NOTE

Age-Grading Adult Tarnished Plant Bugs (Heteroptera: Miridae)[1]

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The ability to determine the age of field-captured insects is valuable when investigating the behavioral ecology and age-structure of a population. Age-grading adult insects has been most commonly done by measuring changes in the somatic and reproductive system with age (Tyndale-Biscoe. 1984. Bull. Entomol. Res. 74: 341-377). Unfortunately, observations of reproductive or cuticular anatomy often require specialized equipment or techniques and are often time-consuming. Easier methods of aging insects have been developed but are generally restricted to a limited number of species.

It is widely believed, though not reported in the literature, that the tarnished plant bug (TPB), *Lygus lineolaris* (Palisot de Beauvois), darkens with age. An easy technique, based on color, which may be useful for age-grading adult TPB is described in this paper.

TPB were reared in the laboratory on store-bought green beans and potatoes. Within 24 h of adult eclosion, subsets (n=3) of males and females were removed and frozen. The other newly-eclosed adults (approximately 75) were aspirated into a separate rearing container that contained green beans and potatoes. These individuals were kept in an environmentally controlled room at a constant temperature, photoperiod of 14:10 (L:D), and humidity (approximately 80% RH). At irregular intervals, additional subsets (n=2 to 4) of males and females were removed and frozen until no TPB remained (approximately 4 weeks). The age of the adult insects, in Celsius degree days (dd), was calculated by subtracting 11.1°C (lower developmental threshold) from the rearing temperature and multiplying by the age of the insect in days (Fleischer and Gaylor. 1988. Environ. Entomol. 17: 246-253). This procedure was replicated twice at two temperatures (22 and 28°C).

To "clear" the insects and extract their pigments into solution, each TPB was individually placed in a screw-cap vial containing 1 ml of 5 molar potassium hydroxide (KOH) solution. One blank vial, containing no insect but otherwise identical, was also prepared for each sample subset. Vials were sealed and held at 28°C for 6 d. The cleared insect remains were then removed, and the extracts were diluted with 2 ml of water.

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The transmittance of light through the prepared TPB extracts was measured using a Beckman-20 spectrophotometer set to a wavelength of 325 nm. The blank was used as a zero reference. Preliminary investigation indicated that the best peak of light absorbance, with an intensity correlated with age, occurred at this wavelength. Regression equations were fitted for the measured percent transmittance versus adult age (SAS Institute. 1985. SAS user's guide: statistics, version 5. Cary, NC.).

The visual color intensity of the prepared extracts was also independently examined by four observers, prior to the dilution, to determine if a satisfactory estimate of TPB age could be made.

Another study was performed to determine host plant effects on light transmittance through TPB extracts. Post second instar TPB were reared on store-bought green beans and potatoes, broccoli tops, or carrots. TPB adults were isolated within 1 day of eclosion, and light transmittance through extracts of 0.5 day-old and 4.5 day-old TPB females was evaluated with a spectrophotometer as before. A t-test was performed to determine if significant differences in light transmittance existed between extracts of TPB reared on different host species.

Percent light transmittance (nm=325) was negatively correlated with increasing TPB age in both males and females, $P < 0.0001$ (Fig. 1). A power function was fit to the data over a wide range of adult ages. The data were more varible in older TPB, and linear regressions correlated better during the first 120 dd of adult life (females: Y = -0.24*X + 57.21, $P < .0001$, r=.85, n=18; males: Y=-0.23*X + 60.52, $P < .0001$, r=.78, n=23). The TPB preoviposition period lasts until about 50 dd of adult age (3-4 days). Thus, we could estimate TPB age well beyond this critical period.

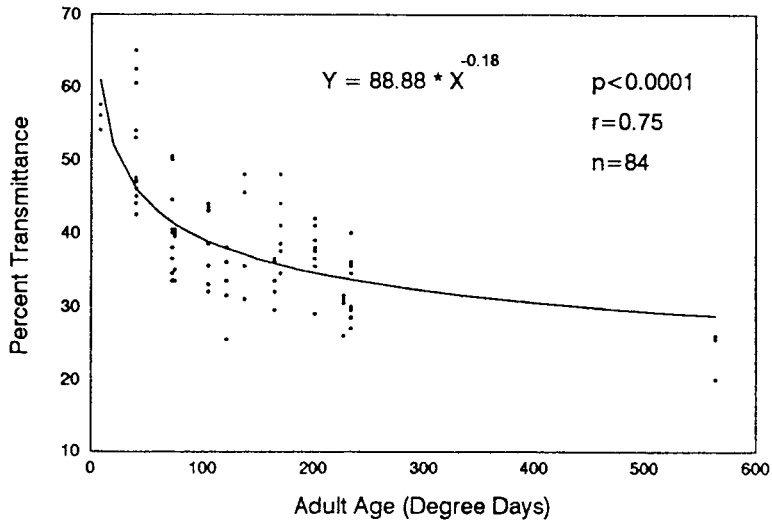

Fig. 1. Regression of light transmittance (nm=325) versus age for extracts prepared from male and female tarnished plant bug adults (Y = percent transmittance, X = age in Celsius degree days).

TPB adults could be seen to darken as they aged. This conclusion was based on visual observations of color intensity in the KOH solutions. However, the observers could not reliably distinguish TPB older than 70 dd or between those within approximately 20-30 dd of each other.

Significantly ($P < 0.01$) more light was transmitted through extracts from 4.5 day-old TPB reared on carrot than through extracts from TPB of the same age reared on other hosts (Table 1). In fact, there was no significant difference in light transmittance between extracts of 0.5 and 4.5 day-old TPB reared on carrots ($P = 0.58$). TPB mortality appeared to be substantially higher on carrots relative to the other hosts, and the developmental rate of immature TPB was considerably reduced. The growth stage of carrot in this test was not the early-reproductive stage that is typically selected by field populations. The lack of light absorbance in the extracts of 4.5 day-old TPB reared on carrots may be an indication of a poor host. Given the opportunity for host selection, color variation between TPB on wild host species may be relatively unimportant. This remains to be tested.

Table 1. The effect of host species on light transmittance (nm = 325) through extracts prepared from 0.5 and 4.5 day-old female TPB adults.

Host	Age (days)	Number of Insects	% Light Transmittance (SE)
Beans and Potatoes	0.5	5	59.5 (1.3) a
	4.5	5	38.0 (2.4) b
Broccoli	0.5	8	63.4 (1.8) a
	4.5	9	35.9 (1.5) b
Carrots	0.5	4	61.9 (4.4) a
	4.5	5	58.1 (3.0) a

Means followed by the same letter are not significantly different ($P < 0.01$, t-test).

Spectrophotometric and visual evaluation can be used to age-grade adult TPB reared in culture and possibly in the field. Further investigation is required to determine the effects of host species on color intensity. Spectrophotometric evaluation appears to be more reliable than visual observation when distinguishing the ages of TPB over a greater range of adult ages. We also believe that clearing insects in KOH solution is more desirable than examining the color of intact specimens because variation in color patterns may be misleading. The accuracy of this method may be improved by using more sophisticated spectrophotometers that would not require dilution of the sample. Spectrophotometric measurements were not directly related to the visual darkening of TPB because the wavelength used in the evaluations was non-visible light. Spectrophotometric evaluation of aging has not been reported for insects which do not appear to darken with age.

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