INCIDENCE OF SPICULES ON THE AEDEAGI OF *CHORISTONEURA FUMIFERANA*, *C. BIENNIS*, AND *C.* UNIDENTIFIED SPECIES (LEPIDOPTERA: TORTRICIDAE)

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Abstract

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Adults of *Choristoneura fumiferana, C. biennis*, and an unidentified species reared from larvae or caught in pheromone traps from British Columbia were used for isozyme analysis to confirm the species identity, and for morphological study to determine the percentage of individuals with spiculate aedeagi. About 82% of *C. fumiferana* and less than 3% of both *C. biennis* and *C.* unidentified species (CPG) have spicules on the left side of their aedeagi. The number of moths that need to be sampled to determine a species is presented, based on the presence or absence of spicules.

Gray, T.G., R.F. Shepherd et G.T. Harvey. La fréquence des spiculé sur l'édéage de trois espèces de *Choristoneura* (Lepidoptera: Tortricidae). *The Canadian Entomologist* **127**: 161–166.

Résumé

Des papillons adultes de *Choristoneura fumiferana,* de *C. biennis* et d'une espèce indéterminée issus de larves ou capturés dans un piège à phéromones en Colombie-Britannique ont été utilisés dans une analyse des isoenzymes pour confirmer l'identité des espèces, et dans une étude morphologique pour déterminer la proportion des individus à édéage spiculé. Environ 82% des papillons de *C. fumiferana* et moins de 3% de ceux de *C. biennis* et de l'espèce indéterminée portaient de spicules du côté gauche de leur adéage. Le nombre de papillons devant être échantillonnés pour déterminer une espèce est indiqué, d'après la présence ou l'absence de spicules.

[Traduit par la Rédaction]

Introduction

Members of the genus *Choristoneura* are difficult to distinguish morphologically at the species level (Harvey 1985; Shepherd 1985). Dang (1985) presented a key for the separation of males of six species. An important feature of this key was the presence or absence of spicules on the aedeagus (Fig. 1). This feature was used to separate the spicule-bearing eastern species, *C. fumiferana* (Clemens) and *C. pinus* Freeman, from the western species with smooth aedeagi, namely, *C. occidentalis* Freeman, *C. biennis* Freeman, *C. orae* Freeman, and *C. lambertiana* (Busck). Subsequently, two unidentified species, *C.* unidentified species (CR) [(CR) = Richmond] collected on *Pinus sylvestris* L. in Richmond, B.C. (Gray and Slessor 1989) and *C.* unidentified species (CPG) [(CPG = Prince George] collected on *Pinus contorta* var. *latifolia* near Prince George, B.C. (Gray and Gries 1993), were also found to have smooth aedeagi. Unfortunately, this criterion was not always reliable, and exceptions were found, particularly with *C. fumiferana*. We undertook this study to determine the proportion of *C. fumiferana* populations that had no spicules based on specimens in collections from Ontario and northern British Columbia and compared the results with the incidence of spicules on the two species it potentially contacts in central British Columbia, *C. biennis* and *C.* unidentified species (CPG). The geographic ranges of *C. biennis* and *C. fumiferana* are essentially allopatric but do come together at Pine Pass, B.C.; *C.* unidentified species (CPG) may have considerable overlap with *C. fumiferana* and the two may be sympatric from Pine Pass to at least Fort Nelson, B.C.

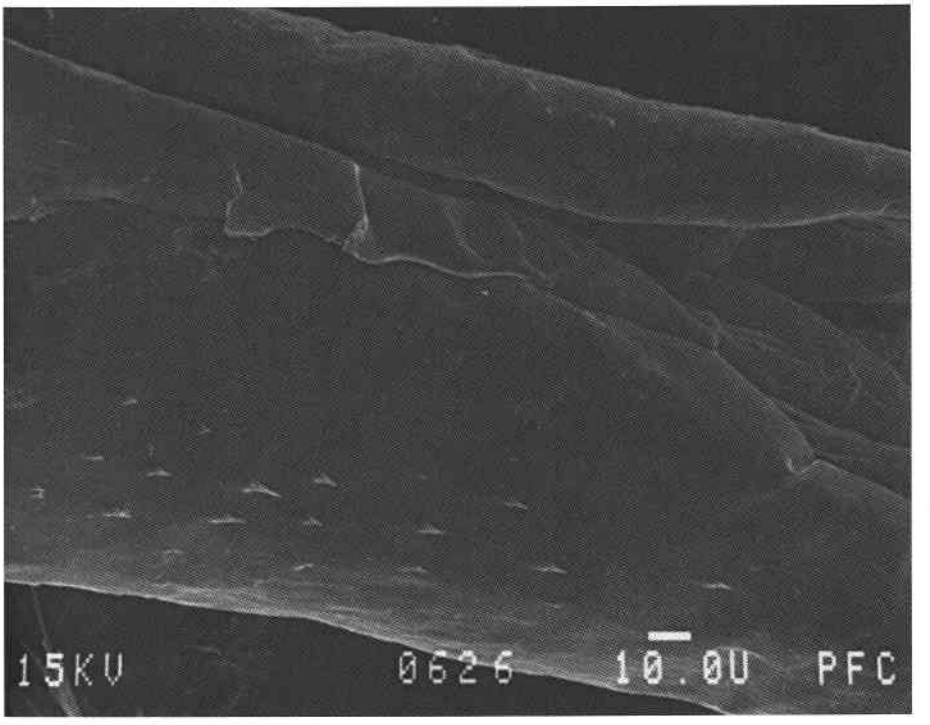

FIG. 1. Scanning electron micrograph of an aedeagus of *C. fumiferana* illustrating the spicules, on the left lateral side, useful in identifying this species.

Materials and Methods

Collections of larvae were made from *Abies* and *Picea* in 1988 and reared to adults; these included three samples of *C. fumiferana* [Ear Falls, Ont. (50°30′N, 94°30′W); Fort Nelson, B.C. (59°25′N, 123°15′W); and Liard Hot Springs, B.C. (59°25′N, 126°07′W)] and one sample of *C. biennis* [MacKay River, B.C. (52°21′N, 120°38′W)]. In addition, G. Harvey collected adults of *C.* unidentified species (CPG) and *C. biennis* in sticky pheromone traps at a number of locations from Davie Lake, B.C. (54°32′N, 122°42′W) to Fort Nelson, B.C. (59°48′N, 122°42′W). *Choristoneura* unidentified species (CPG) responds to an acetate pheromone and *C. biennis* to an aldehyde pheromone [Shepherd, R.F., T.G. Gray, and G.T. Harvey, Geographical distribution of *Choristoneura* species (Lepidoptera: Tortricidae) feeding on *Abies, Picea* and *Pseudotsuga* in western Canada and Alaska, manuscript in preparation]. Moths reared from larvae and those captured in traps were killed and stored in liquid nitrogen until use.

Moths were processed individually by electrophoresis to determine isozymes at nine enzyme loci, following techniques described previously (Harvey and Sohi 1985). Isozyme patterns at most loci measured are similar among all conifer-feeding *Choristoneura* [Stock and Castrovillo 1981; Harvey, G.T., Genetic relationships among *Choristoneura* species (Lepidoptera: Tortricidae) in North America as revealed by isozyme studies, manuscript in preparation]. However, at one locus, aspartate aminotransaminase (AAT-1), the pattern in *C. fumiferana* is unique and allozymes C and D together are present in more than 95% of *C. fumiferana* and allozyme E is always less than 5% in *C. fumiferana*. In the other species, the predominant allozyme E is present in more than 95% of *C. biennis, C. orae*, and other

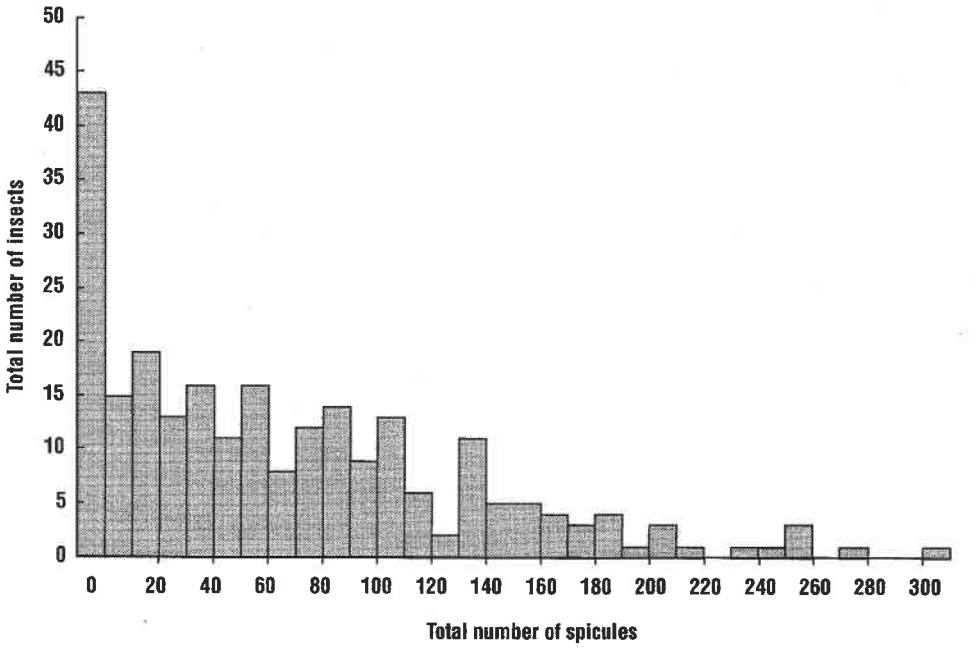

FIG. 2. Frequency distribution of the total number of *C. fumiferana* by spicule density class on the left dorsal, lateral, and ventral sides.

western entities, as well in *C. pinus*. As a result, this enzyme gives better than 95% certainty in separating *C. fumiferana* from any of the other species but is of no use in differentiating the other species.

Abdominal tips containing the genitalia were excised prior to freezing and stored in 70% ethanol. Tips were prepared for analysis by being placed in 20% KOH at room temperature, heated to 90°C, and held at that temperature for approximately 5 min (longer heating destroys the tissues). Each aedeagus was teased away from other genitalic tissues in 70% alcohol, washed in a second bath of 70% alcohol, and mounted using Gurr's medium. The aedeagus was placed with the left side exposed. Spicules on the left, dorsal, lateral, and ventral surfaces were counted using a compound microscope at ×400. Spicule counts were collated with the isozyme genotype information for each individual in the four collections. The isozyme genotype, plus location and host, provided positive species identification; there was no evidence of intermediates between species [Harvey, G.T., Genetic relationships among *Choristoneura* species (Lepidoptera: Tortricidae) in North America as revealed by isozyme studies, manuscript in preparation].

The sample size necessary to distinguish between species with different spicule incidence was determined based on the standard error of the proportion of each species bearing spicules (Snedecor 1980, p. 498).

Results and Discussion

The very low frequencies of allozyme E confirmed that the three larval collections from Ear Falls, Ont., Fort Nelson, B.C., and Liard Hot Springs, B.C., were *C. fumiferana*. Likewise, isozyme patterns of the MacKay River collection confirmed that it was not *C. fumiferana*. Even though the results do not permit differentiation from other western

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TABLE 1. Number of spicules observed on the dorsal, lateral, and ventral surfaces of the aedeagus of two species of *Choristoneura*

	C. fumiferana		*C. biennis*	
Specimen	Left side	Right side	Left side	Right side
1	97	92	0	0
2	36	40	0	0
3	41	40	0	0
4	44	49	0	0
5	224	197	0	0
6	11	15	0	0
7	55	44	0	0
8	6	6	0	0
9	0	0	0	0
10	0	0	0	0
11	71	66	0	0
Average	53	50	0	0

species, the only western species known to feed on *Abies* and *Picea* in this area is *C. biennis*. All specimens collected in the acetate traps from Davie Lake to Fort Nelson were found to be *C.* unidentified species (CPG). It is morphologically and behaviourally different from the other two acetate pheromone responders in northern Canada, i.e. *C. orae* and *C. pinus* (Freeman 1967); no adults of the latter two species have ever been reared from the many larval collections that have been made in this area.

Spicule numbers are in general agreement with expectations (Dang 1985), i.e. many individuals of *C. fumiferana* and very few *C. biennis* had spicules. Within *C. fumiferana* there was an unusual frequency distribution of individuals per spicule density class. A moderate number of individuals had no spicules, but a gradual reduction occurred thereafter in the number of individuals per spicule density up to 307 spicules per half-aedeagus (Fig. 2); there was no secondary peak density. Because of this non-normal frequency distribution, no further analysis was carried out on the basis of spicule density, but the percentage of individuals within a population with or without spicules proved to be a useful criterion. The fact that spicule counts were made on the left side of the aedeagi raised the question "Was there complete symmetry on this organ?" Therefore a subsample of aedeagi was viewed from both sides (Table 1) and showed the occurrence of spicules to be similar on both sides.

There was no significant difference in percentage of individuals having spicules among the three *C. fumiferana* populations: Fort Nelson, B.C.—79% ($n = 84$); Liard Hot Springs, B.C.—83% ($n = 90$); and Ear Falls, Ont.—86% ($n = 69$). The average of all populations was about 82%. This is in contrast to both the *C. biennis* and *C.* unidentified species (CPG) populations in which less than 3% ($n = 90$ and 83, respectively) of individuals had spicules. Dang (1992) stated that 97% of all males of both *C. fumiferana* and *C. pinus* across their geographic range in Canada have spiculate aedeagi but provided no supporting data. Our studies of *C. fumiferana* in both Ontario and northern British Columbia indicated a much lower proportion of males (82%) to have spiculate aedeagi, which decreases the value of this diagnostic character for individual moths although it is useful if a series of adults is available. Near Pine Pass, B.C., where *C. fumiferana* and *C. biennis* come together, isozyme studies indicated that individuals were clearly one species or the other with no identifiable hybrids appearing in the collections [Shepherd, R.F., T.G. Gray, and G.T. Harvey, Geographical distribution of *Choristoneura* species (Lepidoptera: Tortricidae) feeding on *Abies, Picea* and *Pseudotsuga* in western Canada and Alaska, manuscript in preparation].

TABLE 2. Sample sizes required to allow 95% confidence in the conclusion that the proportion of individuals with spicules is the same in the sample as in the general population of that species

	C. biennis or *C.* unidentified sp. (CPG)			
Sample size	5–17	18–27	28–38	39–47
Maximum number of individuals with spicules	0	1	2	3
	C. fumiferana			
Sample size	19	29	45	63
Minimum number of individuals with spicules	15	20	30	40

However, the percentage of males with spiculate aedeagi attracted to the aldehyde bait was only 63% ($n = 35$) and the frequency of the E allozyme was higher than expected indicating that both species are probably present. More work is required to clarify the species present at this location.

Species determinations at low population densities often are made using collections from pheromone traps. The physical condition of such specimens is usually poor, but the aedeagi are usually intact and the proportion of individuals that have spicules is a useful criterion for separating *C. fumiferana* from western species. Assuming the proportions with spicules found in this study to be representative of populations in general, the minimum number of individuals that must be sampled to confirm, with 95% confidence, that a sample is not significantly different from the parameter of a species is given in Table 2. For a specified sample size of suspected *C. biennis* or *C.* unidentified species (CPG) male moths, if the number of individuals with spicules is equal to or less than the corresponding number in Table 2, then the sample is probably *C. biennis* or *C.* unidentified species (CPG). The latter two can be distinguished by the attractant used in the pheromone traps. For a specified sample size of suspected *C. fumiferana* male moths, if the number of individuals with spicules is equal to or greater than the corresponding number in Table 2, then the sample is probably *C. fumiferana*. If the number of individuals with spicules is between the numbers of both species, then there could be a mixture of species at that location, and we currently lack taxonomic means to separate them.

Acknowledgments

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