PRACTICAL TECHNIQUES AND ACCURACY FOR SEXING THE POTATO TUBERWORM, *PHTHORIMAEA OPERCULELLA* (LEPIDOPTERA: GELECHIIDAE)

SILVIA I. RONDON[1] AND LAN XUE[2]
[1]Oregon State University, Hermiston Agricultural Research and Extension Center, Hermiston, OR 97838

[2]Oregon State University, Department of Statistics, Corvallis, OR 97330

Numerous life history studies of the potato tuberworm, *Phthorimaea operculella* Zeller (Lepidoptera: Gelechiidae), a serious pest that affects potato production worldwide, have been done (Langford & Cory 1932; Mukherjee 1949; Bartolini 1951; Stanev & Kaitazov 1962; Al-Ali et al. 1975; Foot 1979, Chauhan & Verma 1991; Rondon 2009). Mukherjee (1949), Bartolini (1951), and Chauhan & Verma (1991) studied dimorphism of this species. Sexual dimorphism does not become evident until yellowish testes become visible in the fourth instar; gradual change in eye pigmentation shows different pupal stages (yellow, early red, middle red, late red, and black eye pupa) (Chauhan & Verma 1991). Mukherjee (1949) found that male pupae have a round "pit-like genitalia aperture" in the middle of the 9th segment, while females have a single aperture on the 8th segment. No one has provided a practical description of pupal differentiation. Our objectives were (1) to sex male and female *P. operculella* pupae and adults by using external morphological characteristics; and (2) to determine the accuracy of sex ratios by comparing 2 different sexing methods.

Rondon et al. (2009) protocol was followed to rear *P. operculella* pupae and adults. Forty-eight-h-old pupae in silken cocoon were collected from the colony, and pupae were freed from the cocoon by immersing pupae in 1% aqueous bleach (Dogramaci et al. 2008) for about 2 min, after which clean pupae were harvested. We used the "width" and "scar" method to sex the pupae. The last abdominal segment is round in males and pointed in females ("width" method), while there is a suture on the 8th or 9th abdominal segment ("scar" method). Twenty pupae were randomly separated into 4 groups and replicated 5 times (n = 400 pupae). Each experiment was conducted by 3 samplers to test accuracy in sampling. Forty-eight-h-old adults were sexed based on dark-spot patterns or the "wing" method (2-3 dots on males; "X" on females) and end of the abdomen shape or the "genitalia" method (thick set of hairs on males). Fifteen adults were randomly separated into 4 blocks replicated 5 times (n = 300 adults). All analyses were conducted with the statistical software program SAS version 9.1 (SAS Institute 1997). In both pupal and adult studies, a paired t test was used to determine if the sex ratios obtained from the 2 sexing methods were significantly different. A generalized estimating equation (GEE) (SAS Software) method was used to compare the number of unclassified cases between 2 methods and to assess whether there was a sampler effect. The GEE was used to account for possible correlation arising from the fact that 3 samplers were checking the same samples. It was performed by PROC GENMOD in SAS.

Male pupae measured 6.4 ± 1.2 mm in length, while female pupae measured 6.6 ± 1.1 mm in length. Females have a suture located approximately 0.59 ± 0.10 mm from the end of the abdomen and males have the suture at 0.38 ± 0.08 mm. The number of females and males determined from both characteristics are summarized in Table 1 for each of 3 different samplers. The "width" method has fewer unclassified cases (P < 0.0001) than the "scar" method with the averaged percentages of unclassified for the "width" and "scar" methods being 0.88% and 20.22%, respectively. A sex ratio of 0.90 was established by averaging "width" sex ratios from 3 samplers. The sexing results based on 2 methods were not different (P = 0.2708) with averaged sex ratio from the "width" and "scar" methods being 0.90 ± 0.01 and 0.83 ± 0.04, respectively. Table 2 shows that only about 83% of the 182 unclassified cases by the "scar" method were classified as female by the "width" method, which explains the lower female to male ratio by the "scar" method.

Adult sexes differ from each other based on dots present in the forewings and tips of the abdomen including genitalia. Females present a characteristic patch of dots in a "cross shape" on the wings, while males present 3 sets of clearly defined dots. The adult male's terminal abdominal segment is broader than that of adult females. The number of females and males determined from both characteristics are summarized in Table 1 for each of 3 different samplers. Similar to the pupal study, the "genitalia" method was found to have fewer unclassified cases (P < 0.0001) than the "wing" method, with the average of unclassified for the "genitalia" and "wing" methods being 0.89% and 12.56%, respectively. A sex ratio of 0.95 was obtained by averaging "genitalia" sex ratios from 3 samplers. The sexing results based on the 2 methods were different (P = 0.0442) with average sex ratios from the "genitalia" and "wing" methods being 0.95 ± 0.01 and 0.82 ± 0.03, respectively. Table 2 shows that about 73% of the 113

TABLE 1. RATIOS OF FEMALE/MALE *P. OPERCULELLA* PUPAE AND ADULTS BASED ON MORPHOLOGICAL CHARACTERISTICS FOR 3 SAMPLERS (N = 400 PUPAE, 300 ADULTS).

		Sampler					Sampler		
		1	2	3			1	2	3
Methods	Sex	Pupae			Methods	Sex	Adults		
Scar	F	162	146	153	Genitalia	F	147	143	145
	M	179	192	186		M	152	153	152
	NA	59	62	61		NA	1	4	3
	F:M	0.91	0.76	0.82		F:M	0.97	0.93	0.95
Width	F	187	187	192	Wings	F	116	114	124
	M	210	210	206		M	147	143	143
	NA	3	3	2		NA	37	43	33
	F:M	0.89	0.89	0.93		F:M	0.79	0.80	0.87

F: Female, M: Male, NA: Unclassified, F:M: Female to Male ratio.

TABLE 2. AGGREGATED RATIOS OF PUPAE AND ADULTS MALES AND FEMALES FROM 3 SAMPLERS FOR *P. OPERCULELLA* TUBERWORM PUPAE.

		Pupae					Adults				
		WIDTH					GENITALIA				
	Frequency	F	M	NA	Total		Frequency	F	M	NA	Total
SCAR	F	409	52	0	461	WINGS	F	352	0	2	354
	M	6	551	0	557		M	0	433	0	433
	NA	151	23	8	182		NA	83	24	6	113
	Total	566	626	8	1200		Total	435	457	8	900

F: Female, M: Male, NA: Unclassified.

unclassified cases by the "wing" method were classified as female by the "genitalia" method.

We thank Chiho Kimoto, Sara Carlson, and Sandra DeBanno for assistance. We thank Mary K. Corp and Arnold P. Appleby (Oregon State University), and Oscar Liburd (University of Florida) for reviews of the manuscript. This research was supported by the Agricultural Research Foundation.

SUMMARY

Sexual dimorphism between *P. operculella* females and males is evident in the pupal and adult stage. Male pupae are slightly smaller than female pupae and have a suture located further away from the end of the abdomen. The "scar"/"width" (pupae) and "wings"/"genitalia" (adults) methods are not entirely consistent. The "width" and the "wings" methods give a lower ratio than the "scar" and "genitalia" method, therefore overestimating the male/female ratio.

REFERENCES CITED

AL-ALI AS AL-NEAMY, I. K., ABBAS, S. A., AND ABDULMASH, A. E. M. 1975. Observations on the biology of the potato tuber moth *Phthorimaea operculella* Zell. (Lepidoptera: Gelechiidae) in Iraq. Z. Ang. Entomol. 79: 345-351.

BARTOLINI, P. 1951. La *Phthorimaea operculella* Zeller (Lepidoptera: Gelechiidae) in Italy. Redia 36: 301-379.

CHAUMAN, U., AND VERMA, L. R. 1991. Biology of potato tuber moth, *Phthorimaea operculella* Zeller, with special reference to pupal eye pigmentation and adult sexual dimorphism. Entomol 16: 63-67.

DOGRAMACI, M., RONDON, S. I., AND DEBANO, S. J. 2008. The effect of soil depth and exposure to winter conditions on survival of the potato tuberworm *Phthorimaea operculella* (Lepidoptera: Gelechiidae). Entomol. Exp. Appl. 129: 332-339.

FOOT, M. A. 1979. Bionomics of the potato tuber moth *Phthorimaea operculella* (Lepidoptera: Gelechiidae) at Pukekohe. New Zealand J. Zool. 6: 623-636.

LANGFORD, G. S., AND CORY, E. N. 1932. Observations on the potato tuber moth. Journal of Economic Entomology 25: 625-634.

MUKHERJEE, A. K. 1949. Life history and bionomics of the potato tuber moth *Gnorimoschema operculella* Zeller, at Allahabad (United Provinces), together with some notes on the external morphology of the immature stages. J. Zool. Soc. India. 1: 57-67.

RONDON, S. I. 2009. The potato tuberworm: a literature review of its biology, ecology, and control. American J. Pot. Res. In press.

RONDON, S. I., HANE, D., BROWN, C. R., VALES, M. I., AND DÖGRAMACI, M. 2009. Resistance of potato germplasm to the potato tuberworm (Lepidoptera: Gelechiidae). J. Econ. Entomol. 102(4): 1649-1653.

SAS INSTITUTE. 1997. SAS User's Guide. SAS Institute, Cary, NC, USA.

STANEV, M., AND KAITAZOV, A. 1962. Studies on the bionomics and ecology of the potato tuber moth, *Gnorimoschema* (*Phthorimaea operculella*) Zeller in Bulgaria and means for its control. Izv. Nauk. Inst. Zasht. Rast. 3: 49-89.