PUPAL AND ADULT PARAMETERS AS POTENTIAL INDICATORS OF COTTONWOOD LEAF BEETLE (COLEOPTERA: CHRYSOMELIDAE) FECUNDITY AND LONGEVITY

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ABSTRACT

Cottonwood leaf beetle, *Chrysomela scripta*, pupae from a laboratory colony were weighed and monitored through adult emergence, oviposition, and mortality to determine if correlations existed between various pupal or adult parameters and fecundity or longevity. Forty-three female cottonwood leaf beetles were monitored. Pupal weight was not a good indicator of fecundity, total oviposition events, number of eggs/beetle/day, or adult longevity. In addition, adult weight showed very low correlation with fecundity, adult longevity, total oviposition events, or number of eggs/beetle/day. However, adult weight was a marginal indicator of the number of eggs/beetle/day, and correlated well with adult body length. Adult longevity could be used to predict fecundity.

Adult insect performance can be measured in various ways, including survival, pupal weight (Augustin et al. 1997), adult emergence, or fecundity (Miller and Ware 1997). Positive correlations have been determined to exist between pupal weight and adult fecundity in Lepidoptera (Miller 1957, Bessin and Reagan 1990, Spurgeon et al. 1995), Diptera (Hawley 1985, Krainacker et al. 1989), and Hymenoptera (Zhang and Wagner 1991, McMillin and Wagner 1995, Eliason and McCullough 1997). Few studies have explored this relationship within Coleoptera.

To our knowledge, either immediate postpupal or sexually mature pupal or adult weights have not been correlated with cottonwood leaf beetle, *Chrysomela scripta* F. (Coleoptera: Chrysomelidae), fecundity or longevity despite this estimate being used and reported previously (Augustin et al. 1997). The objectives of this laboratory study were to determine if size parameters could be used as predictors of fecundity (defined as total eggs per female [Ameen and Story 1997]) and longevity in adult female cottonwood leaf beetles.

MATERIALS AND METHODS

A laboratory colony was established in September 1997 from larvae collected from a hybrid poplar plantation near the Ames Municipal Water Pollution Control Facility near Ames, Iowa. Beetles were reared in plastic crisper

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boxes (27 × 19 × 10 cm) under a constant 16L:8D photoperiod and 24:18°C temperature regime. Beetles were fed greenhouse-grown *Populus* × *euramericana* var. 'Eugenei' foliage, LPI 1–8 (Larson and Isebrands 1971, Bingaman and Hart 1992).

More than 700 pupae were selected systematically from this colony and weighed to the nearest 10^{-4} g at 1 day postpupation using a Mettler AE 100 Analytical Balance (Mettler Instrument Corporation, Hightstown, NJ) in February 1998. Plastic disposable petri dishes (100 × 15 mm) were divided into four sections by using two strips of paper that were cut and placed together to divide the area of the dish into quarters. One piece of moistened Whatman #1 filter paper was placed in each dish to prevent desiccation. One pupa was placed in each of these sections immediately after weighing. Pupae were held under the aforementioned controlled conditions until adult emergence.

Upon emergence, pupal duration, adult weight, and total body length were recorded for each adult beetle. Body weight was measured using a Mettler AE 100 Analytical Balance and body length was measured from the anterior point of the head to the posterior point of the elytra. Cottonwood leaf beetles are sexually dimorphic; therefore, visual estimation of body size was used as an indicator of sex. We assumed that larger beetles were females and smaller beetles were males. Two large and two small adults were placed into small plastic containers (9.5 × 7 2 cm) in hopes of obtaining mating pairs. Many *C. scripta* were sorted and paired unsuccessfully; the only beetles used in this study were those that mated. Waterproof paint was used to paint one, two, three, or four identification dots on the elytra of the beetles within their respective containers. The dots did not cause any behavioral changes or mechanical damage to the beetles (unpublished data). From these containers, 43 mated pairs were selected for observation. Individual pairs were then placed in separate small plastic containers (9.5 × 7 × 2 cm).

Beetle pairs were monitored daily. Fecundity, total oviposition events, and longevity (from emergence to mortality) were recorded for each mated female. Data were analyzed using a correlation and regression analysis (SAS Institute 1985).

RESULTS

Pupal weights averaged 0.0335 g (± 0.0048 SE) and ranged from 0.0242 to 0.0442 g. Adult weights at emergence ranged from 0.0195 to 0.0371 g, and averaged 0.0263 g (± 0.0041). Adult females lived up to 34 days postemergence, with an average adult life span of 22.47 days (± 7.56). All 43 pairs of beetles monitored mated successfully. Total fecundity ranged from 63 to 1003 eggs/female with an average of 484.5 (± 251.3) eggs. Adult females laid 63.2 (±9.69) eggs/day.

Our study showed poor correlation between pupal weight and adult fecundity ($r^2 = 0.08$, n = 43, $F = 3.62$, $P = 0.064$), total oviposition events ($r^2 = 0.05$, n = 43, $F = 2.06$, $P = 0.159$), the number of eggs/beetle/day ($r^2 = 0.12$, n = 43, $F = 5.76$, $P = 0.021$), or female adult longevity ($r^2 = 2.5\ 10^{-3}$, n = 43, $F = 0.14$, $P = 0.715$). Adult weight correlated poorly with fecundity ($r^2 = 0.10$, n = 43, $F = 4.64$, $P = 0.037$), total ovipositional events ($r^2 = 0.07$, n = 43, $F = 3.21$, $P = 0.081$), and longevity ($r^2 = 0.01$, n = 43, $F = 0.56$, $P = 0.456$). Adult length did correlate well with adult weight ($r^2 = 0.55$, n = 43, $F = 50.66$, $P < 0.001$) (Fig. 1). Adult weight showed marginal correlation with the number of eggs/female/day ($r^2 = 0.19$, n = 43, $F = 9.78$, $P = 0.003$) (Fig. 2). Fecundity correlated well with adult longevity ($r^2 = 0.67$, n = 43, $F = 82.4$, $P < 0.001$) (Fig. 3).

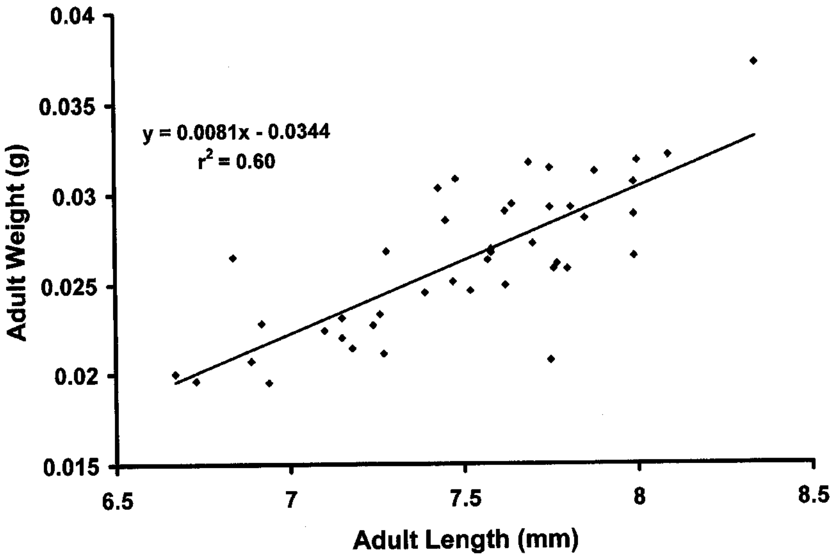

Figure 1. Relationship between adult female *C. scripta* weight and length (within 24 h postemergence).

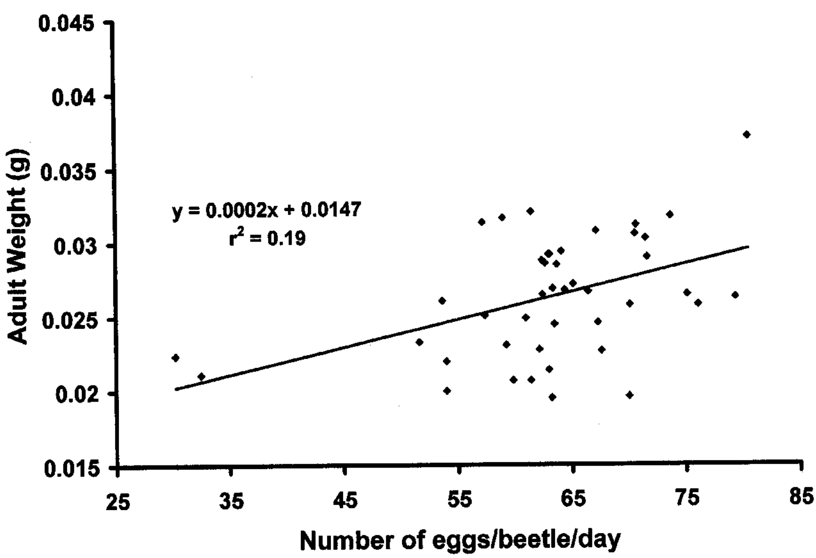

Figure 2. Relationship between adult female *C. scripta* weight and the number of eggs/beetle/day over the entire duration of ovipositional activity.

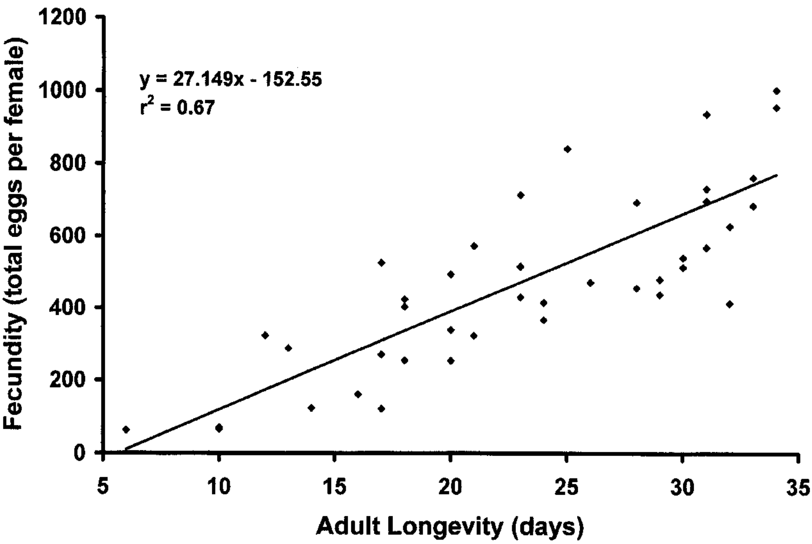

Figure 3. Relationship between adult female *C. scripta* longevity and fecundity.

DISCUSSION

In most insect orders, healthy larvae are often the largest larvae (and subsequently pupae), and emerge into the largest adults (Kamata and Igarashi 1995). For insects that do not feed as adults and lay only one clutch of eggs, larger larval size would allow the potential for more eggs to develop during pupation. However, this hypothesis would not necessarily hold true for insects such as the cottonwood leaf beetle that feed as adults, experience multiple matings, and lay multiple clutches of eggs. The capacity to mate and oviposit multiple times reduces the need for greater size, which also reduces the probability that size would correlate with fecundity. We found this relationship to be true with *C. scripta*.

Average fecundity values in our study (484.5 ± 251.3 eggs per female) were similar to those obtained in a laboratory study by Burkot and Benjamin (1979) (510.0 ± 152.9). Furthermore, the average number of eggs per cluster (63.2 ± 9.69) was nearly identical to that of Burkot and Benjamin (1979) (64.3 ± 14.7). Head and Neel (1973) observed egg masses of 50–100 eggs per cluster in outdoor caged studies. Field studies by Lowe (1898) (45 eggs per cluster) and Haugen (1985) (55.6 ± 11.9 eggs per cluster) are slightly less than those obtained in this study; this could be attributed to the more optimal conditions in the laboratory.

Limited food resources and crowding can impact fecundity in chrysomelids (Zvereva et al. 1995). Intraspecific competition among adults for food resources can lead to reduced leaf area consumed per adult, which subsequently can have an impact on beetle health and reproduction (Zvereva et al. 1995). Adult *C. scripta* are only in competition for food in nature during high population levels (E. R. Hart, personal observation); there was no competition for food resources in this study. All adult beetles were fed ample foliage, and no beetles suffered food shortage or crowding during the course of this experiment. In a study by Bauer et al. (1990) adult *C. scripta* reared on foliage in petri dishes had a shorter life span (33.0 ± 3.4 days) than did adults reared under colony conditions (55 ± 5.8 days). This may have occurred in our study, although life spans for insects in the larger laboratory colony were not recorded. Adults in this study had slightly shorter life spans (22.47 ± 7.56 days) than did the insects in the study by Bauer et al. (1990).

A linear relationship existed between adult female weight and length. These findings parallel results from other studies (Zhang and Wagner 1991), and result from heavier beetles often being larger beetles; increased size often is expressed through increased body length. Fecundity correlated well with adult longevity. This correlation is evident in other leaf beetles as well (Ameen and Story 1997). Presumably, this relationship results because female beetles with longer lives have more opportunities to mate and oviposit than do beetles with shorter life spans. Multiple mating increases fecundity in other insect species (Lamunyon 1997), and the opportunity longer-lived cottonwood leaf beetles have to mate multiple times may be a factor in the increased fecundity. Although the focus of this study was on body size and fecundity correlations of adult females, we recognize that body size can have important implications for adult males as well (Tammaru et al. 1996). Future studies could examine the relationship between male body size and mating success.

Inbreeding can have negative effects on reproduction (Wildt et al. 1987). This laboratory colony had been sustained since October; experiments took place in February. Some inbreeding may have taken place during this time, as this was the fourth generation of cottonwood leaf beetles in the laboratory. Additional factors such as rearing conditions or possible microbial contamination may have influenced longevity and thus total fecundity (Webber and Ferro 1996, Jackson 1997).

Many *Populus* clonal variations and hybrids are being screened for use in short-rotation woody crop systems. Adult cottonwood leaf beetles may live longer on certain genotypes or selections of *Populus* than others. Planting *Populus* clones and hybrids preferred by cottonwood leaf beetle could result in more egg masses and more larvae and could potentially contribute to outbreak conditions. Many characteristics, including growth, hardiness, and pest preference and performance need to be taken into consideration when choosing *Populus* plant material for advancement in regional trials. The performance and life expectancy of adult cottonwood leaf beetles is one of these characteristics.

For insects that feed as adults and have multiple oviposition events, increased larval, pupal, and adult size may be of little benefit to fecundity, because they will have more than one opportunity to oviposit. Therefore, previous measurements of cottonwood leaf beetle larval performance that used pupal weight (Augustin et al. 1997) may not be entirely relevant, and future studies on larval performance should focus on survival, adult longevity, and actual fecundity.

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LITERATURE CITED

Ameen, A. O. and R. N. Story. 1997. Fecundity and longevity of the yellowmargined leaf beetle (Coleoptera: Chrysomelidae) on crucifers. J. Agric. Entomol. 14: 157–162.

Augustin, S., M. R. Wagner, J. Chenault and K. M. Clancy. 1997. Influence of pulp and paper mill wastewater on *Chrysomela scripta* (Coleoptera: Chrysomelidae) performance and *Populus* plant traits. Environ. Entomol. 26: 1327–1335.

Bauer, L. S., J. Meerschaert and O. T. Forrester. 1990. An artificial diet for cottonwood and imported willow leaf beetles (Coleoptera: Chrysomelidae) and comparative performance on poplar foliage. J. Entomol. Sci. 25: 475–480.

Bessin, R. T. and T. E. Reagan. 1990. Fecundity of sugarcane borer (Lepidoptera: Pyralidae), as affected by larval development on gramineous host plants. Environ. Entomol. 19: 635–639.

Bingaman, B. R. and E. R. Hart. 1992. Feeding and oviposition preferences of adult cottonwood leaf beetles (Coleoptera: Chrysomelidae) among *Populus* clones and leaf age classes. Environ. Entomol. 21: 508–517.

Burkot, T. R. and D. M. Benjamin. 1979. The biology and ecology of the cottonwood leaf beetle, *Chrysomela scripta* (Coleoptera: Chrysomelidae), on tissue cultured hybrid *Aigeiros* (*Populus* × *Euramericana*) subclones in Wisconsin. Can. Entomol. 111: 551–556.

Eliason, E. A. and D. G. McCullough. 1997. Survival and fecundity of three insects reared on four varieties of scotch pine Christmas trees. J. Econ. Entomol. 90: 1598–1608.

Haugen, D. A. 1985. Oviposition preference of the cottonwood leaf beetle, *Chrysomela scripta* F., on poplar clones, *Populus* spp. Ph.D. dissertation, Iowa State University, Ames.

Hawley, W. A. 1985. A high-fecundity aedine: factors affecting egg production of the western treehole mosquito, *Aedes sierrensis* (Diptera: Culicidae). J. Med. Entomol. 22: 220–225.

Head, R. B. and W. W. Neel. 1973. The cottonwood leaf beetle: observations on the biology and reproductive potential in Mississippi. J. Econ. Entomol. 66: 1327–1328.

Jackson, J. J. 1997. Biology of *Aphthona nigriscutis* (Coleoptera: Chrysomelidae) in the laboratory. Ann. Entomol. Soc. Am. 90: 433–437.

Kamata, N. and M. Igarashi. 1995. Relationship between temperature, number of instars, larval growth, body size, and adult fecundity of *Quadricalcarifera punctatella* (Lepidoptera: Notodontidae): cost-benefit relationship. Environ. Entomol. 24: 648–656.

Krainacker, D. A., J. R. Carey and R. I. Vargas. 1989. Size-specific survival and fecundity for laboratory strains of two tephritid (Diptera: Tephritidae) species: implications for mass rearing. J. Econ. Entomol. 82: 104–108.

Lamunyon, C. W. 1997. Increased fecundity, as a function of multiple mating, in an arctiid moth, *Utetheisa ornatrix*. Ecol. Entomol. 22: 69–73.

Larson, P. R. and J. G. Isebrands. 1971. The plastochron index as applied to developmental studies of cottonwood. Can. J. For. Res. 1: 1–11.

Lowe, V. H. 1898. Cottonwood leaf beetle, green arsenite. Bull. N. Y. Agric. Exp. Sta. No. 143. 23 pp.

McMillin, J. D. and M. R. Wagner. 1995. Season and intensity of water stress: Host-plant effects on larval survival and fecundity of *Neodiprion gilletti* (Hymenoptera: Diprionidae). Environ. Entomol. 24: 1251–1257.

Miller, C. A. 1957. A technique for estimating the fecundity of natural populations of the spruce budworm. Can. J. Zool. 35: 1–3.

Miller, F. and G. Ware. 1997. Preference for and suitability of Asian elm species and hybrids for the adult elm leaf beetle (Coleoptera: Chrysomelidae). J. Econ. Entomol. 90: 1641–1645.

SAS Institute. 1985. SAS user's guide: basics, version 5 ed. SAS Institute, Cary, NC.

Spurgeon, D. W., P. D. Lingren, T. N. Shaver and J. R. Raulston. 1995. Realized and potential fecundity of the Mexican rice borer (Lepidoptera: Pyralidae) as a function of pupal weight. Environ. Entomol. 24: 94–98.

Tammaru, T., K. Ruohomäke, and K Saikkonen. 1996. Components of male fitness in relation to body size in *Epirrita autumnata* (Lepidoptera, Geometridae). Ecol. Entomol. 21: 185–192.

Webber, D. C. and D. N. Ferro. 1996. Flight and fecundity of Colorado potato beetles (Coleoptera: Chrysomelidae) fed on different diets. Ann. Entomol. Soc. Am. 89: 297–306.

Wildt, D. E., M. Bush, K. L. Goodrowe, C. Packer, A. E. Pusey, J. L. Brown, P. Joslin and S. J. O'Brien. 1987. Reproductive and genetic consequences of founding isolated lion populations. Nature 329: 328–331.

Zhang, Z. Y. and M. R. Wagner. 1991. Cocoon and adult parameters predict fecundity of the pine sawfly *Neodipirion fulviceps*. Southwest. Entomol. 16: 193–198.

Zvereva, E. L., M. V. Kozlov, and S. Neuvonen. 1995. Population density and performance of *Melasoma lapponica* (Coleoptera: Chrysomelidae) in surroundings of smelter complex. Environ. Entomol. 24: 707–715.