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REARING THE OLIGOPHAGOUS CACTOBLASTIS CACTORUM (LEPIDOPTERA: PYRALIDAE) ON MERIDIC DIETS WITHOUT HOST PLANT MATERIALS

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ABSTRACT

Cactoblastis cactorum (Berg) (Lepidoptera: Pyralidae), an oligophagous herbivore from South America, has been used successfully as a biological control agent for several invasive Opuntia species around the world. However, its unintentional arrival in Florida raised serious concern over its possible effect on native Opuntia biodiversity and Opuntia-based industries. Development of control tactics to mitigate the threat of this invasive pest to North America relied upon a constant supply of all life stages of this insect species. Therefore, 3 strains of C. cactorum were established in a laboratory insectary and trials were initiated to optimize rearing methods using an artificial diet. Because monophagous or oligophagous lepidopterans may be sensitive to the balance of nutrients and/or the presence of specific feeding cues and because different strains of an oligophagous lepidopteran may respond differently to various meridic diets, we compared the development and survival of 3 strains of C. cactorum on several meridic diets without host plant materials. Although C. cactorum is an oligophage within the genus Opuntia, it accepted and developed on several diets containing non-host plant ingredients, yeast, and fish meal. The source and balance of non-host nutrients significantly affected all reproductive parameters of C. cactorum. The best performance of C. cactorum was on diets that contained white kidney beans, brewer’s yeast, wheat germ and/or soybeans.

Key Words: Cactoblastis, Opuntia, artificial diet, mass rearing

The South American cactus moth, Cactoblastis cactorum (Berg) (Lepidoptera: Pyralidae), is an oligophagous herbivore whose endophagous larvae feed gregariously on numerous Opuntia species (Caryophyllales: Cactaceae) (Mann 1969; Zimmermann et al. 2004). This feeding behavior of
**C. cactorum** has contributed to its successful use as a biological control agent for several invasive *Opuntia* species around the world (Dodd 1940; Petty 1948; Zimmermann et al. 2004). Although host specificity is desirable for a biological control agent, *C. cactorum* oligophagy within the genus *Opuntia* was not a concern for biological control campaigns in the Old World where *Opuntia* are not indigenous (Dyer 1975). However, following the release of *C. cactorum* on some Caribbean islands beginning in 1957 (Simmonds & Bennett 1966), this herbivore has spread naturally and/or by human intervention to most other islands in the Caribbean, and has been observed attacking most of the 20 *Opuntia* species native to the Caribbean (Zimmermann & Perez Sandi Cuen 2006). In the United States, populations of *C. cactorum* were first observed in the Florida Keys in 1989 (Habeck & Bennett 1990; Dickle 1991) but subsequently spread throughout most of the Florida peninsula and along the Atlantic coast to South Carolina and the Gulf coast to Louisiana (Hight & Carpenter 2009). Because of its rapid spread along the Gulf coast and its reputation as a voracious *Opuntia* feeder, *C. cactorum* is regarded as a serious economic and ecological threat to native and cultivated *Opuntia* spp. in the United States and Mexico (Soberón et al. 2001; Vigueras & Portillo 2001; Garrett 2004; Zimmermann et al. 2004).

In response to the growing threat posed by this invasive pest in North America (Pemberton 1995), an assessment and planning workshop was held in Tampa, Florida, in September 2000 to address the needs for research, education and outreach, risk assessment and regulatory issues, and international collaboration (Mahr 2001). This workshop was followed by a consultants meeting hosted by the Food and Agriculture Organization (FAO) and the International Atomic Energy Agency (IAEA) to review and evaluate the threat of *C. cactorum* to international agriculture and biodiversity (IAEA 2002). During these meetings, an emphasis was placed on the development of mass rearing techniques for establishing a laboratory colony that could be used to support the needed areas of research, such as sex pheromone identification (Heath et al. 2006), dispersal studies (Hight et al. 2002; Bloem et al. 2005a; Savary et al. 2008), developmental biology (Mclean et al. 2006), insecticidal susceptibility (Bloem et al. 2005b), biological control (Paraiso et al. 2012), and the sterile insect technique (Carpenter et al. 2001; Hight et al. 2005). Historically, *C. cactorum* had been reared with great success on excised cladodes in field cages to support the renowned biological control projects against invasive *Opuntia* in Australia (Dodd 1940) and South Africa (Petty 1948). But, addressing the research needs and urgency to mitigate *C. cactorum* as an invasive pest in North America required a constant supply of high quality moths. Consequently, we initiated studies to develop mass rearing protocols for laboratory production of moths, an optimal artificial diet, and a suitable diet presentation. Monophagous or oligophagous lepidopterans may be sensitive to the balance of nutrients and/or the presence of specific feeding cues in their host plants or in an artificial diet (Genc & Nation 2006). Also, different strains of an oligophagous lepidopteran may respond differently to various meridic diets (Carpenter & Bloem 2002). In this study we compared the development and survival of 3 strains of *C. cactorum* on several meridic diets containing different non-host nutrients.

### Table 1. Sources of non-host nutrients, additives and references for the meridic diets evaluated for rearing *Cactoblastis cactorum*.

<table>
<thead>
<tr>
<th>Diet Name (reference)</th>
<th>Source of Non-host Nutrients</th>
<th>Additives</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn-Soy Blend (CSB) (Burton 1970)</td>
<td>Corn-Soy Mix, Torula Yeast</td>
<td>Agar, Ascorbic Acid, Sorbic Acid, Nipagin, Formaldehyde, Vitamin/Mineral Premix</td>
</tr>
<tr>
<td>Pinto Bean (PB) (Burton 1969)</td>
<td>Pinto Bean, Torula Yeast</td>
<td>Agar, Ascorbic Acid, Sorbic Acid, Cholesterol, Nipagin, Sugar, Vitamin/Mineral Premix</td>
</tr>
<tr>
<td>Soybean (SB) (Burton 1969 modified)</td>
<td>Soybean, Torula Yeast</td>
<td>Agar, Ascorbic Acid, Sorbic Acid, Cholesterol, Nipagin, Sugar, Vitamin/Mineral Premix</td>
</tr>
<tr>
<td>White Kidney Bean (WKB+F) (Marti &amp; Carpenter 2008 modified)</td>
<td>White Kidney Bean, Torula Yeast, Fishmeal</td>
<td>Agar, Ascorbic Acid, Sorbic Acid, Cholesterol, Nipagin, Mold Inhibitor, Sugar</td>
</tr>
<tr>
<td>White Kidney Bean (WKB+B) (Marti &amp; Carpenter 2008 modified)</td>
<td>White Kidney Bean, Torula Yeast, Brewer’s Yeast</td>
<td>Agar, Ascorbic Acid, Sorbic Acid, Cholesterol, Nipagin, Mold Inhibitor, Sugar</td>
</tr>
<tr>
<td>Wheat-Soy Blend (WSB) (Burton &amp; Perkins 1972)</td>
<td>Wheat germ, Soybean, Torula Yeast</td>
<td>Agar, Ascorbic Acid, Sorbic Acid, Nipagin, Vitamin/Mineral Premix</td>
</tr>
</tbody>
</table>
Insects

Three different strains of *C. cactorum* were reared at the USDA-ARS Crop Protection and Management Research Unit laboratory, Tifton, Georgia. The “US Cladode” strain was established from *C. cactorum* collected as larvae from infested *Opuntia* spp. along the Florida Gulf coast in 2002 and reared continuously on cactus (*Opuntia ficus-indica* L.) cladodes in the laboratory using the methods described by Marti et al. (2008). The “US Diet” strain was established from the “US Cladode” strain and has been reared continuously on an artificial diet (Marti & Carpenter 2008) for 3 generations at the time these trials were conducted. Another *C. cactorum* strain was established from eggs collected from *Opuntia* spp. plantations near Craddock, South Africa. These eggs were shipped to Tifton, GA, and placed on an artificial diet described by Marti & Carpenter (2008). This South Africa strain was designated as the “SA Diet” strain and had been reared continuously on the artificial diet for 3 generations at the time these trials were conducted.

Rearing Methods

*Cactoblastis cactorum* were reared following the general methods described by Marti et al. (2008). A walk-in environmental chamber maintained at 29 ± 2 °C, 14:10 h L:D, and 60-80% RH was used to hold all rearing containers for the diet trials.

Diet Treatments

Several meridic diets that had been used successfully to rear other lepidopteran species were selected for this study. The diets varied and/or were modified so that different sources and combinations of non-host nutrients could be evaluated (Table 1). All the diets were similar in that they contained a measure of torula yeast, agar,
ascorbic acid, sorbic acid, and nipagin (methyl p-hydroxybenzoate). Cholesterol and sugar were added to all the diets except the Corn-Soy Blend (CSB) (Burton 1970) and the Wheat-Soy Blend (WSB) (Burton & Perkins 1972) diets. The CSB diet included formaldehyde as a mold inhibitor, and the White Kidney Bean with fishmeal (WKB+B) and White Kidney Bean with brewer’s yeast (WKB-B) diets (Marti & Carpenter 2008 modified) included a mold inhibitor solution (5 mL/liter of diet); each mL consisting of 418 μL propionic acid, 42 μL phosphoric acid, and 540 μL water. The Soybean (SB) diet was a modification of the Pinto Bean (PB) diet (Burton 1969) where the pinto bean meal was replaced with an equal amount of soy flour. The diet used to rear C. cactorum at the time of this study (Marti & Carpenter 2008) was adapted from a diet developed in South Africa for Chilo partellus (Swinhoe) (Lepidoptera: Pyralidae), the maize stalk borer, by Kfir (1992), and initially evaluated in South Africa for C. cactorum by Zimmermann (2003). The diet designated WKB+F in this study was similar to the diet described by Marti & Carpenter (2008) except that it contained torula yeast (96 g) instead of brewer’s yeast (186 g), 540 g of white kidney beans (instead of 630 g), and 180 g of fishmeal. The diet designated WKB+B in this study was similar to the WKB+F diet except that the fishmeal was replaced with 90 g of brewer’s yeast. A vitamin/mineral premix was added to all diets except the WKB+B and WKB+F diets.

Diets were prepared according to the published protocols, and then the hot diets were poured into 90 × 15 mm Petri dish bottoms. After cooling, the round diet cakes were removed from the Petri dishes and dipped into molten paraffin to provide a thin waxy coat simulating a wax-covered cactus cladode (Marti et al. 2008). An eggstick containing approximately 100 eggs was placed on the surface of each diet cake or cactus cladode in a 20 × 30 × 10 cm plastic container fitted with a ventilated lid and reared according to the methods of Marti et al. (2008). Five containers (replications) were used for each strain on each diet treatment. Data were recorded on the developmental time (neonate to adult), percent survival, and the weight and gender of each pupa.

Fig. 2. The mean (± S.E.) weight of Cactoblastis cactorum female pupae reared on Opuntia cladodes and on 6 meridic diets containing different non-host proteins showed significant interactions between strain and diet. Insect strain is indicated in the caption and larval diet is indicated on the x-axis. For each strain, diet means with the same lower case letter are not significantly different. For each diet, strain means with the same upper case letter are not significantly different. Diet name abbreviations are as follows: CSB (Corn-Soybean Blend), PB (Pinto Bean), SB (Soybean), WKB+B (White Kidney Bean), WSB (Wheat-Soy Blend). The Cactoblastis strains were (1) “US Cladode” strain collected in Florida and reared continuously on cactus (Opuntia ficus-indica L.) cladodes, (2) the “US Diet” strain established from the “US Cladode” strain and reared continuously on artificial diet, and (3) the “SA Diet” strain obtained from South Africa and reared on artificial diet.
Data were analyzed by PROC-ANOVA (SAS Institute 1989) as a 2 factor ANOVA. Factors for analysis included larval diet (6 treatments) and insect strain (3 strains). Dependent variables were percent survival, developmental time, and pupal weight. Because female pupae have a greater mass and longer developmental times than male pupae, data for developmental time and pupal weight were sorted by gender before analysis was conducted. When significant differences were indicated, means were separated by the Tukey-Kramer statistic at $P = 0.05$ (SAS Institute, 1989). The rate of increase for each strain on cladodes or one of the diets was calculated using a modification of the formula by Birch (1948) ($r = \ln R_0 / T$). For our calculations, $R_0 =$ mean number of female progeny reaching adulthood from each female parent, and generation time ($T$) = egg incubation time (21 d) + 2 d for oviposition + female developmental time (neonate to adult). Data from the “US Cladode” colony were used to calculate a regression equation for the relationship between the number of eggs laid and the female pupal weight ($\#$ eggs $= -27.817 + 1040.3 \times$ female pupal weight). This equation was used to estimate the fecundity of females from each strain reared on each diet treatment (Honek 1993; Tammaru et al. 1996; Marti & Carpenter 2008). The time (d) required for a population to double in size, or doubling time (DT), was calculated ($DT = \ln (2)/r$) for each strain on each diet.

**RESULTS AND DISCUSSION**

Survival of *C. cactorum* was significantly influenced by an interaction between strain and type of diet ($F = 30.1, df = 6, 84, P < 0.0001$) (Fig. 1). There were no significant differences among the 3 strains for survival on each diet treatment, however, relative survival for the different strains varied between diet treatments. For example, the mean survival for the US Diet strain was highest among the 3 strains when reared on PB and WKB+B diets but US Diet strain survival was lower than the...
other 2 strains when reared on the SB and WSB diets. As a result, survival of the US Diet strain when reared on PB and WSB diets was not significantly different, but the survival of US Cladode and SA Diet strains when reared on PB and WSB diets was significantly different. Other examples of interaction between strain and diet treatment can be seen when comparing the survival of the different strains on WKB+B and WSB, SB and WKB+F, Cactus and WSB, Cactus and WKB+F, and WKB+F and WKB+B. When larvae were reared on the SB and WKB+B diets the survival of all 3 strains was not significantly different from the survival of these strains when reared on cactus cladodes. Overall survival was lowest when larvae were reared on the CSB and PB diets.

Pupal weight of *C. cactorum* females was significantly influenced by an interaction between strain and type of diet ($F = 19.27, df = 6, 1850, P = 0.0006$) (Fig. 2). There were no differences among strains for female pupal weights when reared on CSB and WSB diets, but differences were observed among strains when larvae were reared on the other meridic diets and cactus cladodes. For all diet treatments except CSB, the female pupal weight for the US Diet tended to be higher than the female pupal weight of other strains. Overall, female pupal weights were lowest for CSB and PB diets, though not always significantly different. Pupal weight of *C. cactorum* males also was significantly influenced by an interaction between strain and type of diet ($F = 11.1, df = 6, 2235, P < 0.0001$) (Fig. 3). Mean weights of male pupae from each strain reared on cactus cladodes were not significantly different from mean weights of pupae reared on SB, WKB+F, WKB+B, and WSB meridic diets. Similar to the female pupal weights, male pupal weights for each strain were lowest when larvae were reared on the CSB and PB diets, though not always significantly different.

The developmental time of *C. cactorum* females reared on meridic diets and *Opuntia* cladodes was significantly influenced by an interaction between strain and type of diet ($F = 223.29, df = 6, 1734, P < 0.0001$) (Fig. 4). The mean num-
The number of days required for development was not significantly different among strains when compared within each diet treatment, except for the CSB diet where development of US Diet females was significantly longer than the development of US Cladode females. For females from all 3 strains, developmental time was significantly longer on CSB and PB diets compared with other diets. The meridic diets on which the females required the shortest developmental time were SB, WKB+B, and WSB. Females from each strain developed most rapidly on the cactus cladodes. The developmental time of *C. cactorum* males also was significantly influenced by an interaction between strain and type of diet ($F = 252.6$, $df = 6$, $1863$, $P < 0.0001$) (Fig. 5). Similar to the females, mean developmental times for males from all 3 strains were significantly longer on CSB and PB diets compared with other diets. The meridic diets on which the males required the shortest developmental time were SB, WKB+B, and WSB. Males from each strain developed most rapidly on the cactus cladodes.

The net reproductive rates ($R_0$) for *C. cactorum* reared on cactus cladodes were 19.4, 28.7, and 36.6 for the SA Diet, US Diet, and US Cladode strains, respectively. The WKB+B diet yielded the highest $R_0$ among the meridic diets with values of 18.7, 26.1, and 33.8 for the SA Diet, US Diet, and US Cladode strains, respectively. The CSB and PB diets yielded the lowest $R_0$ values, and for the CSB diet, the $R_0$ values were too low (0.66-0.81) to sustain a population. The doubling times (DT) for each strain reared on the different diets and cactus cladodes are presented in Fig. 6. The DT values for the US Cladode and US Diet strains were lower when reared on the WKB+B diet than when reared on the other meridic diets. The SA Diet strain had the lowest DT value when reared on the WSB diet. Although the DT was lowest for *C. cactorum* when reared on cactus cladodes, the DT for WKB+B was only about 2 d greater.

Laboratory-reared insects have been crucial to the advancement of many aspects of entomology including ecology, behavior, physiology,
genetics, insecticide screening, host plant resistance, production of biological control agents and biopesticides, the study of semiochemicals, and the sterile insect technique (Knipling 1984). Diet may be the most important component of laboratory rearing of insects (Parker 2005) for the production of quality insects at an acceptable cost. The importance of diet is indicated by the nearly 2,000 references compiled by Singh (1977) for rearing 750 species of insects, spiders, and mites. Many of these diets are acceptable for rearing multiple species for several continuous generations (Singh 1985). However, even if the diet is nutritionally sound, it may not be optimal for some monophagous or oligophagous lepidopterans that are sensitive to the balance of nutrients and/or the presence of specific feeding cues in their host plants or in an artificial diet (Moore 1985; Genc & Nation 2004). Also, different strains of an oligophagous lepidopteran may respond differently to various meridic diets (Carpenter & Bloem 2002). Protein and the accompanying amino acids are critical elements to any insect diet, and the protein content, protein source, and amino acid balance are often modified to optimize a standard diet (Moore 1985).

Although *C. cactorum* is an oligophage of plants within the genus *Opuntia*, it accepted and developed on several non-host plant, yeast, and animal nutrients. However, we found that the source and balance of non-host nutrients significantly affected all reproductive parameters. The use of brewer’s yeast as an additive source of protein and other nutrients (WKB+B) was superior to the use of fishmeal (WKB+F), and the use of soybean (SB) was superior to the use of pinto bean (PB). The CSB diet that was comprised of corn, soybean, yeast, and casein was not a suitable diet for *C. cactorum*. The poor performance of *C. cactorum* on the CSB diet may have been influenced by the presence of formaldehyde as an antimicrobial agent. While *C. cactorum* reared on *Opuntia* cladodes reproduced more rapidly than *C. cactorum* reared on the artificial diets, *C. cactorum* also performed well on the WKB+B, WSB, and SB diets.

As a result of this study, the current mass rearing diet for *C. cactorum* is based on the WKB+B.
diet. This diet has produced high quality insects for 25 generations in the Tifton, Georgia laboratory and for 15 generations at Florida’s Division of Plant Industry, Gainesville, Florida. Mass reared C. cactorum continue to be used for a variety of experiments (life table studies, pheromone improvement, and trap calibration) and control technologies (the SIT, biological control, and mating disruption), all of which could not be conducted without a reliable supply of quality insects.

ENDNOTE

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