *S. liquefaciens* to sterile water (100 mL) in 250 mL Erlenmeyer flasks, and the bacterial concentration in each case was diluted to 1×10^6 bacteria mL⁻¹ based on measurements with a Petroff-Hausser counting chamber (Hausser Scientific Partnership, Horsham PA) and a microscope (1000 X). Equal parts of the bacterial suspensions were combined to make a third combined bacterial suspension, resulting in a concentration of 0.5×10^6 bacteria mL⁻¹ for each bacterial species. Each of the three suspensions was aseptically transferred with a sterile transfer loop to an individual set of nine sterile aqueous cultures in 250 mL Erlenmeyer flasks containing 0.1 g (d.w.) of substrate. In each culture set, the substrate in three flasks consisted of autoclaved hyphae of *G. clavigera*; autoclaved hyphae of *O. montium* was included as the substrate in three more flasks, and the three remaining flasks contained chitin derived from crab shells (poly-N-acetyl-glucosamine, practical grade, catalogue number C-7170, Sigma Chem. Co., St. Louis, MO, U.S.A.). The hyphae of *G. clavigera* and *O. montium* used in this assay were collected from 250 mL liquid (malt extract broth) cultures, each of which was inoculated with a 5mm diam. agar plug from a stock culture and incubated on an orbital shaker (100 r.p.m., 20°C) for 7d. The hyphae were rinsed in distilled water, autoclaved, rinsed again, and dried prior to incorporation into the substrate assay. A set of nine controls containing only water was also inoculated. The flasks were secured at a random upright position on the platform of a rotary shaker. After 24 h incubation on the shaker (100 r.p.m.) at 20°C, bacterial populations were assessed as before with the Petroff-Hausser counting chamber and the microscope. The microscope (1000X) was also used to ob-

serve the degree of bacterial adhesion and aggregation on solids in the liquid cultures. The growth data were analyzed using analysis of variance; no statistical analysis of aggregation or adhesion was performed because there was no variance in the results.

Statistical analysis. Aside from FAME analysis, all statistical analyses were performed with Statistica 6.1 (Statsoft Inc., Tulsa, OK, U.S.A.). For data corresponding to larval sampling versus larval mortality, separate one-way analyses of variance (ANOVAs) were performed. Trunk aspect (degrees from North) was used as a categorical factor in one-way ANOVA, while site was nested as a random factor within moisture type for a two-factor ANOVA. In these and subsequent similar analyses, Levene's test was used to check for homogeneity of variance, and the Newman-Keuls multiple range test was used to compare means. Where Levene's test indicated significant ($P < 0.05$) heterogeneity of variance, ANOVA was performed after appropriate data transformation ($\arcsin\sqrt{Y}$). Where transformations were unable to remove significant ($P < 0.05$) heterogeneity of variance, non-parametric comparisons

were employed. Larval mortality was subjected to regression analysis, using percent mortality as the dependant variable and either elevation or percentage of green trees attacked as independent variables. Student's *t* test for dependent samples was also used to compare hypothetical (expected) mortality values with mortality associated with the five most dominant bacteria. The expected values, derived from observed overall mortality rates, were generated by apportioning 50% of expected larval observations as dead, and 97% of expected adult observations as dead. For the experiment assessing fungal growth vs. bacterial inoculations, data were subjected to an ANOVA for nested factors. Bacterial isolates were treated as a random categorical variable nested within bacterial species, with five isolates per bacterial species, each replicated five-fold. Bacterial species was treated as a categorical variable nested within fungal species (3 per fungal species). A series of chi-square tests were utilized to compare the effect of chitinous substrates on the growth of *S. liquefaciens* and *R. aquatilis*, wherein the expected values were the mean growth in controls or mean growth in each of the substrates.

RESULTS

Isolation of bacteria from beetles. Characteristics of the sample sites are provided in Table 1. The slabs provided a total of 67 dead MPB adults, 2 living adults, 110 living larvae, and 112 dead larvae. All adults were mature (parental), and there were no exit holes apparent in the bark. A typical living larva is shown in Figure 2. Of the forty-four slabs containing insects, 1 lacked larvae and seven lacked adults. Larval mortality in the slabs ranged from 0 to 100%. Larval mortality at hydric sites (54.3%) was not significantly different from mortality in mesic sites (60.4), but mortality in the both of these site types was significantly greater ($p = 0.018$) than mortality in the xeric sites (24.1%). There was also a significant ($p = .039$) effect of site location on mortality (Figure 3). There were

no significant differences in mortality attributable to trunk aspect ($p = 0.63$). There were no significant regression trends for mortality versus elevation ($p = 0.32$) or mortality versus the percentage of green trees attacked ($p = 0.686$).

Identification of bacteria. Bacterial colonies were produced in 55% of the beetles sampled. This resulted in 161 pure cultures, of which 130 provided sufficient growth on TSBA medium for FAME analysis. Twenty-seven species of bacteria were identified in the beetles based on matches to reference strains in the MIDI TSBA40 library (Table 2). Eleven isolates had SI values less than 0.300, and were categorized as unidentified. Dendrogram cluster analysis separated the unidentified isolates into 5 species-related unknown groups based on



Figure 2. A typical living mountain pine beetle larva included in the cultural isolations.

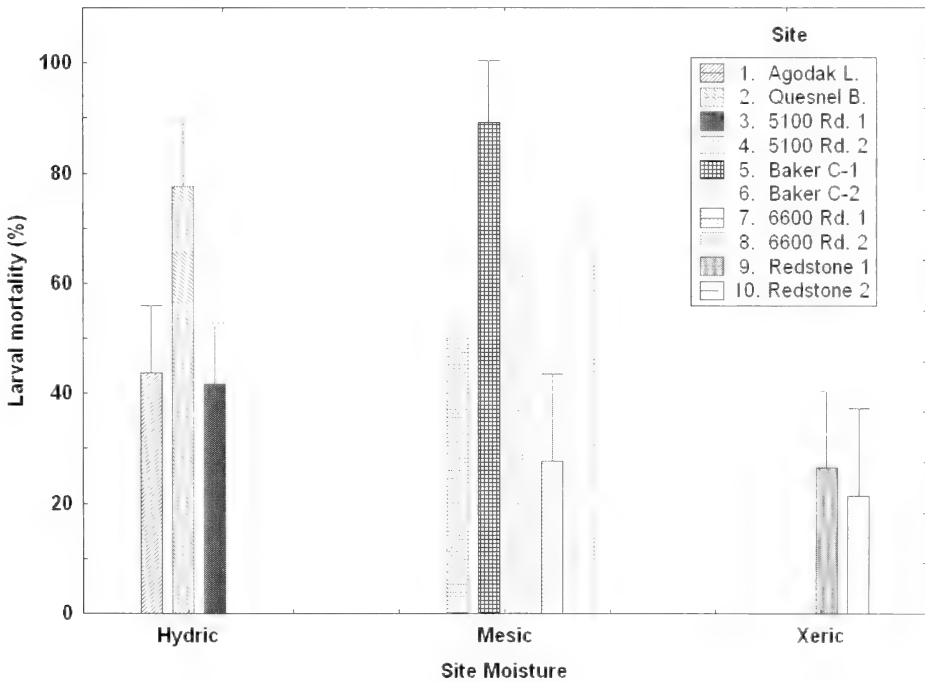


Figure 3. The effect of site location and moisture on the mortality of 232 mountain pine beetle larvae in the Southern Interior of B.C., March, 2003. Site numbers correspond to the sites listed in Table 1.

their FAME profiles. Five of six isolates in one of the unidentified species groups were found in dead larvae. *Serratia* spp. were frequently isolated from larvae (living and dead), possibly indicating a role in the insect's normal gut microflora. *R. aquatilis* was also frequently isolated (3 adults, 9 larvae). The incidence of *R. aquatilis* was widespread, occurring at eight of the ten

sites and in 11% of the insects, but it was mainly (93%) found in larval cadavers. Other bacteria occurring principally in dead insects were less frequently isolated, although mortality associated with *Pseudomonas syringae* var. *tabaci* (Wolf & Foster) Young et al. was significant ($p = 0.01$) in comparison to expected mortality (Table 2). Twelve bacterial species were isolated from

Table 2.

The incidence of bacterial species isolated from mountain pine beetle, and the mortality of associated adults and larvae.

| Species | Similarity index (%) ¹ | Incidence (%) ² | Isolates in living (and dead) larvae (no.) ³ | Isolates in living (and dead) adults (no.) ³ | Total sites (no.) | Beetle mortality (%) ⁴ |
|--|-----------------------------------|----------------------------|---|---|-------------------|-----------------------------------|
| <i>Serratia liquefaciens</i> | 75 ± 8 | 22 | 11 (10) | 0 (7) | 8 | 61 |
| <i>Serratia plymuthica</i> | 76 ± 8 | 17 | 7 (8) | 1 (6) | 7 | 62 |
| <i>Rahnella aquatilis</i> | 89 ± 5 | 11 | 1 (10) | 0 (3) | 8 | 93 * |
| <i>Pseudomonas syringae</i> v. <i>tabaci</i> | 91 ± 2 | 5 | 0 (4) | 0 (2) | 3 | 86 * |
| <i>Hafnia alvei</i> | 79 ± 6 | 5 | 1 (3) | 0 (2) | 4 | 83 |
| <i>Pantoea citrea</i> | 76 ± 4 | 4 | 1 (2) | 0 (2) | 4 | 60 |
| <i>Enterobacter pyrinus</i> | 71 ± 5 | 3 | 2 (2) | 0 (0) | 3 | 50 |
| <i>Erwinia chrysanthemi</i> biotype IV/VI | 75 ± 9 | 3 | 0 (0) | 0 (4) | 3 | 100 |
| <i>Proteus vulgaris</i> GC supgp. A | 74 ± 7 | 2 | 1 (0) | 0 (1) | 2 | 60 |
| <i>Kluyvera ascorbata</i> | 62 ± 8 | 2 | 1 (0) | 0 (2) | 2 | 67 |
| <i>Brevibacillus agri</i> | 86 ± 0 | 2 | 0 (3) | 0 (0) | 2 | 100 |
| <i>Pseudomonas syringae</i> v. <i>phaseolicola</i> | 91 ± 2 | 2 | 2 (1) | 0 (0) | 1 | 33 |
| <i>Paenibacillus lentimorbus</i> | 64 ± 1 | 2 | 1 (1) | 0 (0) | 1 | 50 |
| <i>Salmonella typhimurium</i> GC subgp. B | 72 ± 13 | 2 | 2 (0) | 0 (0) | 2 | 0 |
| <i>Sphingobacterium multivorum</i> | 84 ± 6 | 2 | 0 (1) | 0 (1) | 1 | 100 |
| <i>Kluyvera intermedia</i> | 73 | 1 | 0 (0) | 0 (1) | 1 | 100 |
| <i>Pseudomonas fluorescens</i> biotype G/C | 92 | 1 | 0 (1) | 0 (0) | 1 | 100 |
| <i>Serratia grimesii</i> Grimont | 81 | 1 | 0 (1) | 0 (0) | 1 | 50 |
| <i>Bacillus megaterium</i> subgp. A | 92 | 1 | 0 (1) | 0 (0) | 1 | 100 |
| <i>Cedecea davisae</i> | 65 | 1 | 0 (1) | 0 (0) | 1 | 100 |
| <i>Chromobacterium violaceum</i> | 89 | 1 | 0 (0) | 0 (1) | 1 | 100 |
| <i>Pantoea agglomerans</i> GC subgp. 1 | 75 | 1 | 0 (1) | 0 (0) | 1 | 100 |
| <i>Klebsiella pneumoniae</i> var. <i>ozaenae</i> | 85 | 1 | 0 (1) | 0 (0) | 1 | 100 |
| <i>Raoultella terrigena</i> | 59 | 1 | 0 (0) | 0 (1) | 1 | 100 |
| <i>Pseudomonas putida</i> biotype B | 85 | 1 | 0 (1) | 0 (0) | 1 | 100 |
| <i>Salmonella cholerasuis</i> v. <i>arizonae</i> | 67 | 1 | 1 (0) | 0 (0) | 1 | 0 |
| <i>Sphingomonas paucimobilis</i> | 97 | 1 | 0 (1) | 0 (0) | 1 | 100 |
| <i>Xenorhabdus nematophilus</i> | 71 | 1 | 0 (0) | 0 (1) | 1 | 100 |

¹ Where there was more than one observation, indices in this column are shown as the mean ± s.d.

² The incidence percentage is calculated as (number of beetle hosts / number of beetles tested) × 100.

³ In this column, the number of isolates found in living insects is followed by the number of isolates found in dead insects, in parentheses.

⁴ Percentages followed by an asterisk are significantly different ($p < 0.01$, Student's t test for dependent samples) than expected mortality (larvae = 51%; adults = 100%).

bark samples also producing insects with *R. aquatilis*; these included three isolates of *P. syringae* var. *tabaci* (Table 3).

Bacterial inhibition of bluestain fungi.

There were significant differences in growth inhibition for parameters corresponding to fungal species ($F_1 = 107.4$, $p = 0.000000$) and bacterial species ($F_2 = 3.2$, $p = 0.045270$). Bacterial isolates within species had no significant impacts ($F_{12} = 1.5$, $p = 0.125199$). Bacterial inhibition of *O. montium* was nearly twice the inhibition experienced by *G. clavigera*. Bacterial species produced similar levels of inhibition against the growth of *O. montium*. *S. liquefaciens* was significantly ($p < 0.05$) less inhibitory towards the growth of *G. clavigera* (Table 4). Caution is warranted in quantitative interpretation of these results, in that volatile compounds from the microbes could permeate throughout the plates, affecting the scale of the response in both controls and treatments. The relative responses, however, indicate a potential for inhibition.

Interactions between bacteria and chitin-containing substrates.

Chi-square comparisons indicated that *S. liquefaciens* and *R. aquatilis* thrived on autoclaved mycelium and, to a greater degree, on practical grade chitin (Table 5). All cultures with combined inocula appeared to have approximately equal proportions of each bacterial species when cell morphology was observed under a compound microscope. Combined cultures generally performed as well as individual cultures, with no clear indication of a competitive advantage for either of the two bacteria. The bacteria did not adhere to the smooth surfaces of the autoclaved mycelia, but *R. aquatilis* would occasionally adhere to minute rough areas on particles of the practical grade chitin. These rough areas appeared to serve as loci for adhesion and aggregation of the bacteria. When *R. aquatilis* was combined with *S. liquefaciens*, bacteria adhered to many more sites on the chitin particles, and numerous small bacterial aggregates formed (Table 5).

DISCUSSION

Although it was not isolated from every dead larva, *R. aquatilis* appears to be associated with mortality in MPB. Parental adults had an expected mortality of 100% during the winter; *R. aquatilis* is therefore not expected to have caused any increased mortality in adults. *R. aquatilis* is an enteric bacterial species that occurs widely in water and soil environments (Berge *et al.* 1991, Heulin *et al.* 1994, Horie *et al.* 1985), and it has been detected from a variety of insects, including bark beetles (Vasanthakumar *et al.* 2006; Delalibera *et al.* 2005). There are reports of this species linked to opportunistic bacterial infections in humans (Caroff *et al.* 1998, Lebessi *et al.* 1990, Maraki *et al.* 1994, Matsukura *et al.* 1996, Oh and Tay 1995), and some strains are reported to possess antagonistic properties against bacterial plant diseases (Laux *et al.* 2002, 2003). Regarding quantification, the frequencies reported here are for surface-sterilized insects receiving a moderate amount of com-

pression during placement on agar. This was sufficient to express hindgut contents onto the isolation medium, but a specific method for extraction of gut contents could have generated higher incidence statistics for bacteria tending to aggregate inside the larvae. Using other isolation methods, for example different isolation media or serial dilutions of ground tissue, would also yield other non-dominant bacteria and a greater diversity of species. The results thus pertain to the proportion of insects where the particular bacterial species are dominant, rather than quantifying the actual proportion of insects associated with the bacteria.

Some genotypic and phenotypic heterogeneity is reported for *R. aquatilis* (Brenner *et al.* 1998, Pokhil 1998, Selenska-Pobell *et al.* 1995, Varbanets *et al.* 2004); the biological role of isolates found in MPB remains an open question. For example, further study would be needed to determine whether the bacterium is pathogenic, or

Table 3.

Bacterial incidence in living and dead larvae of Mountain Pine Beetle, where larvae in the same bark sample were also infested with *Rahnella aquatilis*.

| Species | Class | Order | Isolates | Site(s) ¹ |
|--|---------------------|-------------------|----------|----------------------|
| <i>Paenibacillus lentimorbus</i> | Bacilli | Bacillales | 2 | 3 |
| <i>Pantoea agglomerans</i> GC subgroup | Gammaproteobacteria | Enterobacteriales | 1 | 8 |
| <i>Enterobacter pyrinus</i> | Gammaproteobacteria | Enterobacteriales | 1 | 6 |
| <i>Erwinia chrysanthemi</i> biotype VI | Gammaproteobacteria | Enterobacteriales | 3 | 5, 6, 10 |
| <i>Hafnia alvei</i> | Gammaproteobacteria | Enterobacteriales | 1 | 10 |
| <i>Proteus vulgaris</i> GC subgroup | Gammaproteobacteria | Enterobacteriales | 1 | 6 |
| <i>Pantoea citrea</i> | Gammaproteobacteria | Enterobacteriales | 1 | 3 |
| <i>Pseudomonas fluorescens</i> biotype G | Gammaproteobacteria | Pseudomonadales | 1 | 10 |
| <i>Pseudomonas syringae</i> var. <i>tabaci</i> | Gammaproteobacteria | Pseudomonadales | 3 | 6 |
| <i>Serratia liquefaciens</i> | Gammaproteobacteria | Enterobacteriales | 5 | 3, 6, 8 |
| <i>Serratia plymuthica</i> | Gammaproteobacteria | Enterobacteriales | 7 | 3, 6, 8 |
| <i>Sphingomonas paucimobilis</i> | Alphaproteobacteria | Sphingomonadales | 1 | 5 |

¹ Original collection sites listed in Table 1

Table 4.

The effect of bacterial species with fungal species assayed for growth inhibition.

| Bluestain Fungus | Bacterial species | % Inhibition of fungal growth ¹ |
|---------------------|------------------------|--|
| <i>O. montium</i> | <i>R. aquatilis</i> | 74.1 a |
| | <i>S. liquefaciens</i> | 73.2 a |
| | <i>S. plymuthica</i> | 68.2 a |
| <i>G. clavigera</i> | <i>R. aquatilis</i> | 46.0 bc |
| | <i>S. liquefaciens</i> | 33.7 c |
| | <i>S. plymuthica</i> | 46.2 bc |

¹ Means in this column followed by the same letter are not significantly different according to the Newman-Keuls multiple range test ($p > 0.05$). The test was performed on transformed data ($\arcsin\sqrt{Y}$); actual %Inhibition is shown.

simply an opportunist that flourishes after the larvae succumb to viruses, nematodes, or other stresses not screened in this study.

The three most dominant bacteria inhibited the growth of the bluestain fungi. None of the bacterial species were significantly more inhibitory versus either bluestain fungal species. None of the bacterial species

aggregated on autoclaved mycelia of the fungi. These results are interesting, in that bacteria are thought to mediate or inhibit the growth of fungi associated with two other *Dendroctonus* spp.: *D. frontalis* Zimmermann (southern pine beetle) and *D. rufipennis* (Kirby) (spruce beetle) (Cardoza *et al.* 2006, Scott *et al.* 2008). Further tests

Table 5.

The effect of chitin-containing substrates on the growth, adhesion and aggregation of *Serratia liquefaciens* and *Rahnella aquatilis*.

| Substrate | <i>S. liquefaciens</i> ¹ | <i>R. aquatilis</i> ¹ | Density (10 ⁶ bacteria mL ⁻¹) ² | Adhesive cultures (no.) | Aggregating cultures (no.) |
|--|-------------------------------------|----------------------------------|---|-------------------------------|----------------------------------|
| None | + | | 0.36 a | 0 | 0 |
| | | + | 0.32 ac | 0 | 0 |
| | + | + | 0.19 a | 0 | 0 |
| <i>G. clavigera</i> (autoclaved hyphae) | + | | 5.24 bc | 0 | 0 |
| | | + | 4.06 bc | 0 | 0 |
| <i>O. montium</i> (autoclaved hyphae) | + | + | 5.22 bc | 0 | 0 |
| | + | | 7.88 c | 0 | 0 |
| Crab shell | + | | 6.09 bc | 0 | 0 |
| | | + | 8.23 c | 0 | 0 |
| | + | + | 10.44 d | 0 | 0 |
| | | + | 15.34 d | 3 | 0 |
| | + | + | 16.69 d | 3 | 3 |

¹ A plus sign (+) in these columns indicates cultures were inoculated with the species listed in the column heading.

² Means in this column followed by the letter 'a' are not significantly different from the expected control concentration of 0.29x10⁶ bacteria mL⁻¹ using the Chi-square test ($p > 0.05$). Means followed by the letter 'b' are not significantly different from the expected concentration for growth on hyphae of *G. clavigera* (4.77x10⁶ bacteria mL⁻¹), means followed by the letter 'c' are not significantly different from the expected concentration for growth on hyphae of *O. montium* (7.73 x10⁶ bacteria mL⁻¹), and means followed by the letter 'd' are not significantly different from the expected concentration for growth on Crab shell (15.167.73 x10⁶ bacteria mL⁻¹), also using Chi-square tests ($p > 0.05$).

would be necessary to understand the cause of the inhibition. Nutrient depletion, pH changes, or various secondary compounds are all examples of possible inhibitory factors. Although *R. aquatilis* is reported to grow well on wood (Kallioinen *et al.* 2003), proliferation of bluestain fungi in the beetle galleries suggests that the enteric bacteria primarily inhabit the insect gut, where they would have limited impact on the spread of the fungi. Another study has shown that the growth of *O. montium* is stimulated by microbes isolated from the galleries of MPB, including three yeasts (*Candida* sp., *Pichia scolyti*, and an unidentified basidiomycete) and a bacterial species (*Micrococcus* sp.). However, the same study also found that *Candida* sp., the basidiomycete yeast, and *Micrococcus* sp. were inhibitory to the

growth of *G. clavigera*. Isolated from fresh phloem near MPB attacks, *Bacillus pumilus* is also reported to be inhibitory to the growth of both fungi (Adams *et al.* 2008). There is probably a diverse range of antagonistic and mutualistic interactions among the various microbes that associate with MPB.

The adherence and aggregation of *R. aquatilis* and *Serratia* spp. on arthropod chitin could indicate a role for microbial consortia in the observed beetle mortality. Insect hindguts can possess chitinous structures that facilitate digestion through bacterial aggregation (Hackstein and Stumm 1994). *R. aquatilis* and *Serratia* spp. are involved in biofilm formation (Steidle *et al.* 2001). Further research is needed to understand the prevalence of *R. aquatilis/Serratia*

spp. consortia in the environment and how *R. aquatilis* becomes established in MPB populations. The abundant growth of *S. liquefaciens* and *S. plymuthica* on autoclaved mycelium of *Ophiostoma* spp. agreed with the reported chitinolytic properties of these species (Berg *et al.* 1999, Joshi *et al.* 1988). Presumably, larval MPB could benefit from any digestive action of these bacteria on bluestain fungi. However, other bacterial species could also persist in the *R. aquatilis*/*S. liquefaciens* consortium, potentially creating chronic stress in the insect host or affecting cold-tolerance. In this study, slabs producing specimens with *R. aquatilis* also produced specimens containing *Pseudomonas* spp., an ice-nucleating species (Lee *et al.* 1998).

The association of *Serratia* spp. with living beetles agrees with a previous study of *D. frontalis*, where bacteria isolated from the gut of healthy insects included *Serratia* spp. (Moore, 1971). Earlier research on southern pine beetle correlated the occurrence of several bacterial species with beetle mortality, including *Serratia marcescens* Bizio, *Pseudomonas aeruginosa* (Schroeter) Migula, *Pseudomonas fluorescens* Migula, *Bacillus thuringiensis* Berliner, *Bacillus cereus* Frankland & Frankland, and *Flavobacterium* sp. (Moore 1971). In further tests on southern pine beetle, pathogenicity was demonstrated for *S.*

marcescens, the *Pseudomonas* spp., and the *Bacillus* spp. (Moore 1972b).

Without further *in vivo* testing, it is difficult to assign a definite pathogenic or opportunistic role for *R. aquatilis* in larvae or adults of MPB. A pathogenic form of *R. aquatilis* might have utility as a biological control agent, since the species is motile and could potentially penetrate beetle galleries during wet weather. On the other hand, even if *R. aquatilis* is simply an opportunist that aggregates with species such as *S. liquefaciens*, this mechanism of action might still be exploited to reduce the cold tolerance of the insect. Answers are needed regarding the natural frequency of particular bacterial associations, but the deliberate introduction of aggregating bacteria along with ice-nucleating species could reduce the cold tolerance of MPB. This could be especially beneficial if climate warming continues to exacerbate the beetle impacts. In any eventual effort to develop biological control agents, the occurrence and influence of other pathogens in larvae (viruses, nematodes, etc.) should also be explored. The geographic occurrence of *R. aquatilis* should also be surveyed and its mode of distribution within brood trees elucidated. A more complete understanding of microbial influences and constraints on MPB may help us deal with future outbreaks.

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Cereal leaf beetle, *Oulema melanopus* (L.) (Coleoptera: Chrysomelidae), attraction to oat plantings of different ages

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ABSTRACT

The cereal leaf beetle (CLB), *Oulema melanopus*, a serious pest in oats, barley and wheat, is a relatively new pest on the west coast of North America. To determine if adults showed a preference for oat stands of different ages, we examined adult and egg densities in four sequentially planted oat stands in the Willamette Valley, Oregon, in 2005 and 2006. Adults moved from earlier to later plantings (from older to younger oats) during the growing season, particularly once the flag leaf had emerged in earlier plantings. In 2005, the seasonal pattern in egg counts tended to match that of adult counts in the first three oat plantings. The egg to adult ratio was greater in the earlier planted (older) oats, particularly the first planting. The egg to adult ratio was more variable in 2006. Adults spent the most physiological time (degree-days) in the second oat planting, and total egg numbers were highest in the second and third plantings. Data suggest that delayed planting as a trap crop management tool for CLB is complex and potentially ineffective.

Key Words: cereal leaf beetle, *Oulema melanopus*, plant age, host attraction, egg production, management

INTRODUCTION

The cereal leaf beetle (CLB), *Oulema melanopus* (L.) (Coleoptera: Chrysomelidae), is a new pest in cereals in the western United States (Rao *et al.* 2003). CLB was first detected in Michigan in 1962 and soon became a serious pest of small grains in the Midwest, the Atlantic States, and eastern Canada. Wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), and oats (*Avena sativa* L.) were damaged (Haynes and Gage 1981), with yield reductions in oats reaching 30% (Wilson *et al.* 1969) to 48.8% (Merritt and Apple 1969). Incorporation of trichome resistance in experimental wheat varieties reduced CLB numbers, although effective trichome resistance was not present in other grains (Haynes and Gage 1981). Biological control efforts were initiated and, once the gregarious larval parasitoid *Tetrastichus julis* (Walker) (Hymenoptera: Eulophidae) and the egg parasitoid *Anaphes flavipes* (Foerster)

(Hymenoptera: Mymaridae) became established, damaging populations requiring chemical control measures were substantially reduced (Haynes and Gage 1981). CLB was first detected in Oregon and Washington in 1999 (Rao *et al.* 2003), where it caused direct damage to cereal crops. In addition, quarantine restrictions were established on movement of hay and forage from infested counties in Oregon and Washington to neighboring California and Canada due to potential transport of adult CLB in baled straw. As the pest moved into the region, attention was directed towards developing improved monitoring tools (Rao *et al.* 2003) and control tactics. The parasitoid *T. julis* is not widespread in the Pacific Northwest and *A. flavipes* has not yet established.

CLB has one generation per year. Eggs are laid in spring and the larvae develop through four instars by early summer. Pupa-

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tion occurs in earthen cells in the upper 5 cm of soil and adults emerge after two to three weeks after pupation begins. The overwintering adult population dies by mid-June, several weeks before emergence of the first summer adults. New adults feed to a limited extent but do not mate. Adults overwinter within fields if there is a large amount of crop residue, or outside fields in sheltered fencerows and woodlots (Haynes and Gage 1981).

There is no consistent pattern in spring movement of CLB in relation to the age of the host plant. Adults typically move from overwintering sites to grasses and winter wheat and then to emerging spring-planted grains. In winter wheat, which is planted in fall, CLB adults prefer later plantings (Casagrande *et al.* 1977) and more eggs and larvae are found on these plants (Gage

1974). The opposite is found in oats, which are planted in spring. Early plantings of oats have more CLB adults (Casagrande *et al.* 1977), eggs and larvae (Gage 1974). For unknown reasons, adult preference for winter wheat versus spring grains (primarily oats) can change over several years (Casagrande *et al.* 1977), suggesting that relationships between plant species, plant age, and CLB adult preference are not static (Haynes 1973).

In this study we examined the dynamics of CLB adult and egg densities in oat stands of four ages in the Willamette Valley, Oregon. To assess the potential impact of CLB in these stands, we determined the total time adults spent in each stand and the total number of eggs laid. The potential role of host-plant selection behaviours in CLB management strategies is discussed.

MATERIALS AND METHODS

The study was conducted in 2005 and 2006 at the Hyslop Field Laboratory, Oregon State University, in a field of oats (variety Cayuse). This field was adjacent to an oat nursery used by USDA-APHIS and Oregon Department of Agriculture (ODA) for propagating *T. julis*. Four stands of oats (Cayuse) were planted sequentially on 15 March, 5 April, 26 April, 2 June, 2005; and 7 April, 20 April, 10 May and 6 June, 2006. In 2005, the fourth planting was delayed due to the high rainfall in May. In 2006, limited rainfall in late April and May delayed germination of the second planting, so the second and third plantings were irrigated until rains started again in late May. In both years, the four treatments (called planting dates PD1 through PD4 in chronological order) were set up as a randomized complete block design with four replicates. Each plot was 6.85 m wide and approximately 42.5 m long, and consisted of 45 oat rows,

We monitored CLB adult and egg populations in the plots throughout the overwintered adult activity period (mid-April through late June). Sampling began as soon as the first adults were seen in the plots and

continued at weekly intervals until adults were no longer present in the field. In 2005, adults and eggs were counted in ten randomly located subsamples (30.5 cm of row) per block. In 2006, we took five subsamples per block, and low CLB populations necessitated increasing the subsample area for adults to five adjacent rows (30.5 cm sections), and two adjacent rows (30.5 cm sections) for eggs. The sampling regime ended before the emergence of new adults of the next generation. Adult females and males were not differentiated in 2005. In 2006, a representative number of adults was collected from the plots for identification of sex. In 2006, we recorded the plant development stage using the Zadok's Scale (Zadoks *et al.* 1974) to allow for comparisons based on oat plant growth stage.

To calculate the total developmental time adults spent in each of the planting date treatments we transformed the data to a centigrade degree-day (CDD) time scale. Expressing the data on this scale eliminates the effect of variable temperatures on behavior and oviposition, whether seasonal or weekly. Cumulative centigrade degree-days (CCDD) were calculated using temperature

data from the Hyslop weather station, and the CLB development thresholds of 7°C (minimum) and 30°C (maximum) (Guppy and Harcourt 1978). The biofix (start) dates, 6 March in 2005, and 2 April in 2006, for CDD accumulation were based on the date of first adult emergence as predicted by the CLB IPM weather model derived from a synthesis of 10 data sets (IPPC 2009). Adult sample counts were plotted on the CCDD scale, and the graphical method (Southwood 1978) with 20-DD intervals was used to plot the points. The values of cumulative adult degree-days (CADD) per 30.5 cm of row were calculated by summing the area under the curve.

CLB eggs take about two weeks to hatch at spring temperatures in the Willamette Valley. Hence, the same egg could be counted at two or three weekly sampling periods. To calculate the actual total number of eggs laid in each plot over the ovipositional period, egg counts were plotted using the graphical method (Southwood 1978) at 20-DD intervals. The cumulative egg degree-days was divided by the number of degree-days it takes for an egg to hatch (105 CDD; Guppy and Harcourt 1978) to obtain the total number of actual eggs per sample unit (Southwood 1978).

Egg production per adult was calculated by dividing the number of eggs at a given number of accumulated degree-days by the number of 20-DD intervals over which the eggs could have accumulated (5 intervals maximum). This number was then divided by the running average of adults over corresponding time period. This value was not calculated for the fourth planting because of the minimal adult and egg density data.

Adults can move between plots, which raised the question of whether these experimental units were independent. This question was addressed by examining the spatial autocorrelation variogram for egg and adult count data on each sampling date (SAS 9.1).

Repeated-measures analysis was used to

adjust the p-values for temporal autocorrelation present in the within-sampling-date comparisons for differences in egg and adult counts among planting dates. Because counts of adults and eggs increase and then decrease over time we are not interested in the main effects of Julian date and planting date; and effects of planting date are more appropriately analyzed using the cumulative degree-day approach. Count data often fit the Poisson distribution and the comparison of adult counts among planting dates was conducted using PROC Genmod, with parameter options link=log dist=poisson (SAS 9.1). However the low power of this analysis due to the small number of data clusters, and the deviance from the Poisson that occasionally resulted when using the subsample means, led us to analyze the egg count data differently. Egg counts were transformed using the variance-stabilizing transformation developed for CLB egg spatial distributions ($\log_{10}(\text{counts} + 0.13)$) (Logan 1980) and analyzed using repeated measures in PROC Mixed (SAS 9.1). The lack of a similar stabilizing transformation for adults, and the small number of adults in 2006, precluded our using the more powerful mixed model approach for the adult data. For the PROC Mixed and PROC Genmod analysis, we compared planting date means within sampling dates using unadjusted probabilities after first testing for the main effect difference.

To adjust for the statistical problem of modeling zero variance for adult and egg counts where we recorded zero individuals, we dropped these 'planting date by sampling date' entries from the data sets before analysis. Instead, we compared the other counts on this date to zero counts by determining if the 95% confidence limits of the means included zero.

Statistical comparisons of CADD and total eggs among planting dates were performed using ANOVA in PROC GLM with the Tukey adjustment for the number of comparisons (SAS 9.1).

RESULTS

There was minimal spatial autocorrelation in CLB stages among plots in both years. Significant autocorrelation among plots occurred on only one sampling date for adults and one sampling date for egg counts. On all other sampling dates there was either no spatial autocorrelation or the correlation was negative (plots at further distances were more correlated than closer plots). This indicates that the plots were independent from each other and statistics based on the assumption of independent experimental units can be used. It also suggests that significant movement was taking place between the oat plots and adjacent grain fields.

In 2005, overwintered adults appeared after the first two plantings had emerged, and most died by 15 June (Table 1). On each sampling date, CLB adults had a choice of oats of two or more planting date (PD) treatments. No adults were counted in the fourth planting (PD4) on 15 and 24 June, due to the late emergence of oats and declining population of adults, however, a few adults were seen outside the rows sampled. Counts of adults in each planting date treatment generally increased until the following planting date treatment became more attractive, then decreased in the older treatment. Statistical differences among mean counts of adults per planting date treatment occurred on six of eight sampling dates where adults were present (Table 1). Countering this general trend was the decline in adults in the third planting and increase in the second planting during the third week of May (19 May). This was a week of unusually intense rainstorms and we speculate that the adults were seeking refuge in areas of greater plant biomass. At this time the plant height of PD2 was 31.4 cm versus 12.0 cm for PD3. The total number of adults decreased at this time (Table 1) suggesting that some were leaving the oat plantings to seek refuge in other fields or fencerows.

In 2006, sampling started just as the first oat planting emerged. The 2006 adult population was approximately one-tenth that in

2005 (Table 1). The rise and decline in adults over time in each of the plantings was similar to that in 2005, although the trends were not as uniform, perhaps due to the greater variability in the much smaller populations. There were no statistical differences among mean adult counts at $P < 0.05$. However trends in the 2006 count data were similar to 2005 (Table 1). There was a declining trend in adult counts in the oat plantings on the 25 May sampling date. Sampling that week occurred after three days of high rainfall.

When adult population counts over calendar time are transformed to a CDD scale, the sequential movement of adults from early- to late-planted oats through the season can be seen in 2005 and 2006 (Fig. 1a, b). The decline in total adults in the plots each year after several days of heavy rain in late May is notable. It occurred at approximately 310 and 350 CDD in 2005 and 2006, respectively. Expressing the data on a CDD scale also allows calculation of total adult residency time, and total egg numbers, in stands planted at different times. The analysis of CADD in 2005 showed that PD2 had the highest CADD, followed by PD3 and PD1 (Table 2). In 2006, the relative CADDs of the four planting dates were similar to 2005, although the differences were not significant (Table 2). Total CADD in 2005 was 13.88 versus 1.58 in 2006.

On a calendar scale, CLB adults first appeared in the oat plots during the same week in 2005 and 2006. In 2006, the large movement of adults into PD3 coincided with PD1 and PD2 reaching the Zadok's scale of 43 and 33, respectively (boot stage and 3rd node stage) (Table 3). On a degree-day scale (developmental minimum of 7°C) the adults appeared 76.4 CDD earlier in 2006 (Fig. 1). This translates to 12 days at the daily temperatures at this time of year. An even larger difference occurred, 17 days, when a developmental minimum of 9°C (Fulton and Haynes 1975) was used. In 2005, approximately 110 CDD occurred between the time overwintering adults were predicted to emerge, and when they ap-

Table 1.

Weekly mean CLB adult counts in each sequentially planted oat stand.

| Planting date | Sampling dates 2005 ¹ | | | | | | | | | |
|---------------|----------------------------------|------|------|-------|-------|-------|-------|-------|------|------|
| | 21-4 | 29-4 | 6-5 | 12-5 | 19-5 | 24-5 | 31-5 | 9-6 | 15-6 | 24-6 |
| 15 March | 0.03b | 0.13 | 0.15 | 0.18b | 0.10b | 0.10b | 0.00b | 0.00b | 0.00 | 0.00 |
| 5 April | 0.30a | 0.30 | 0.30 | 0.28b | 0.40a | 0.25b | 0.20b | 0.00b | 0.00 | 0.00 |
| 26 April | | | 0.18 | 0.60a | 0.38a | 0.58a | 1.33a | 0.28a | 0.00 | 0.00 |
| 2 June | | | | | | | | | 0.00 | 0.00 |
| Total | 0.33 | 0.43 | 0.63 | 1.06 | 0.88 | 0.93 | 1.53 | 0.28 | 0.00 | 0.00 |
| Planting date | Sampling dates 2006 | | | | | | | | | |
| | 21-4 | 26-4 | 2-5 | 10-5 | 18-5 | 25-5 | 1-6 | 8-6 | 15-6 | 23-6 |
| 7 April | 0.02 | 0.03 | 0.03 | 0.02 | 0.04 | 0.02 | 0.00 | 0.00 | 0.00 | 0.00 |
| 20 April | | | 0.02 | 0.04 | 0.10 | 0.01 | 0.05 | 0.01 | 0.01 | 0.00 |
| 10 May | | | | | | 0.06 | 0.09 | 0.03 | 0.03 | 0.00 |
| 6 June | | | | | | | | | | 0.00 |
| Total | 0.02 | 0.03 | 0.05 | 0.06 | 0.14 | 0.08 | 0.14 | 0.04 | 0.04 | 0.00 |

¹ Planting date means within year and sampling date with different letters are statistically different at $P < 0.05$.

Table 2.

Cumulative adult degree-days and total egg numbers in the four oat planting dates

| Planting date | Cumulative adult degree-days (CADD) ¹ | Total number of eggs ² |
|---------------|--|-----------------------------------|
| 2005 | | |
| 15 March | 1.53 ± 0.40 b | 49.0 ± 5.5 b |
| 5 April | 7.78 ± 1.50 a | 64.7 ± 6.0 a |
| 26 April | 4.49 ± 0.38 b | 63.0 ± 2.7 a |
| 2 June | 0.00 ± 0.00 c | 1.7 ± 0.4 c |
| Total | 13.88 | 178.4 |
| 2006 | | |
| 7 April | 0.37 ± 0.10 a | 7.0 ± 1.2 a |
| 20 April | 0.65 ± 0.23 a | 5.1 ± 0.7 a |
| 10 May | 0.54 ± 0.05 a | 4.9 ± 0.4 a |
| 6 June | 0.00 ± 0.00 b | 0.3 ± 0.1 b |
| Total | 1.56 | 16.3 |

¹ Means ± SE of PD entries. Within years PDs with different letters are statistically different at $P < 0.05$.

² Total number of eggs per planting date estimated from the area under the curve analysis.

peared in the oat plots. In 2006, this interval was 90 CDD (Fig. 1). Thus there are either errors in the models or CLB adults are on other hosts (winter grains) for several

weeks before migrating into the oats. We did not monitor CLB adults on other hosts early in the season.

In 2005, the temporal pattern in egg

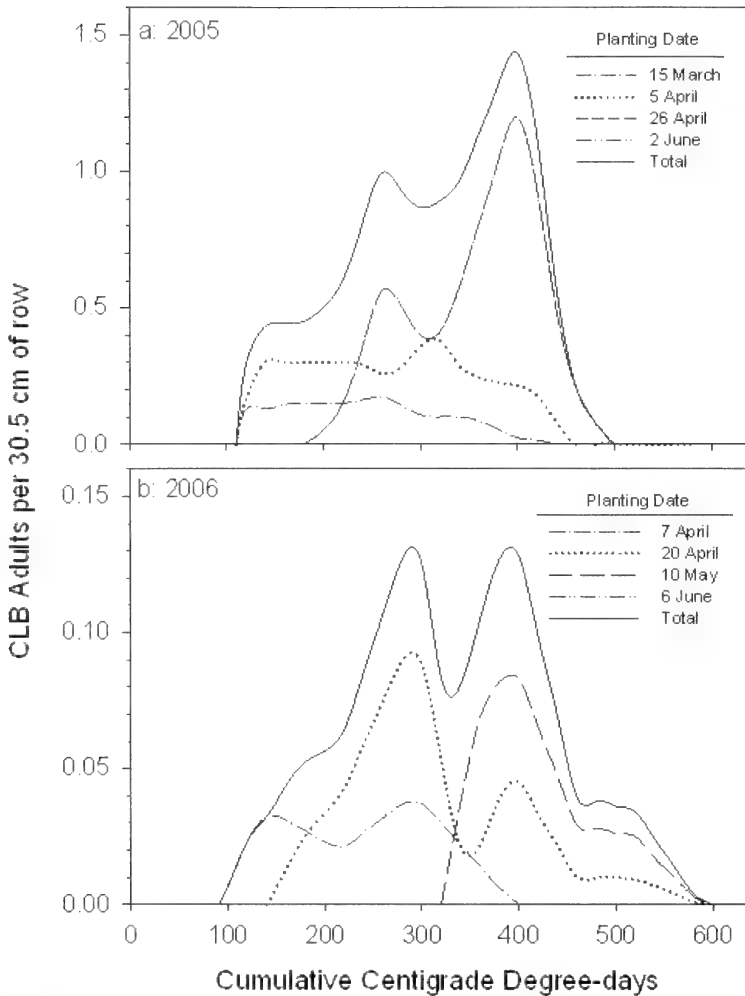


Figure 1. The mean density of CLB adults expressed on a cumulative centigrade degree-day scale, in oat stands of four age classes, i.e., planting dates (PD) 1 – 4: (a) 2005; (b) 2006. The biofix dates were 6 March in 2005, and 2 April in 2006, the dates of first adult emergence predicted by the CLB weather model (IPPC 2009).

density among planting dates was similar to that of the adult populations, with egg counts in each consecutive planting increasing to a peak and then declining as counts increased in the later plantings. There were statistical differences in egg counts on eight of the sampling days (Table 4). The similar egg counts on 19 May are a consequence of the movement of adults out of the younger (smaller) oats, and movement into the older (larger) oats which occurred that week. Quantitative changes in egg density did not always mirror changes in adult density. The egg density in PD2 was only slightly greater than that PD1 (Table 4), even

though the adult population was twice as great in PD2 (Table 1). Similarly, even though the population of adults in PD3 was greater than that in PD2, the number of eggs laid was approximately the same. These relationships between egg and adult counts resulted in the eggs per adult values in PD1 being at times much greater than in PD2 and PD3 (Table 5).

When expressed on a CDD scale, the pattern of egg density over time in the four planting date treatments in 2005 was similar to the calendar day scale (Fig. 2a). The total number of eggs was greater for PD2 and PD3 compared to PD1, while few eggs

Table 3.

Oat plant growth stages (Zadok's scale) at three representative periods during the spring sampling period in 2006

| Sampling date | Planting date | | | |
|---------------|---------------|----------|------------------|--------|
| | 7 April | 20 April | 10 May | 6 June |
| 2 May | 14 | 11.5 | n/a ¹ | n/a |
| 1 June | 45 | 33 | 22.5 | n/a |
| 23 June | 87 | 55 | 33 | 14 |

¹ n/a - plants not yet emerged. Zadok's Scale of 14 is a young plant with 4 leaves unfolded; 87 is the hard dough stage of the developing seed.

Table 4.

Weekly mean CLB egg counts in each sequentially planted oat stand

| Planting date | Sampling dates 2005 ¹ | | | | | | | | | |
|---------------|----------------------------------|------|-------|-------|------|-------|-------|-------|------|-------|
| | 21-4 | 29-4 | 6-5 | 12-5 | 19-5 | 24-5 | 31-5 | 9-6 | 15-6 | 24-6 |
| 15 March | 0.5 b | 16.1 | 30.0a | 28.3b | 17.5 | 16.0b | 4.0c | 2.0c | 0.2c | 0.0 b |
| 5 April | 2.5 a | 18.4 | 37.4a | 34.4a | 21.0 | 22.9a | 11.0b | 3.9b | 1.5b | 0.1b |
| 26 April | | 0.0 | 4.5b | 9.6c | 19.3 | 23.6a | 40.3a | 30.1a | 8.7a | 0.3b |
| 2 June | | | | | | | | | 0.4c | 2.0a |
| Planting date | Sampling dates 2006 ¹ | | | | | | | | | |
| | 21-4 | 26-4 | 2-5 | 10-5 | 18-5 | 25-5 | 1-6 | 8-6 | 15-6 | 23-6 |
| 7 April | 0.3 | 1.9 | 2.1a | 2.6 | 3.7 | 2.2 | 1.0 | 0.1b | 0.4b | 0.0 b |
| 20 April | | | 0.2b | 0.9 | 3.7 | 1.9 | 1.6 | 0.1b | 0.2b | 0.1b |
| 10 May | | | | | | 2.3 | 1.8 | 0.9a | 2.5a | 1.0a |
| 6 June | | | | | | | | | | 0.3b |

¹ Planting date means within year and sampling date with different letters are statistically different at $P < 0.05$.

Table 5.

Mean CLB eggs per adult at 100 CDD intervals during the adult activity period¹

| Planting date | Accumulative CDD from overwintering adult emergence | | | | | |
|---------------|---|------|------|------|------|------------------|
| | 160 | 260 | 360 | 460 | 560 | |
| 2005 | | | | | | |
| 15 March | | 33.1 | 38.2 | 23.0 | 16.3 | n/a ² |
| 5 April | | 13.2 | 24.2 | 13.5 | 4.6 | n/a |
| 26 April | | -- | 21.5 | 9.5 | 7.1 | 11.0 |
| 2006 | | | | | | |
| 7 April | | 22.1 | 23.7 | 13.1 | 2.1 | n/a |
| 20 April | | 26.9 | 12.0 | 6.1 | 0.6 | 2.4 |
| 10 May | | -- | -- | 20.1 | 3.1 | 15.3 |

¹ Number of eggs from Fig. 2 divided by the running average of adults from the previous five 20 CDD periods in Fig. 1 (adults that could have laid those eggs).

² n/a - adults not found in plots

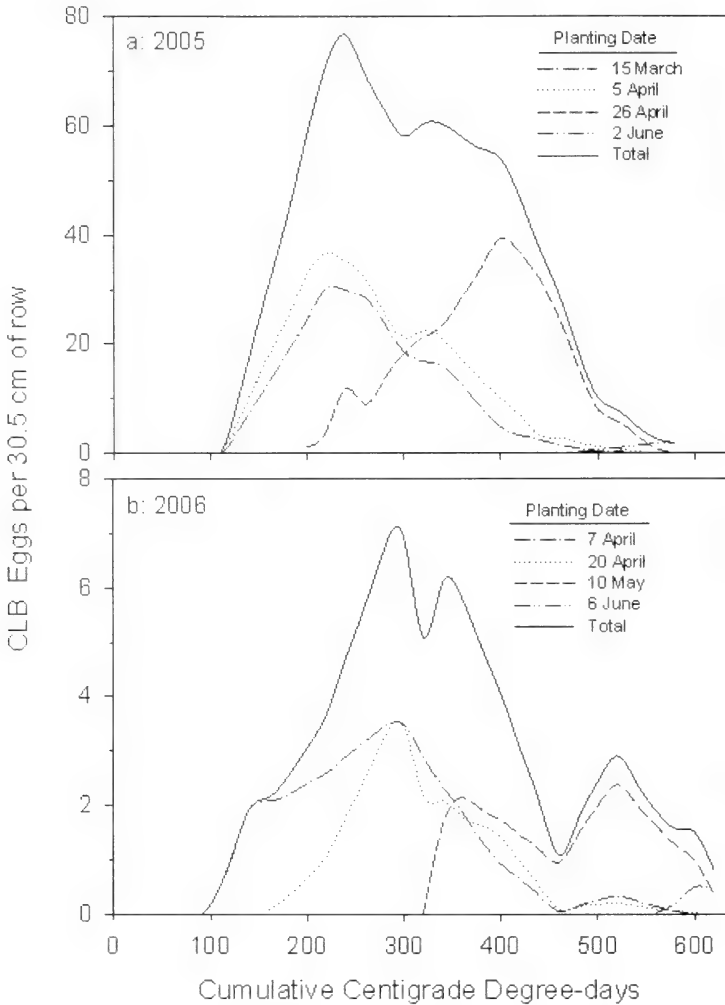


Figure 2. The mean density of CLB eggs expressed on a cumulative centigrade degree-day scale, in oat stands of four age classes, i.e., planting dates (PD) 1 – 4: (a) 2005; (b) 2006. The biofix dates were 6 March in 2005, and 2 April in 2006, the dates of first adult emergence predicted by the CLB weather model (IPPC 2009).

were laid in PD4 (Table 2).

In 2006, the temporal pattern of egg counts did not follow adult densities as well as in 2005. The sequential increase and decrease in the three planting dates was not as uniform. There were significant differences among planting dates in egg counts on four sampling days, mostly at the end of the CLB activity period when PD3 counts were greater than those in the other planting dates (Table 4). The movement of adults from the younger (smaller) oats during the rains of the week of 25 May disrupted the sequential pattern of egg increases in the

younger oats. In 2006, the relative difference among the first three planting date treatments in the eggs per adult value was variable (Table 5), reflecting the lack of correlation between adults and egg counts.

When 2006 egg densities are plotted on a CDD scale (Fig. 2b), the most noticeable difference is that the single peak in total egg density separates into two peaks that correspond to the peaks in total adults (Fig. 1b). There were no statistical differences in the total number of eggs in the first three planting date treatments (Table 2).

DISCUSSION

When CLB adults have a choice of oat stands of different age they tend to prefer younger plants. Overwintered adults move into newly emerged oats after spending time on other hosts, and adult populations increase over time. Populations on older oats decline at the time adult counts are increasing in younger oats. This behaviour is particularly evident once the older oats reach the flag leaf stage. The age of oat leaves affects CLB oviposition, with a dramatic decline in oviposition occurring when a plant approaches the flag leaf stage (GDH unpublished data). This is in contrast to the work of Casagrande *et al.* (1977) that found higher adult populations in older (earlier planted) oats. This contrasting information suggests that adult preference for young plants is not static, and that factors such as weather conditions and the relative differences in plant maturity and size can influence host preference.

There were some differences in the data between 2005 and 2006, in part due to the approximately 10-fold smaller CLB populations in 2006. The 45% parasitism rate of late season larvae by *T. julis* in 2005 (GDH unpublished data) probably accounts for the population decline in 2006. Samples in 2006 contained 100% parasitized CLB larvae after the second generation of *T. julis* (R. Worth unpublished data). One difference between 2005 and 2006 that may have influenced adult and egg counts is the spring drought in 2006. While we used irrigation to get the second and third plantings germinated and established, these later planted stands were probably under greater water stress than the deeper rooted first planting. CLB adults may have been responding to a possible difference in plant water status.

Observations during greenhouse studies showed that many CLB adults leave the oat plants and collect on cage sides between 1000 h and 1600 to 1700 h (unpublished), and the present study documented periodic disappearances of a portion of the within-field population to other habitats. These

observations suggest that CLB adults are moving within and between fields on a regular basis and can respond to changing host plant and environmental cues.

The eggs per adult values calculated from the CDD data are much greater than the eggs per female obtained from laboratory cage experiments. Laboratory data ranged from 8.7 to 12.2 eggs per day at 26.7 °C (Wellso *et al.* 1973). Eight to 18 eggs per day were laid over the first half of post-aestival adult life (Wellso *et al.* 1975). The data from the present study, greenhouse observations, and other studies (Gutierrez *et al.* 1974, Casagrande *et al.* 1977) indicate that CLB adults move frequently within and between fields, and raise the possibility that a significant portion of the adults were not on the oat plants during our mid-day sampling.

In 2005, the impact of CLB was greatest on the second and third oat plantings. More cumulative adult degree-days were recorded in the second planting, and the highest total egg numbers occurred in PD2 and PD3. The fourth planting was minimally affected by CLB due to its late emergence in relation to adult phenology. Similar trends were found in 2006.

While CLB adults were attracted to younger oat plants, preference was not absolute. The variation in adult attraction and egg production in stands of different ages is in part because older oats are not a uniform resource for adults or their developing offspring. Older oats are actually a composite of both young and old leaves, so adult CLB can find young leaves in an older oat stand. In a greenhouse study, the majority of eggs were laid on the softer older leaves, or young tiller leaves of older oat plants (unpublished).

This study suggests that using oat plantings of different ages as a trap crop to help control damage for CLB infestations will be unpredictable and potentially unprofitable. Damage to the flag leaf causes the greatest loss in yield (Yoshida 1972) so the optimum timing of consecutively planted stands

to draw CLB adults away from the primary crop will need to be modeled using input from oat plant growth models, CLB adult preference related to oat phenology, and CLB developmental thresholds. In addition,

late-planted oats are likely to have significantly reduced yields compared to early planted oats (Ciha 1983). Therefore, a late-planted trap crop of oats, even if sprayed to control CLB, will suffer yield loss.

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Effect of sex pheromone and kairomone lures on catches of codling moth

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ABSTRACT

Field studies were conducted in sex pheromone-treated apple orchards to evaluate the performance of a clear 0.11 m² vertical interception trap coated with oil and baited with either (*E,E*)-8-10-dodecadien-1-ol (codlemone), ethyl (*E,Z*)-2,4-decadienoate (pear ester), or both attractants (combo) for adult codling moth, *Cydia pomonella* (L.). Interception traps baited with codlemone or pear ester caught significantly more males only or both sexes than unbaited traps, respectively. Interception and delta traps baited with codlemone caught similar numbers of males. Interception traps baited with pear ester caught up to 8-fold more males and 30-fold more females than similarly baited delta traps, respectively. Seasonal catches of females did not differ between light and pear ester-baited interception traps. Delta traps caught significantly more males, fewer females, and a similar number of total moths as the interception trap when both were baited with the combo lure. These data suggest that new clear trap designs can be developed to increase catches of female codling moth which may enhance seasonal monitoring and establish more useful predictive population models.

Key Words: apple, *Cydia pomonella*, traps, colour, monitoring

INTRODUCTION

Passive interception traps constructed of clear plastic, coated with an oil film, and hung vertical in the canopy were developed to study the behaviors of male and female codling moths *Cydia pomonella* (L.), in orchards treated with sex pheromone (Weissling and Knight 1994). While, moth catches on individual interception traps were not comparable to either sex pheromone-baited or light traps, two of the key attributes of these passive traps were the capture of nearly equal numbers of each sex and in providing an unbiased estimate of the proportion of mated females (Knight 2000). Passive interception traps have been used to experimentally demonstrate the occurrence of mating delay (Knight 1997) and to estimate the level of mating in sex pheromone-treated orchards (Knight 2006). These traps have also been used to study the distribution of moths within an orchard canopy (Weissling and Knight 1995) and to

examine patterns of adult movement into sex pheromone-treated orchards (Knight 2007a). In addition to their use as a research tool, passive interception traps have been evaluated as monitoring aides to predict the seasonal phenology of female codling moth (Knight 2000). Their use demonstrated that female versus male moth captures can improve the prediction of the start of egg hatch and were more closely correlated with levels of fruit injury at both mid-season and prior to harvest.

Yet, despite these many benefits derived from using interception traps to monitor codling moth, a number of drawbacks have limited their adoption by growers; such as their relatively low moth capture rate compared with sex pheromone-baited traps, their non-specificity, the short useful life of the oil coating, especially during hot or wet periods, and an overall greater level of difficulty and higher cost of servicing these

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traps versus the standard plastic or cardboard traps. Alternative trap and lure designs that could alleviate some of these issues could enhance the benefits provided to growers from monitoring female codling moths within their orchards. One approach may be to use the bisexual attractant, ethyl (*E, Z*)-2, 4-decadienoate (pear ester) to further increase the catch of female moths on interception traps. Capture of female codling moth in delta traps baited with pear ester have been reported to improve prediction of first egg hatch and result in more accurate action thresholds (Knight and Light 2005a, b). However, the performance of pear ester relative to codlemone with standard traps has been inconsistent across a number of geographical regions with a broad range in its attractiveness for females reported (Ioriatti et al. 2003, Thwaite et al. 2004, Il'ichev 2004, Trimble and El-Sayed

2005, Kutinkova et al. 2005, Mitchell et al. 2008).

Growers within the western United States have widely adopted a lure (combo lure) loaded with both pear ester and (*E, E*)-8-10-dodecadien-1-ol (codlemone) because of its higher male and total moth catch than codlemone lures (Knight et al. 2005). Unfortunately, the combo lure catches a low proportion of female moths and few pest managers have been willing to identify the sex of trapped moths (Hawkins 2008). Thus, the full potential value of utilizing pear ester to monitor female codling moth has not been realized. Studies are reported here that evaluated the effectiveness of baiting interception traps with codlemone, pear ester, or both attractants. Results suggest that opportunities exist with codling moth to develop more efficacious monitoring systems that include adult female densities.

MATERIALS AND METHODS

General methods. Studies were conducted in 2003 and 2006 in a 20-ha commercial apple orchard, *Malus domestica* (Borkhausen) situated near Moxee, WA (46° 33' N, 120° 23' W). This orchard was a mixed planting of 'Delicious' and 'Golden Delicious' with a 4.0 – 4.5 m canopy height, and a 4.8 x 5.5 m (tree x row) spacing. The orchard was certified organic and no supplemental insecticide sprays were applied during either season, except for the use of 2 – 6 applications of 1.0% horticultural oil (Orchex 796, Exxon, Houston, TX). The orchard was treated with 500 – 1,000 Isomate™ C-Plus dispensers ha⁻¹ loaded as per label with 182 mg of a 53:30:6 blend of codlemone, dodecanol, and tetradecanol (Pacific Biocontrol, Vancouver, WA).

Interception traps (0.33 x 0.33 m) were cut from rolls of 0.25 mm semi-rigid UV-stabilized film (#10SR36150, W. J. Dennis Co., Elgin, IL). A 0.5 x 2.0 cm slit was cut in the top center of each trap 1.5 cm from the edge. A 17.0 cm piece of 1.4-cm wide yellow tie-strapping (Postal Products Unlimited, Milwaukee, WI) was threaded

through this slit and used to attach each trap to an orange plastic clip (Suterra LLC, Bend, OR). Traps were coated with oil (STP Oil Treatment, STP, Fort Lauderdale, FL) using a standard paint roller (smooth texture). Interception traps were replaced every 3-7 d during studies. Interception traps were baited with proprietary codlemone, pear ester, or pear ester and codlemone lures provided by manufacturers. Septa were attached to interception traps by piercing lures with a standard paper clip and hooking the clip to the yellow strapping ca. 1-4 cm above the center top edge of the trap. The membrane lure was attached to the strapping with an adhesive pad provided on the back surface of the lure. White delta-shaped traps (28.5 x 20.0 cm) with sticky inserts (17.0 x 17.0 cm) were included in these studies for comparison (Trécé Inc., Adair, OR). Sticky liners were replaced either weekly or up to a 4-wk interval depending on their condition. Lures were replaced after 8 wks during the two seasonal studies in 2003 and 2006. All traps were placed in the upper third of the canopy, ca. 3-m. Interception traps were

clipped with the use of a pole to small branches, while delta traps were attached to a 1.3 m schedule 40 pvc pipe (Knight et al. 2006). Traps within each study were evenly randomized and spaced 15 – 30 m apart in a grid. Moths were removed from traps in the field and sexed with the aid of a microscope in the laboratory.

Baiting interception traps. Two tests were conducted to evaluate the attractiveness of interception traps baited with codlemone during 2003. The first test was conducted from 10 – 18 July with a red rubber septum loaded with 10.0 mg codlemone (Pherocon[®] CM 10X, Trécé Inc.). The baited delta-shaped trap was replicated 6-times and 10 replicates of baited and unbaited interception traps were included. In addition, six unbaited delta traps were included in the study, but none of these traps caught any moths and these data were not included in the analysis. Delta trap liners and interception traps were replaced on 15 July. A second study was conducted from 19 – 29 July using a proprietary plastic membrane lure (Biolure[®] 10X, Suterra LLC, Bend, OR). Baited delta traps were replicated nine times and 15 baited and unbaited interception traps were included. Delta trap liners and interception traps were replaced on 22 July.

Seasonal evaluation, 2003. A portion of the orchard was subdivided into eight 100 x 100 m replicate blocks. Five baited (pear ester) and unbaited interception traps, one baited (pear ester) delta-shaped trap, and one light trap (6 W blacklight bulb) baited with Dichlorvos (18.6% active ingredient, No-pest Strip[™], United Industries, St. Louis, MO) were randomly placed in a grid with a 25 x 25 m spacing within each block. The study was initiated on 13 June and all traps were checked 21 times (2-7 d

intervals) until 29 August. Data were summarized across dates based on the accumulation of degree days (lower threshold of 10 °C) from first moth flight (5 May) to the completion of the first (456 degree days) and second moth flight (1044 degree days) (Knight 2007b). Moth catch recorded after 10 July was included in the second flight period.

Seasonal evaluation, 2006. The orchard was divided into six 100 x 100 m blocks. Unbaited interception traps and interception and delta-shaped traps baited with either pear ester or the combo lure were compared. One delta-shaped trap with each lure and three interception traps of each type were placed within each block in a grid with a 30 x 30 m spacing. Traps were initially placed in orchards on 13 June and checked 20 times during the season. Cumulative moth counts for each flight were based on the accumulation of degree days from the start of moth flight (4 May). Moth catch after 6 July was included in the second flight.

Statistical analysis. The mean moth catches from each group of interception traps placed within each block (5 traps per block in 2003 and 3 traps per block in 2006) were calculated and used in the subsequent comparison with other trap types. Count data were transformed with a square root transformation and proportional data with the angular transformation to stabilize variances (Snedecor and Cochran 1967). Analysis of variance (ANOVA) was used to compare the main treatment effect for the various trap and lure combinations (Analytical Software 2003). Tukey's method was used to detect significant ($P < 0.05$) pair-wise comparisons within significant ANOVA's.

RESULTS

Baiting interception traps. Significant differences in catches of both sexes and total numbers of moths occurred among the three trap-lure combinations in tests with two different codlemone lures (Table 1).

Codlemone-baited delta and interception traps caught similar numbers of male and total moths. Both traps caught significantly more male and total moths than the unbaited interception trap. The baited and

Table 1.

Comparison of mean (SE) codling moth catches in (2003) unbaited and baited interception and baited delta traps using high-load codlemone lures.

| Lure | Trap | Mean (SE) moth catch per d ¹ | | |
|-----------|-----------------------|---|-----------------------------|----------------------------|
| | | Male | Female | Total |
| Red septa | Baited delta | 5.8 (0.5)a | 0.0 (0.0)b | 5.8 (0.5)a |
| | Unbaited interception | 1.4 (0.3)b | 1.0 (0.1)a | 2.4 (0.3)b |
| | Baited interception | 5.6 (1.7)a | 0.9 (0.1)a | 6.5 (1.7)a |
| | ANOVA: df = 2,23 | $F = 8.75$, $P < 0.01$ | $F = 111.5$ $P < 0.0001$ | $F = 6.62$, $P < 0.01$ |
| Membrane | Baited delta | 4.8 (0.4)a | 0.02 (0.01)b | 4.8 (0.4)a |
| | Unbaited interception | 0.5 (0.1)b | 1.1 (0.2)a | 1.6 (0.3)b |
| | Baited interception | 3.4 (0.9)a | 0.7 (0.2)a | 4.1 (1.1)a |
| | ANOVA: df = 2,36 | $F = 33.7$ $P < 0.0001$ | $F = 24.2$ $P < 0.0001$ | $F = 10.5$ $P < 0.001$ |

¹ Column means for each lure followed by a different letter were significantly different, $P < 0.05$, Tukey's.

unbaited interception traps caught significantly more females than the baited delta trap. Results were similar in tests using either a rubber septum or membrane lure (Table 1).

Seasonal evaluation, 2003. Significant differences in the cumulative male, female, and total moth catches during each moth flight occurred among four trap-lure combinations (Table 2). Light traps caught significantly more male and total numbers of codling moth than interception and delta traps baited with pear ester and unbaited interception traps. Pear ester-baited interception and light traps caught similar numbers of females. The baited interception traps caught significantly more female and total moths than the pear ester-baited delta traps. The unbaited interception trap caught significantly more moths than the delta trap during the first but not the second flight. The interception traps baited with and without pear ester caught similar numbers of male moths in the first flight but the baited trap caught significantly more female and total moths in the second flight. The proportion of females caught by the different lure-trap combinations varied significantly, $F_{3, 28} = 3.45$, $P < 0.05$. The light trap

caught a significantly lower proportion of female moths than the baited interception trap over the entire season. The unbaited interception and delta traps caught an intermediate proportion of female moths.

Seasonal evaluation, 2006. Significant differences in the catches of male, female, and total codling moths occurred between unbaited interception and interception and delta traps baited with either pear ester or the combo lure during both flights in 2006 (Table 3). The combo-baited delta trap caught significantly more male moths than all other trap types during both flights. The other four traps did not differ during the first moth flight in male moth captures. However, during the second moth flight the baited interception traps caught significantly more males than the unbaited interception and the pear ester-baited delta traps. The unbaited interception trap also caught significantly more male moths than the pear ester-baited delta trap.

The baited interception traps caught significantly more female codling moth than the baited delta traps during both flights (Table 3). The unbaited interception trap caught similar numbers of female moths as the pear ester-baited interception

Table 2.

Comparison of seasonal codling moth catches (2003) in interception and delta traps baited with pear ester and unbaited interception and light traps, n = 8.

| Lure - trap | Cumulative mean (SE) moth catch per trap ¹ | | | | | |
|-----------------------|---|-------------------------------------|--------------------------------------|--------------------------------------|--------------------------------------|--------------------------------------|
| | 1st moth flight | | | 2nd moth flight | | |
| | Male | Female | Total | Male | Female | Total |
| Unbaited interception | 15.8 (1.9)b | 10.8 (1.5)a | 26.5 (2.5)b | 74.4 (6.30bc) | 44.4 (5.9)b | 118.8 (7.9)bc |
| Baited interception | 18.1 (1.3)b | 19.9 (2.5)a | 38.0 (2.7)b | 159.1 (5.6)b | 128.9 (15.3)a | 288.0 (16.6)b |
| Baited delta | 1.9 (1.0)c | 0.8 (0.4)b | 2.6 (0.9)c | 21.0 (4.4)c | 16.3 (4.5)b | 37.3 (8.5)c |
| Unbaited light | 71.6 (13.3)a | 27.1 (8.1)a | 98.8 (18.6)a | 526.1 (138.9)a | 172.1 (43.7)a | 698.3 (179.0)a |
| ANOVA: df = 3,28 | <i>F</i> = 33.8 <i>P</i> < 0.0001 | <i>F</i> = 16.9 <i>P</i> < 0.001 | <i>F</i> = 34.2 <i>P</i> < 0.0001 | <i>F</i> = 22.4 <i>P</i> < 0.0001 | <i>F</i> = 17.8 <i>P</i> < 0.0001 | <i>F</i> = 22.4 <i>P</i> < 0.0001 |

¹ Column means followed by a different letter were significantly different, *P* < 0.05, Tukey's .

Table 3.

Comparison of seasonal moth catches (2006) in unbaited interception and baited interception and delta traps with pear ester and pear ester + codlemone (combo) lures, n = 6.

| Lure - trap | Cumulative mean (SE) moth catch per trap ¹ | | | | | |
|--------------------------------|---|-------------------------------------|--------------------------------------|--------------------------------------|--------------------------------------|---------------------------------------|
| | 1st moth flight | | | 2nd moth flight | | |
| | Male | Female | Total | Male | Female | Total |
| Unbaited interception | 0.6 (0.2)b | 0.8 (0.2)bc | 1.3 (0.3)bc | 9.0 (1.2)c | 6.1 (0.8)b | 15.1 (1.9)b |
| Pear ester-baited interception | 1.0 (0.2)b | 1.6 (0.4)ab | 2.7 (0.5)ab | 17.5 (1.7)b | 27.4 (2.8)a | 44.9 (4.2)a |
| Combo-baited interception | 1.3 (0.3)b | 1.8 (0.4)a | 3.2 (0.6)ab | 17.9 (1.7)b | 23.5 (2.5)a | 41.4 (4.0)a |
| Pear ester-baited delta | 0.0 (0.0)b | 0.2 (0.2)c | 0.2 (0.2)c | 2.0 (0.7)d | 0.8 (0.5)c | 2.8 (0.9)c |
| Combo-baited delta | 7.5 (2.3)a | 0.2 (0.2)c | 7.7 (2.4)a | 32.7 (6.5)a | 3.7 (1.1)c | 36.3 (7.5)a |
| ANOVA: df = 4,25 | <i>F</i> = 9.06 <i>P</i> < 0.0001 | <i>F</i> = 5.27 <i>P</i> < 0.001 | <i>F</i> = 9.01 <i>P</i> < 0.0001 | <i>F</i> = 23.2 <i>P</i> < 0.0001 | <i>F</i> = 45.0 <i>P</i> < 0.0001 | <i>F</i> = 30.2, <i>P</i> < 0.0001 |

¹ Column means followed by a different letter were significantly different, *P* < 0.05, Tukey's .

and both delta traps during the first moth flight. The unbaited interception trap caught significantly more female moths than either delta trap during the second flight.

The combo-baited delta trap caught similar numbers of total moths as the baited interception traps in both moth flights. The pear ester-baited delta caught significantly

fewer total moths than these three traps during both moth flights. The unbaited interception traps caught an intermediate number of total moths: fewer moths than the combo-baited delta in the first flight and fewer moths than the baited interception and the combo-baited delta, but significantly more moths than the pear ester-

baited delta in the second flight. The proportion of females caught by the different lure-trap combinations varied significantly, $F_{4,24} = 4.56$, $P < 0.01$. The combo-baited delta trap caught a significantly lower pro-

portion of female moths than either of the baited interception trap during 2006. The unbaited interception and pear ester-baited delta traps caught an intermediate proportion of female moths.

DISCUSSION

The clear, oil-coated unbaited interception trap has proved to be an effective tool to monitor the mating status of female codling moths and the density, distribution, and movement of both sexes in experimental orchards (Weissling and Knight 1994, 1997; Knight 1997, 2000, 2006, 2007a). Studies reported here demonstrate that baiting the interception trap with codlemone can increase male catches to levels comparable to standard delta traps and with the use of pear ester creates a more effective trap than the delta for monitoring female codling moth. Further studies with the interception trap should evaluate the use of the more potent, acetic acid and pear ester combination lure (Landolt et al. 2007).

Trap effectiveness is strongly influenced by the anemotactic flight and close-range behaviors of adult moths to both the lure and the physical structure of the trap (Foster and Muggleston 1993). Trap reflectance and moth vision appear to be critical factors influencing the capture of male codling moths in traps of various colours (Knight and Miliczky 2003, Knight and Fisher 2006). Multiple field observations of adult codling moth inside screened cages suggest that both sexes fly accidentally into the clear interception trap while moving within and through tree canopies (unpubl. data). Surprisingly, an unbaited interception trap caught significantly more total moths than a delta trap baited with pear ester.

Flight tunnel studies have revealed that a significant proportion of male codling moths orienting to codlemone lures placed inside of various white sticky traps land on the outside of the trap first, walk inside, and then become stuck (Knight et al. 2002). Switching from white to orange-colour traps increased male moth catches in the field, and flight tunnel assays suggested this

was primarily caused by increasing the proportion of males that flew directly inside the orange versus white trap, especially under low light conditions (Knight and Fisher 2006). Interestingly this difference in moth behavior between trap colours became greater as the light level was increased. This may reflect the male's response period to both codlemone and pear ester occurring primarily during scotophase (Knight and Light 2005c).

Female codling moths respond to trap colour differently than males. For example, orange and white delta traps baited with pear ester had similar catches of females in both field and flight tunnel experiments (Knight and Fisher 2006). While, direct observations of female's orientation and contact with pear ester-baited delta traps have not been reported, the diurnal response of females to pear ester-baited traps begins in the late afternoon and occurs on average earlier than the response of males (Knight and Light 2005c), and coincides with the peak timing of oviposition, 1800 – 2200 h (Riedl and Loher 1980). Thus, trap's reflectance over the UV or visible spectrum may be a more critical factor affecting female than male capture on interception traps.

The relatively high cost of maintaining interception traps likely will continue to interfere with grower adoption despite the enhanced benefits which can be derived from monitoring female codling moth. Reducing the size of traps and placing them lower in the canopy would improve their handling and servicing but would also significantly reduce moth catches (Ioriatti et al. 2003, Knight and Light 2005c). One alternative that should be explored is the use of clear delta traps. The operational advantages of a clear delta versus interception trap are that its profile is smaller so that

traps can more easily be placed in the canopy and fewer non-target insects are caught, and that the sticky liner is placed horizontally inside the trap so the coating does not run off and is protected from precipitation. Clear delta traps with clear liners are available from at least one supplier in Europe (PRI, Wageningen, The Netherlands), but

its use with pear ester for codling moth has not been reported. Studies are needed to assess whether a smaller horizontal sticky surface placed inside of a clear trap with a restricted opening would be as effective as the larger vertical surface of the interception trap.

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Aphids (Hemiptera: Aphididae) associated with rhubarb (*Rheum* spp.) in the Matanuska Valley, Alaska: species composition, seasonal abundance, and potential virus vectors

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ABSTRACT

Culinary rhubarb, *Rheum* spp., is one of the priority crop species curated by the United States Department of Agriculture (USDA) Agricultural Research Service (ARS) in Palmer, Alaska. Water-pan traps in commercial rhubarb in the Matanuska-Susitna River Valley near Palmer and in the USDA ARS *Rheum* germplasm collection caught aphids belonging to eight species: *Aphis helianthi* Monell; *Chaitophorus neglectus* Hottes and Friso, *Euceraphis betulae* (Koch); *Hayhurstia atriplicis* (L.); *Macrosiphum euphorbiae* (Thomas); *Myzus persicae* (Sulzer); *Pemphigus* spp.; and *Rhopalosiphum padi* (L.). Only three of the species (*M. euphorbiae*, *M. persicae*, and *R. padi*) collected in water-pan traps were also handpicked from rhubarb plants. The bird cherry-oat aphid, *R. padi*, was the most abundant species collected in water-pan traps and from rhubarb plants. Based on their disease transmission capability, *A. helianthi*, *M. euphorbiae*, *M. persicae*, and *R. padi*, can be considered to be of potential economic importance to rhubarb production in Alaska.

Key Words: aphids, rhubarb, *Rheum*, Alaska, vectors, germplasm

INTRODUCTION

Culinary rhubarb, *Rheum* spp. (Polygonaceae), is one of the priority crop species curated at the Subarctic Agricultural Research Unit (SARU) of the United States Department of Agriculture (USDA) Agricultural Research Service (ARS). This site in Palmer, Alaska, is the primary rhubarb repository for the USDA ARS National Plant Germplasm System (NPGS 2010) which maintains a diverse collection of plant genetic material. Currently, the SARU

Rheum collection has 41 clonal accessions (Kuhl and DeBoer 2008), some of which are infected with *Turnip mosaic virus* (TuMV) (Robertson and Ianson 2005), one of the most important diseases affecting rhubarb in Britain (Tomlinson and Walkey 1976) and in Alaska. *Turnip mosaic virus* has a large plant host range and a worldwide distribution (Stobbs and Sterling 1990, Walsh and Jenner 2002, Plant Viruses on Line 2009). The virus spreads me-

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chanically and by aphid transmission. The insect fauna associated with *Rheum* spp. is little known and there is no consensus on the aphid species associated with this plant. In a bibliography of rhubarb and other *Rheum* species, Marshall (1988) listed nine aphid species from seven genera affecting rhubarb throughout the world. Other authors reported three (Capinera 2001), or nineteen species (Blackman and Eastop

2006) of aphids associated with *Rheum* species.

There are no known published reports on aphids associated with rhubarb in Alaska. The present work was initiated to identify the aphids associated with rhubarb in the Matanuska-Susitna River Valley, Alaska, USA and to identify potential vectors of TuMV.

MATERIALS AND METHODS

Aphids associated with rhubarb were surveyed on a commercial farm in the Matanuska-Susitna River Valley near Palmer (N 61.53°, W 149.08°), Alaska. Samples were also taken from the SARU *Rheum* collection in Palmer (N 61.57°, W 149.25°). Habitat types surrounding field sites varied. The commercial farm is located in a developed rural area adjacent to large-scale vegetable production. The SARU collection is located in an isolated area surrounded by grassland and forest. Rhubarb foliage and stems were harvested weekly on the farm, while no harvesting occurred in the SARU germplasm collection. Rhubarb inflorescences were removed at both sites.

To construct a voucher collection, aphids were collected from both sites. Samples were taken weekly or bi-weekly by examining rhubarb plants selected at random from fields on the commercial farm (2005 to 2007) and in 2008 by inspecting all plants in the SARU *Rheum* collection. Collected aphids were placed in 95% ethanol, and stored for slide mounting and eventual identification by the authors using various references (Palmer 1952, Footitt and Richards 1993, Footitt and Maw 1997, Pike *et al.* 2003, Blackman and Eastop 2000, 2006) and museum vouchers. The abaxial and adaxial sides of the top three leaves of every plant in the collection were inspected every seven days during the months of August and September 2008. Aphids were also captured in water pan traps similar to those described by Stoltz *et al.* (1997). Traps were constructed by plac-

ing a 7-mm thick, yellow-green acrylic square (10 x 10 cm, Yellow 2037, United States Plastic Corp., Lima, OH, USA) in a 750-ml plastic Rubbermaid® dish (Newell Rubbermaid Company, Fairlawn, OH, USA) filled with a 0.05% soap solution (Ultra Dishwashing Liquid, Planet®, Victoria, BC, Canada). Traps were maintained at canopy height with the aid of adjustable stands (Villanueva and Peña 1991; Stoltz *et al.* 1997). Traps were placed around field perimeters just prior to rhubarb emergence and maintained until all plants were harvested (commercial field) or at first frost (SARU *Rheum* collection). A total of 33 trap stations were set (six traps/year in the commercial field and five traps/year in the germplasm collection) from May to October, 2005-2008. Traps were changed weekly and brought back to the laboratory where insects were strained from the soap solution and preserved in 95% ethanol for identification. The numbers of aphids per trap per week were combined to calculate the total number of aphids per 14-day period over the three years.

Additionally, the University of Alaska Museum of the North (UAM) insect collection was examined for aphids. The UAM collection includes the Washburn insect collection (Washburn 1972; UAM 2009), which was compiled by USDA entomologists J.C. Chamberlin, R.H. Washburn, and others during the 1940's and 1950's. This collection is considered to be the only large general insect collection maintained in the state (Pantoja *et al.* 2009).

RESULTS AND DISCUSSION

A total of 3,325 specimens representing eight species and genera were collected from water-pan traps in commercial rhubarb and the SARU *Rheum* collection (Table 1). The species include: *Aphis helianthi* Monell; *Chaitophorus neglectus* Hottes and Frison; *Euceraphis betulae* (Koch); *Hayhurstia atriplicis* (L.); potato aphid, *Macrosiphum euphorbiae* (Thomas); green peach aphid, *Myzus persicae* (Sulzer); *Pemphigus* spp.; and bird cherry-oat aphid, *Rhopalosiphum padi* (L.). All species were collected in both locations, but more aphids (72% of total) were collected from the SARU *Rheum* collection than from the commercial field. Approximately 18% of the aphids collected could not be identified. Two species, *R. padi* (34.1%) and *Pemphigus* spp. (21.3%) represented 55% of the overall number of aphids collected. *Rhopalosiphum padi* was the most abundant species, representing 26% and 37% of the aphids collected from the commercial field and the SARU *Rheum* collection, respectively. The difference in aphid counts between sites can be explained by crop association. The SARU *Rheum* collection is located in an isolated site surrounded by forest and grasses, while the commercial rhubarb field was surrounded by vegetables providing additional alternate hosts for the aphids. There were no aphid colonies on rhubarb plants, indicating that this crop does not support development and is not a preferred host for the aphid species reported here.

Examination of the UAM insect collection revealed a total of 38 specimens representing nine identified species from eight genera, but none of the specimens were associated with *Rheum* spp. (Table 2). To our knowledge, the present study represents the first report on aphids from *Rheum* spp. in Alaska.

The seasonal abundances of the two most prevalent species and of three less numerous species that are potential virus vectors (discussed below) are shown in Figures 1 and 2. Both *R. padi* and *Pemphigus* spp. were trapped from late June until

mid October, with *Pemphigus* reaching a peak in early July, and *R. padi* peaking in late August (Fig. 1). *Aphis helianthi*, *M. euphorbiae* and *M. persicae* were trapped from early June until mid October, with *A. helianthi* peaking in early July, and the other two species being present at low numbers throughout (Fig. 2).

The majority of the species collected in our study probably represent migratory aphids moving from other plant species. The second most abundant genus, *Pemphigus*, is represented by several species not easily identifiable (Footit and Maw 1997). Although the *Pemphigus* spp. complex is commonly collected in agricultural fields in Alaska (Stoltz *et al.* 1996, 1997), the distribution and biology of the complex is poorly known and there are no reports on virus transmission studies with this group (Stoltz *et al.* 1997). *Pemphigus* spp. was the prevalent species collected in potato fields in the Matanuska-Susitna River Valley of Alaska representing 23% of the water-pan trap catches (Stoltz *et al.* 1997). An unidentifiable species of the *Pemphigus* spp. complex has been reported affecting rhubarb (*R. rhaponticum*) roots in New Zealand (Savage 1982). The agricultural importance of the *Pemphigus* species complex needs attention and revision (Savage 1982, Footit and Maw 1997, Stoltz *et al.* 1997, Blackman and Eastop 2000).

Although present in low numbers, *A. helianthi*, *M. euphorbiae*, and *M. persicae* are of potential economic importance to rhubarb production. These three species along with *R. padi* are known vectors of potyviruses (Kortier and Grafius 1994, Footit and Maw 1997, Blackman and Eastop 2000). *Myzus persicae* is a known vector of TuMV on Cruciferae (Dombrovsky *et al.* 2005). *Aphis helianthi* has been associated with crops of the Compositae and Umbelliferae families, but its biology and vector capacity are not well known (Kortier and Grafius 1994, Blackman and Eastop 2006). *Macrosiphum euphorbiae* and *M. persicae* have been associated with *Rheum* spp., suggesting that they might be vectors

Table 1.

Sums and percentages of aphids captured in water-pan traps in a commercial rhubarb field and in the USDA ARS *Rheum* germplasm collection at the Subarctic Agricultural Research Unit in Palmer, Alaska, USA, during 2005-2008.

| Species | Commercial | | Collection | |
|---|------------|------|-------------|------|
| | Sum | % | Sum | % |
| <i>Aphis helianthi</i> Monell | 29 | 3.1 | 79 | 3.3 |
| <i>Chaitophorus neglectus</i> Hottes and Frison | 18 | 1.2 | 94 | 3.9 |
| <i>Euceraphis betulae</i> (Koch) | 167 | 17.7 | 247 | 10.0 |
| <i>Hayhurstia atriplicis</i> (L.) | 33 | 3.5 | 128 | 5.4 |
| <i>Macrosiphum euphorbiae</i> (Thomas) | 32 | 3.4 | 54 | 2.3 |
| <i>Myzus persicae</i> (Sulzer) | 21 | 2.2 | 24 | 1.0 |
| <i>Pemphigus</i> spp. | 189 | 20.1 | 520 | 21.8 |
| <i>Rhopalosiphum padi</i> (L.) | 244 | 25.9 | 891 | 37.4 |
| Unknown | 209 | 22.2 | 346 | 14.5 |
| Total | 942 | | 2383 | |

Table 2.

Sums and percentages of aphid species present at the University of Alaska Museum of the North in Fairbanks, Alaska, USA.

| Species | Sum | % | Host |
|--|-----------|------|--|
| <i>Aphis helianthi</i> Monell | 7 | 18.4 | <i>Cornus stolonifera</i> Michx. |
| <i>Aphis varians</i> Patch | 1 | 2.6 | <i>Epilobium angustifolium</i> L. |
| <i>Bornerina variabilis</i> Richards | 1 | 2.6 | <i>Alnus crispa</i> (Aiton) Turrill |
| <i>Euceraphis</i> sp. | 1 | 2.6 | <i>Betula resinifera</i> Britton |
| <i>Macrosiphum euphorbiae</i> (Thomas) | 17 | 44.7 | <i>Malus</i> sp., <i>Lactuca sativa</i> L. |
| <i>Nasovonia</i> sp. | 2 | 5.3 | <i>Delphinium</i> sp. |
| <i>Nearctaphis bakeri</i> (Cowen) | 1 | 2.6 | <i>Malus</i> sp. |
| <i>Nearctaphis yohoensis</i> Bradley | 1 | 2.6 | <i>Sorbus</i> sp. |
| <i>Pterocoma populifoliae</i> (Fitch) | 4 | 10.5 | <i>Populus</i> sp. |
| <i>Rhopalosiphum padi</i> (L.) | 1 | 2.6 | <i>Prunus padus</i> L. |
| Unknown | 2 | 5.3 | <i>Lonicera tatarica</i> L., <i>Cornus</i> sp. |
| Total | 38 | | |

of viruses on rhubarb plants (Marshall 1988, Capinera 2001, Blackman and Eastop 2000, 2006). To our knowledge, our report represents the first time *A. helianthi* and *R. padi* are linked with rhubarb.

Fifty-nine alate specimens representing three species, *M. euphorbiae* (n = 18), *M. persicae* (n = 6), and *R. padi* (n = 31), were handpicked from rhubarb plants, suggesting that these aphid species might serve as virus

vectors (Kortier and Grafius 1994, Footitt and Maw 1997, Blackman and Eastop 2000).

Although not collected in this study, the melon aphid, *Aphis gossypii* Glover, and the turnip aphid, *Lipaphis pseudobrassicae* (Kaltenbach), have been previously reported in Alaska (Stoltz *et al.* 1997). Both species have been associated with over 50 plant viruses, including TuMV

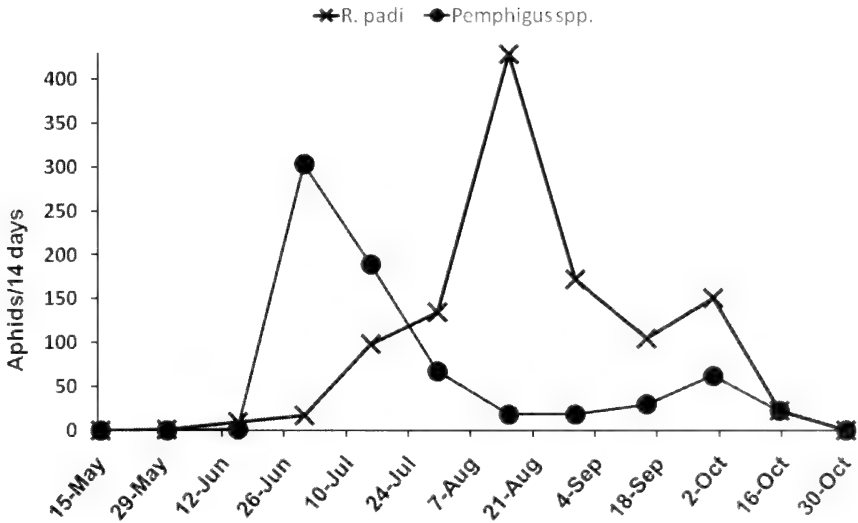


Figure 1. Sums of aphids per 14 days for the two most abundant species, *R. padi* and *Pemphigus* spp. collected with water-pan traps at two sites in proximity to Palmer, Alaska, USA, from 2005 to 2008.

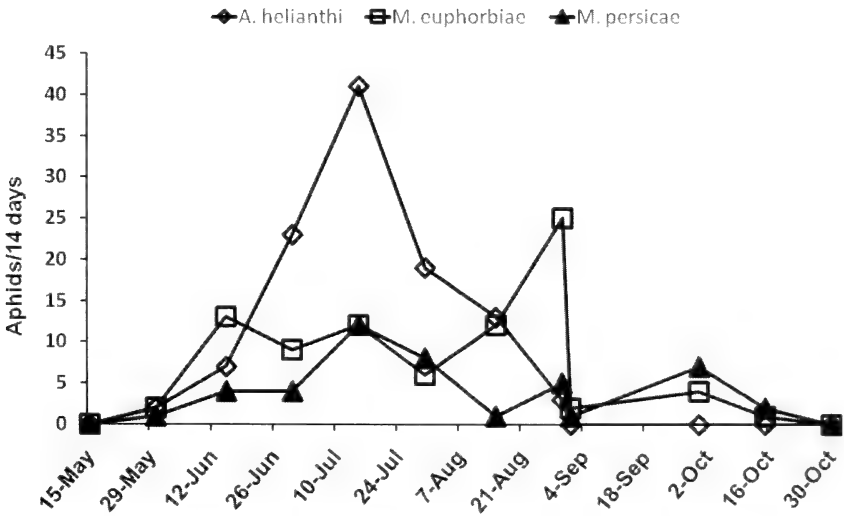


Figure 2. Sums of aphids per 14 days for the three aphid species that are potential virus vectors, *A. helianthi*, *M. euphorbiae*, and *M. persicae*, collected with water-pan traps at two sites in proximity to Palmer, Alaska, USA, from 2005 to 2008.

(Dombrovsky *et al.* 2005, Blackman and Eastop 2006). Pantoja (unpublished data) collected 48 specimens of the cabbage aphid, *Brevicoryne brassicae* (L.) in water pan traps from commercial rhubarb in Palmer, Alaska, in 2004, after a nearby cabbage field was harvested. *Brevicoryne brassicae* is another potential vector of TuMV to rhubarb (Blackman and Eastop 2000). To

our knowledge, the presence of *B. brassicae* on rhubarb in the Palmer area represents a new record for agricultural crops in the state as this species is not listed by previous reports (Chamberlin 1949, Washburn 1974, Robinson 1979, Stoltz *et al.* 1996, 1997, UAM 2009) from agricultural settings in Alaska.

Future research should investigate the

correlation between the aphid species present in Alaska and their potential association with TuMV in rhubarb fields, alternate hosts of the abundant species, and overwintering habits of the economically important

species. Research is also needed to establish the potential contribution of aphids to the spread of TuMV in the SARU germplasm collection in Palmer.

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SCIENTIFIC NOTE

New distribution records for United States Lygaeoidea (Hemiptera: Heteroptera)

G.G.E. SCUDDER¹

New state records are given for 17 lygaeoid Heteroptera species in the United States. Henry and Froeschner (1988) summarized the known state distribution for the species of Heteroptera in Canada and the continental United States. A number of new state records have been noted during recent identification of specimens from various sources. These are detailed below. I am indebted to the Museum curators listed for loan of specimens

Data cited are those on specimen labels. Museum abbreviations used in the text are as follows:

CNC: Canadian National Collection of Insects, Agriculture and Agri-Food Canada, Ottawa, ON (R.G. Foottit).

ROM: Royal Ontario Museum, Toronto, ON (D. Currie).

UCB: Essig Entomological Collection, University of California, Berkeley, CA (C. Barr).

UBC: Spencer Entomological Collection, Beaty Biodiversity Museum, University of British Columbia, Vancouver, BC (K.M. Needham).

UIM: W.F. Barr Entomological Collection, University of Idaho, Moscow, ID (W.F. Barr).

USNM: National Museum of Natural History, Smithsonian Institution, Washington, DC (T.J. Henry).

UNITED STATES NEW RECORDS.

Family GEOCORIDAE

Geocoris atricolor Montandon

MT: 1♀, Missoula, 9.vi.1985 (G.G.E. Scudder) [USNM].

Family HETEROGASTRIDAE

Heterogaster flavicosta Barber

AR: Yell Co., Mt. Nebo St. Pk., ca 10

mi SW Russellville on Ark. Hwy. 155, along trail, 1800', 9.v.1984 (R. Jaagumagi, R. Vineyard) [ROM #840015c].

Family LYGAEIDAE

Nysius angustatus Uhler

MT: 1♀, Apgar, Glacier, 9.ix.1966 (G.G.E. Scudder) [USNM]; 1♂, Bozeman, 19.vii.1929 (W. Downes) [UBC]; 3♂ 3♀, *id.*, 17.vii.1936 (W. Downes) [UBC]; 3♀, Logan Pass, 6664', 9.ix.1966 (G.G.E. Scudder) [CNC].

Nysius raphanus Howard

GA: 1♂, Millen, 25.viii.1957 (J.G. Chillcott) [CNC]. NH: 1♀, Lakes of the Clouds, Mt. Washington, 5000', 4.viii.1954 (Becker, Munroe & Mason) [CNC]; 1♂, *id.*, 9.viii.1954 (Becker, Munroe & Mason) [CNC]. SC: 1♀, Aiken, at light, 11.vi.1957 (J.R. Vockeroth) [CNC]; 1♂, *id.*, 12.vi.1957 (J.R. Vockeroth) [CNC]; 1♀, *id.*, 12.vi.1957 (W.R.M. Mason) [CNC]; 1♂ 1♀, *id.*, 23.vi.1957 (W.R.M. Mason) [CNC]; 1♀, Kirksey, 24.vi.1957 (W.R.M. Mason) [CNC]; 9♂ 6♀, Seneca, 20.viii.1957 (L.A. Kelton) [CNC]; 2♂ 2♀, *id.*, (W.R. Richards) [CNC].

Xyonysius californicus (Stål)

TN: 1♀, Indian Gap, 5200', Great Smoky Mt. N.P., 8.vii.1957 (W.R.M. Mason) [CNC].

Family OXYCARENIDAE

Crophius scabrosus Uhler

NV: 4♂ 4♀, Carson City, 25.vi.1929 (R.L. Usinger) [UCB]; 6♂ 8♀, Washoe County, Pyramid, *Juniperus* sp., 4-5.vii.1947 (R.L. Usinger) [UCB].

Family RHYPAROCHROMIDAE

Subfamily PLINTHISINAE

Plinthisus americanus Van Duzee

ID: 1♀, Lemhi Co., 8 mi S. Tendoy,

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- 10.ix.1969 (W.F. Barr) [UIM].
 Subfamily RHYPAROCHROMINAE
 Tribe DRYMINI
Scolopostethus diffidens Horváth
 NV: 1♂ 1♀, Lake Tahoe, Zephyr Cove, 1900 m., 9.xii.1986 (A. Smetana) [CNC].
Scolopostethus thomsoni Reuter
 NV: 1♂ 1♀, Lake Tahoe, Zephyr Cove, 1900 m., 9.xii.1986 (A. Smetana) [CNC].
 Tribe GONIANOTINI
Trapezonotus arenarius Linnaeus
 ID: 1♀, Kootenai Co., Worley, sweeping alfalfa, 30.iv.1953 (W.F. Barr) [UIM].
 Tribe LETHAEINI
Xestocoris nitens Van Duzee
 AR: 1♂, Logan Co., Cove Lk., 9 mi SE Paris, ex debris at edge of lake, 25.v.1986 (J.M. Campbell) [CNC].
 Tribe MEGALANOTINI
Megalonotus sabulicola (Thomas)
 MT: 1♀, Glacier Nat. Park., L. McDonald, 13.vi.1985 (G.G.E. Scudder) [CNC]; 1♂ 1♀, Missoula, 9.vi.1985 (G.G.E. Scudder) [CNC].
Spragisticus nebulosus (Fallén)
 VT: 1♂, Essex Jct., 15.x.1952 (J.F. Brimley) [CNC].
 Tribe MYODOCHINI
Ligyrocoris sylvestris (Linnaeus)
 NM: 1♀, Jemez Sprs., 23.viii.1972 (L.A. Kelton) [CNC]. WA: 1♀, Newport, Pioneer Park, 8.ix.1966 (G.G.E. Scudder) [CNC].
Perigenes constrictus (Say)
 MN: 2♂ 1♀, Minneapolis, 28.viii.1969 (A.B. Acton) [USNM].
 Tribe RHYPAROCHROMINI
Cordillonotus stellatus Scudder
 ID: 1♂, Ada Co., Black's Cr. Res., 30.vi.1963 (A.R. Gittins) [UIM].
 Tribe UDEOCORINI
Neosuris castanea (Barber)
 WA: 1♂, Oroville, E. Osoyoos L., 48° 58'N 119°25'W, *Purshia* assoc., AN BGxh1, Pitfall trap 04-4, 5.v-30.v.1994 (G.G.E. Scudder) [CNC]; 1♀, *id.*, trap 04-5 (G.G.E. Scudder) [CNC]; 1♀, *id.*, trap 05-1 (G.G.E. Scudder) [CNC]; 1♀, *id.*, trap 04-4, 2.viii-6.ix.1994 (G.G.E. Scudder) [CNC].

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SCIENTIFIC NOTE

The Schizopteridae (Hemiptera), a family new to CanadaG.G.E. SCUDDER¹

The Schizopteridae is a very small family of minute bugs belonging to the Infraorder Dipsocoromorpha (Stys 1995). These insects are typically extremely small, and have antennae with the first two segments very short, while the third and fourth antennal segments are longer and thinner, with many long, thin, erect or semi-erect setae.

Keyed by both Slater and Baranowski (1978) and Stys (1995), the Schizopteridae are 0.8 to 2.00 mm long. The forewings are convex, strongly sclerotized and beetle-like, but they overlap slightly along the midline. Characteristically, these bugs have the prosternum inflated and produced ventrally so as to enclose the fore coxae and the ventral surface of the head. The hind coxae are also peculiar in having the inner surface provided with a pair of roughened pads which are used in conjunction with a metasternal spine as a jumping organ.

The family has a worldwide distribution and is primarily tropical or subtropical, with at least 35 genera and about 120 described species (Stys 1995). Only four genera and four species are reported to occur in North America (Henry 1988; Henry et al. 2010). Males have three tarsal segments on each leg, while females have two segments on the fore and middle legs, and three segments on the hind legs. The four known North American taxa have been keyed by Baranowski and Slater (1978).

While *Glyptocombus saltator* Heide-mann is reported from several of the United States (AR, DC, GA, MD, MI, TN, VA,

WA), *Corixidea major* McAtee & Malloch is known from Arkansas, Florida, Oklahoma, Tennessee and Virginia, while *Nannocoris arenarius* Blatchley is recorded from Florida, Georgia, North Carolina and Virginia (Henry et al. 2010). *Schizoptera bispina* McAtee & Malloch is restricted to Florida in North America (Henry 1988). This note records the first occurrence of the family in Canada. The record is based on 1♂ with the data: "CAN: BC: Vancouver, Pacific Spirit Pr. Pk., 26.vi.1997, Colls. J. Lea, A. Klimaszewski, ex forest edge". The specimen (Fig. 1) is 1.33 mm long and is deposited in the Canadian National Collection of Insects, Agriculture and Agri-Food Canada, Ottawa, ON.

This British Columbia specimen is not any of the genera or species keyed in Slater and Baranowski (1978). Dr. T.J. Henry has examined the specimen and informs me that it appears to be new to science. However, not being an expert on the family involved, I am inclined not to describe it at the present time. The elucidation of the correct identity must remain a future task.

Elsewhere, the Schizopteridae most frequently occur in damp soil and in forest litter. Little is known of their biology and feeding habits, but they are thought to be predators (Slater and Baranowski 1978).

I am indebted to Dr. T.J. Henry for his advice. Don Griffiths kindly took the photograph presented as Figure 1. Launi Lucas processed this note.

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Figure 1. Family Schizopteridae, 1♂, CAN: BC: Vancouver, Pacific Spirit Pr. Pk., 26.vi.1997, Colls. J. Lea, A. Klimaszewski, ex forest edge. Photograph by D. Griffiths.

SCIENTIFIC NOTE

Range expansion and hosts of *Ctenophthalmus pseudagyrtis* Baker (Siphonaptera: Ctenophthalmidae) in central Alaska

G.E. HAAS¹, N. WILSON², J.R. KUCERA³, T.O. OSBORNE⁴,
J.S. WHITMAN⁵ and W.N. JOHNSON⁵

Traub (1980, 1985) noted that Eocene mammal data account for *Ctenophthalmus* in the western hemisphere. The Nearctic flea *Ct. pseudagyrtis* originated from an African ancestor that accompanied its host rafting in the south Atlantic from Africa to South or Middle America while continents were much closer together in the early Eocene epoch (Traub 1980: pp. 144-145, 161; 1985: pp. 368-406). Traub (1980: p. 119) commented "The host versatility exhibited by *Ctenophthalmus* is exceptional...". He gave examples of "Extra Ordinal fleas within Two or More Orders of Host" including "*Ctenophthalmus* (*C.*) *agyrtis*, etc." on "microtines, murids, sorcids, etc." (Traub 1985: Table 8.15). Traub (1980: pp. 142-143) had observed "The range of its main host, *Microtus*, greatly exceeds that of *C. (N.) pseudagyrtis*." Traub (1980: pp. 142-145) was especially puzzled by the anomalous range of the only *Ctenophthalmus* species in North America being limited in the West by the Rocky Mountains and the North by Southern Canada. He and Holland overlooked collections in central Alaska, Arizona, and New Mexico. Only preliminary records for Alaska were published (Haas *et al.* 1989). Five new Alaska *Ct. pseudagyrtis* and two new host *Myodes rutilus* (Pallas) records are given in New Material Examined and Fig. 1 of the present report.

New material examined: USA, Alaska, Galena, 15 km WNW, N bank Yukon River: 1♀ ex *Sorex cinereus*, 21-vii-1988, T. O. Osborne 4520. McGrath vicinity, Kuskokwim River watershed, 1♂ ex *My. rutilus*, 29-vi-1989, J. S. Whitman; 1♂ same data but pool of 8 *My. rutilus*, 7-21-vii-1989. Nowitna NWR, NW bank Little Muddy River, Hades Lake vicinity, Nowitna River watershed, 64°38'N, 154°00'W, 1♂, 1♀ ex *Microtus xanthognathus* (Leach), 9-ix-1995, W. N. Johnson 421; same data but 1♀, 9-ix-1996, WNJ 383.

One interior Alaska watershed with *Ct. pseudagyrtis* reported by Haas *et al.* (1989, Fig. 1) increased to three, i.e. from north bank Yukon River west of Galena to Nowitna River and Kuskokwim River above McGrath. Tributaries of the latter two rivers interdigitate at elevations below 305 m to provide potential for small mammal populations to move between these watersheds.

This topography can account for the range of *Mi. xanthognathus* having a southwest marginal record near the mouth of the Takotna River (Hall 1981, Map 460). That location is directly across from the Kuskokwim River oxbow in which McGrath is located. The ectoparasite study area was within 1.6 km of the city.

The habitat was second-growth deciduous forest interspersed with upland grassy areas. Understory was a heavy grass/forb type. At the Nowitna NWR 1985 burn study areas, *Mi. xanthognathus* habitat was "tall shrub-sapling stage of early successional forest," with some patches of mature conifers, moss and herbs (Paragi *et al.* 1996). Similarities between the two study areas suggest that both provided habitats favored by taiga voles for their post-fire vegetation (Conway and Cook 1999).

Myodes rutilus stands apart from the other hosts by being amphiberingean and without previous *Ct. pseudagyrtis* records. However, the southern-ranging Nearctic congener southern red-backed vole, *My. gapperi* (Vigors) is listed as a major host of *Ct. pseudagyrtis* in Canada by Holland (1949, 1985) with 43 collections. In contrast, the northern red-backed vole ranges from Alaska across Canada to Hudson Bay north of the southern red-backed vole range (Holland 1985, Map 86), without any record of being a host in Canada (Holland 1985, p. 489, Map 34). Updated distribution maps of the two red-backed voles are in MacDonald and Cook (2009: Maps 20, 21).

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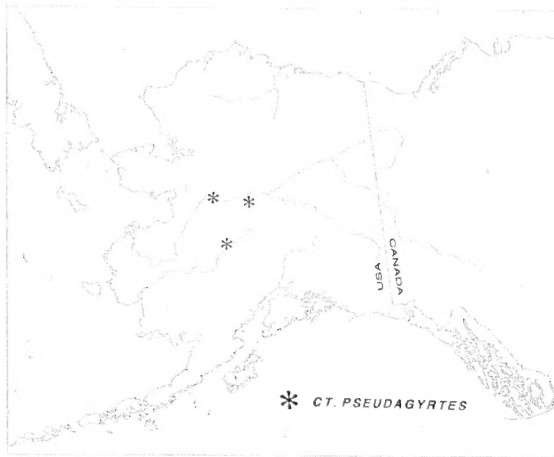


Figure 1. Locations of three study areas in Alaska where *Ctenophthalmus pseudagyrtis* specimens were collected. Upper left symbol marks north bank Yukon River, 15 km WNW of Galena, lower symbol marks McGrath, Kuskokwim River and upper right symbol marks Hades Lakes, Nowitna NWR.

Ctenophthalmus pseudagyrtis had a large literature with many genera and species of small mammal hosts, mainly Nearctic shrews, mice and voles in North America north of Mexico (Benton 1980, Holland 1985, Haas *et al.* 1989, Fagerlund *et al.* 2001, etc.) and in certain biogeographic provinces of Mexico (Morrone *et al.* 2000). *Sorex cinereus* and especially *Microtus pennsylvanicus* are hosts that range widely across the continent. The taiga vole, however, despite ranging from Hudson Bay across much of Alaska (Hall 1981, Map 460; MacDonald and Cook 2009: Map 19) only has *Ct. pseudagyrtis* records in Alaska. Adult specimens of *Ct. pseudagyrtis* were scarce and no larvae were found. Yet 160 of 291 vole nests were positive for 2420

adult fleas (Haas 1982). These were vole fleas of 14 taxa with inclusion of 618 adults of 10 taxa reared from 29 of the nests. Specimens of *Ct. pseudagyrtis* were not found.

New records of three more *Ct. pseudagyrtis* ex two taiga voles in Nowitna NWR remind us of the hypothesis (Haas *et al.* 1989) that fossil records of Zakrzewski (1985) show where taiga voles shifted from northeast and Midwest US states northwest to Alaska and could have carried *Ct. pseudagyrtis* with them. A recent review of *Mi. xanthognathus* fossils in Alaska and a new taiga vole distribution map are presented by MacDonald and Cook (2009: pp. 97-98, Map 19).

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