This item is the archived peer-reviewed author-version of:

Pollen as an alternative or supplementary food for the mirid predator *Macrolophus pygmaeus*

Bjorn Vandekerkhove & Patrick De Clercq

BIOLOGICAL CONTROL 53 (2): 238-242, 2010

1   Pollen as an alternative or supplementary food for the mirid predator *Macrolophus pygmaeus*

2

3   Bjorn Vandekerkhove[1] and Patrick De Clercq[1,*]

4

5 *[1] Laboratory of Agrozoology, Department of Crop Protection, Faculty of Bioscience Engineering, Ghent University,*

6 *Coupure Links 653, B-9000 Ghent, Belgium*

7

8

9

10

11

12

13

14

15

16

17

18

19

20

21

22 * Corresponding author: e-mail: Patrick.DeClercq@UGent.be

23   Fax: 32 (0) 9 264 62 39

24   Phone: 32 (0) 9 264 61 58

25   Abstract

26

27   For the mass production of the mirid predator *Macrolophus pygmaeus* eggs of the Mediterranean

28   flour moth *Ephestia kuehniella* are routinely used as an effective but expensive factitious food. In

29   the current study, the potential of pollen as a supplementary food for *M. pygmaeus* was

30   investigated. In a first experiment, the minimum amount of *E. kuehniella* eggs needed for optimal

31   development and reproduction was determined to be 40 eggs per individual per 3 days. Then,

32   different amounts of *E. kuehniella* eggs were offered to individual nymphs, supplemented or not

33   with frozen moist honeybee pollen. Insects reared on only 10 *E. kuehniella* eggs per 3 days

34   suffered higher mortality, developed slower and had lower adult weights and oocyte counts than

35   insects reared on 40 *E. kuehniella* eggs or 10 eggs supplemented with pollen. When the nymphs

36   were fed only pollen, survival rates and oocyte production were lower than when both pollen and

37   flour moth eggs were provided. On pollen alone, ca. 80% of the nymphs successfully reached

38   adulthood; their adult weights and oocyte counts were respectively 12% and 32% lower

39   compared with individuals fed optimal amounts of flour moth eggs. When an egg yolk-based

40   artificial diet was supplemented with bee pollen, development and fecundity were better than on

41   the artificial diet alone. The practical implications of pollinivory for the mass production and the

42   use of this beneficial insect in augmentative biological control programs are discussed.

43

44   *Keywords*: *Macrolophus pygmaeus*; Pollen; Artificial diet; Predator; *Ephestia kuehniella*

45

46

47

48

2

49   <u>1. Introduction</u>

50

51   *Macrolophus pygmaeus* (Rambur) (Hemiptera: Miridae) is a natural enemy of major economic

52   importance for the biological control of whiteflies and other small arthropod pests in protected

53   cultivation of temperate and Mediterranean Europe (Perdikis et al., 1999; Perdikis &

54   Lykouressis, 2000; Margaritopoulos et al., 2003). *M. pygmaeus* is a zoophytophagous predator

55   and is able to complete its development in the absence of prey on several crop and non crop

56   plants such as tomato, pepper, cucumber and eggplant (Lykouressis et al., 2001; Perdikis &

57   Lykouressis, 2004). It has been shown by Perdikis & Lykouressis (2000) that pollen can

58   stimulate development of *M. pygmaeus* when combined with other food sources, such as eggplant

59   leaves and aphids.

60

61   The current study investigated the effect of pollen as a sole or supplementary food on the

62   developmental and reproductive fitness of *M. pygmaeus*. Eggs of the Mediterranean flour moth

63   *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) are currently widely used as a factitious food

64   for rearing *M. pygmaeus*. These eggs are highly effective in supporting nymphal and adult

65   cultures of the predator but are also costly, with current market prices in the range of 600-800

66   EUR/kg. Partial replacement of *E. kuehniella* eggs in the production process of *M. pygmaeus*

67   could lead to a cost reduction, which may further stimulate the use of the predator in biological

68   control programs (De Clercq, 2008). In the present study, the effect of supplementing suboptimal

69   amounts of *E. kuehniella* eggs with frozen moist honeybee pollen on the developmental and

70   reproductive performance of *M. pygmaeus* was investigated. The potential of pollen to

71   supplement a nutritionally suboptimal artificial diet was also tested.

72

3

73

74

75   ## 2. Materials and methods

76

77   *2.1 Insect colony*

78

79   The test insects of *M. pygmaeus* were taken from a laboratory colony started with eggs supplied

80   by Koppert B.V. (Berkel & Rodenrijs, The Netherlands). Nymphs were reared in plastic

81   containers (24 x 16 x 8 cm), furnished with paper towels and covered with nylon netting. The

82   nymphs were reared on leaves of *Nicotiana tabacum* L. cv. Xanthi and were fed frozen eggs of *E.*

83   *kuehniella*, also supplied by Koppert B.V.; flour moth eggs were replenished every two days.

84   Water was provided via a soaked paper plug fitted into a plastic dish (4.3 cm diameter). The

85   insects were maintained in growth chambers at 23±1 °C, 60±5% RH and a 16L:8D h photoperiod

86   for the rearing of the stock colony and for all experiments described below.

87

88

89   *2.2 Preparation of the artificial diet*

90

91   The artificial diet used in the experiments is based on the diet described by White et al. (2000). It

92   contained 2 g Primatone®, 20 g soy flour, 10 g maltose (all from MPX Biomedicals, Asse-

93   Relegem, Belgium), 25 g tap water and 20 g fresh hen's egg yolk. The ingredients were mixed at

94   high speed with a Virtis mixer (SP Industries Inc., Gardiner, New York) for two minutes. The

95   diet was stored in a refrigerator at 4°C and new diet was prepared every week. The semi-liquid

96   artificial diet was encapsulated using a diet encapsulation device (Analytical Research Systems,

4

97   Gainesville, Florida) into 70 µL hemispherical domes, consisting of stretched Parafilm[®]'M' and

98   sealed with adhesive tape (Scotch[®] 3M packaging super tape).

99

100

101   *2.3 Determining the minimum amount of E. kuehniella eggs needed for optimal development of*

102   *individual nymphs*

103

104   In order to appreciate the value of pollen in supplementing suboptimal amounts of *E. kuehniella*

105   eggs, the minimum amount of these eggs to be administered to individual nymphs for optimal

106   development had to be determined. First instars (< 24h old) were taken out of the stock culture

107   and were placed individually in plastic cups (4 cm diameter, 2.5 cm high) on paraffin

108   impregnated wax paper substrates. Two individual Parafilm[®] domes filled with tap water were

109   offered to the insects to serve as a moisture source. Predator nymphs were offered 5, 10, 20, 40 or

110   80 eggs of *E. kuehniella* (respective sample sizes: 28, 27, 28, 59, 30 and 33 first instars). The

111   flour moth eggs and water domes were replaced every 3 days. Nymphs that died on the first day

112   of the experiment were replaced by new ones, as it was assumed that their death was due to

113   handling rather than to treatment effects. Nymphal development and survival were monitored on

114   a daily basis. Nymphs that successfully reached the adult stage were sexed and weighed at

115   emergence on a Sartorius Genius balance type 'ME215P' with 0.01 mg precision (Sartorius,

116   Goettingen, Germany). Unmated females were kept individually for one more week on the same

117   diet after which they were dissected; oocytes were counted following a weighted counting

118   method described by Vandekerkhove et al. (2006): late vitellogenic to mature oocytes in

119   ovarioles were scored 1, early to mid vitellogenic oocytes 0.5 and previtellogenic oocytes 0.25;

120   mature oocytes present in the oviducts were also scored 1. The scores for all ovarioles were then

121   summed yielding a weighted sum of oocytes. Escaped individuals were omitted from analysis.

122

123

124   *2.4 Pollen as a supplement or substitute for E. kuehniella eggs*

125

126   In a subsequent experiment it was investigated if supplementing pollen could compensate for a

127   suboptimal amount of *E. kuehniella* eggs. Also developmental and reproductive fitness of *M.*

128   *pygmaeus* fed solely on bee pollen was assessed. The pollen used in the experiments was frozen

129   moist honeybee pollen (i.e. pollen placed in the freezer at the day of collection), consisting of a

130   mixture of pollen mainly from Brassicaceae (67%) and Anacardiaceae (11%). The pollen pellets

131   were thawed and finely ground with a Nova type 20 grinder before being offered to the insects.

132   First instars (< 24h old) of the predator were taken out of the stock cultures and were placed in

133   the same cups as described above on paraffin impregnated wax paper substrates. Two Parafilm®

134   domes filled with tap water were offered to the insects to provide for moisture. Predator nymphs

135   were fed one of the following diets: 10 *E. kuehniella* eggs, 40 *E. kuehniella* eggs, 10 *E.*

136   *kuehniella* eggs supplemented with 15 mg pollen, 40 *E. kuehniella* eggs supplemented with 15

137   mg pollen, 80 *E. kuehniella* eggs supplemented with 15 mg pollen or 15 mg pollen only

138   (respective sample sizes: 28, 64, 31, 26, 26 and 29 first instars). All foods and water domes were

139   replaced every three days. Development and survival of nymphs were daily monitored and

140   resulting adults were weighed at emergence. Unmated females were transferred to new individual

141   cups, offered the same diet as in their nymphal life and dissected after one week of adult life to

142   count oocytes.

143

144

145 *2.5 Pollen as a supplement or substitute for an artificial diet*

146

147 A third experiment was carried out to investigate whether a suboptimal artificial diet (White et

148 al., 2000) could be supplemented with bee pollen to support development and reproduction of *M.*

149 *pygmaeus*. The experimental setup was similar to that in the previous experiment. Besides

150 Parafilm® domes with tap water, individual nymphs now received one of four diets: 40 eggs of *E.*

151 *kuehniella*, an artificial diet dome, an artificial diet dome supplemented with 15 mg pollen or 15

152 mg pollen alone (respective sample sizes: 38, 36, 39 and 36 first instars). All foods and water

153 domes were replaced on Mondays, Wednesdays and Fridays. Development and reproductive

154 potential were assessed as described above.

155

156

157

158 *2.6 Statistical analysis*

159

160 One-way analysis of variance (ANOVA) was performed to determine differences in nymphal

161 development times, adult weights, oocyte counts and predation rates among treatments. Means

162 were separated using a Tukey test (variables with homoscedastic data) or a Tamhane test

163 (variables with heteroscedastic data). Nymphal survival rates were compared among treatments

164 by a two-way contingency table with Pearson's Chi-square test. All data were statistically

165 analysed using SPSS 15.0 (SPSS Inc., 1989-2009).

166

167

168

3. Results

170

3.1 Determining the minimum amount of E. kuehniella eggs needed for optimal development of

individual nymphs

173

Nymphal survival was significantly affected by treatment (χ^2=16.67*; df=5; P*=0.005; Pearson's

Chi-square test). Survival was high in treatments supplying at least 10 flour moth eggs to

individual nymphs per 3 days, ranging from 86.4 to 96.3% (Table 1). When nymphs were fed

only 5 *E. kuehniella* eggs per 3 days, survival averaged 64.3%.

178

There were significant differences in developmental times of predators offered different amounts

of flour moth eggs (*F*=42.6; *df*=5,172; *P*<0.001; ANOVA). Nymphs that were fed 20 eggs or

more every 3 days developed to adults within 17.8 to 18.0 days. Those reared on 10 *E. kuehniella*

eggs needed a significantly (*P*<0.05) longer time (19.5 days) to complete their development,

whereas those reared on 5 eggs took 23.5 days to reach adulthood.

184

Supplying 40 or more flour moth eggs every 3 days resulted in significantly higher adult female

body weights than when 20, 10 or 5 flour moth eggs were given (*F*=65.6; *df*=5,81; *P*<0.001;

ANOVA). Male body weights were significantly higher on 20 eggs or more than on 10 or 5 eggs

(*F*=18.3; *df*=5,82; *P*<0.001; ANOVA).

189

190   Females fed 40 or more flour moth eggs every 3 days had higher oocyte counts (13.8-14.9) than

191   those fed 20 eggs (9.6). When females only received 10 or 5 flour moth eggs, the oocyte counts

192   dropped to 3.1 and 0.2, respectively (F=39.2; df=5,77; P<0.001; ANOVA).

193

194

195   *3.2 Pollen as a supplement or substitute for E. kuehniella eggs*

196

197   Nymphal survival was similar on all diets (χ^2=7.15; df=5; P=0.21; Pearson's Chi-square test),

198   and ranged from 71% (10 *E. kuehniella* eggs) to 92% (40 *E. kuehniella* eggs + pollen) (Table 2).

199

200   Duration of nymphal development was comparable for predators reared on 40 or more flour moth

201   eggs with or without a pollen supplement. When nymphs were reared on 10 flour moth eggs

202   supplemented with pollen, development was significantly slower than when they were offered 80

203   flour moth eggs with pollen, but was similar to that when 40 flour moth eggs were offered with

204   or without pollen (F=19.6; df=5,168; P<0.001; ANOVA). Developmental rate of nymphs fed on

205   pollen alone was similar to that of nymphs fed 40 flour moth eggs or 10 flour moth eggs with

206   pollen.

207

208   Body weights of female and male adults that had developed on 10 *E. kuehniella* eggs were

209   significantly lower than of those in the other treatments, averaging 0.84 mg and 0.63 mg,

210   respectively (females: F=24.6; df=5,83; P<0.001; males F=7.9; df=5,77; P<0.001; ANOVA).

211   When females were fed 10 flour moth eggs with pollen, their body weights were not different

212   from those of females fed 40 flour moth eggs (1.26 mg) or 40 flour moth eggs with pollen (1.28

213   mg).

214

215 Oocyte counts were similar for females reared on a diet of 40 *E. kuehniella* eggs or those given

216 any diet that combined *E. kuehniella* eggs with pollen, but females reared on pollen without flour

217 moth eggs had a significantly lower oocyte count. A diet of 10 *E. kuehniella* eggs resulted in the

218 lowest oocyte counts (F=32.6; df=5,77; P<0.001; ANOVA).

219

220

221 *3.3 Pollen as a supplement or substitute for an artificial diet*

222

223 Survival rates of nymphs did not differ among treatments (χ^2=7.43*; df=5; P*=0.059; Pearson's

224 Chi-square test), ranging from 72% (artificial diet without pollen) to 92% (pollen only) (Table 3).

225

226 Nymphs fed on flour moth eggs reached adulthood faster than those fed on pollen or artificial

227 diet with pollen (F=30.1; df=3,124; P<0.001; ANOVA). Nymphs fed on the artificial diet alone

228 developed significantly slower than those in all other treatments.

229

230 Fresh body weights of adult females were significantly higher when they were reared on flour

231 moth eggs than when reared on pollen and artificial diet or on pollen alone (F=17.9; df=3,56;

232 P<0.001; ANOVA). When reared on the artificial diet alone, female body weights were

233 significantly lower than in the other treatments. When fed on *E. kuehniella* eggs, adult males had

234 superior body weights compared to those on the other diets (F=7.8; df=3,62; P<0.001; ANOVA).

235

236 Oocyte counts were higher for females reared on a diet of flour moth eggs than for those reared

237 on pollen and artificial diet or on artificial diet alone (F=22.6; df=3,53; P<0.001; ANOVA).

238 Females supplied with pollen alone had similar oocyte numbers as those given flour moth eggs or

239 artificial diet and pollen.

240

241

242

243 4. Discussion

244

245 At least 40 *E. kuehniella* eggs (ca. 1 mg fresh weight) per three days were needed per individual

246 to fully support nymphal development and reproductive capacity of young adults. Yano et al.

247 (2002) estimated that for the anthocorid predator *Orius sauteri* (Poppius) (Hemiptera:

248 Anthocoridae) 30 *E. kuehniella* eggs per 4 days were a sufficient number for one individual to

249 successfully complete its nymphal development. This information can be useful for optimising

250 cost efficiency of mass production, although additional experiments are needed to confirm if our

251 results obtained with individual nymphs can be extrapolated to communal rearing.

252

253 Adding pollen to a suboptimal amount of flour moth eggs had a positive effect on nymphal

254 survival, nymphal developmental rate, adult weight and oocyte development. Biological

255 parameters of predators reared on a suboptimal amount of flour moth eggs supplemented with

256 pollen were similar to those of predators that were supplied with an excess of *E. kuehniella* eggs.

257 This suggests that the bee pollen was nutritionally adequate for *M. pygmaeus* nymphs to

258 compensate for a quantitatively suboptimal diet. Although the pollen may have degraded to some

259 extent during the 2-3 day period that it remained in the test containers, there were no evident

260 signs of bacterial or fungal contamination; this may be related to the fact that relative humidity in

261 the containers remained moderate as a result of the absence of leaf materials. Pollen of

262 entomophilous plants is known for its high levels of proteins, amino acids, starch, lipids and

263 some minor nutrients such as vitamins and minerals (Patt et al., 2003; Lundgren and

264 Wiedenmann, 2004; Lundgren, 2009). Different species of pollen may differ in their nutritional

265 value, and particularly amino acid and lipid contents of pollen grains vary greatly among plant

266 species (Richards and Schmidt, 1996). In the current study, pollen pellets collected by honeybees

267 were used, containing mainly pollen from Brassicaceae and Anacardiaceae. Both plant families

268 mainly contain entomophilous taxa (Singh and Jain, 1987). Pollen of *Brassica* spp. is known to

269 be a valuable food source for honeybees (e.g. Cook et al., 2003) and bumble bees (e.g. Rasmont

270 et al., 2005), but little is known on the value of Anacardiaceae pollens for insects. Besides

271 interspecific differences in the nutritional value of pollens, intraspecific variability can also have

272 a significant influence on the biological performance of a pollen feeding insect: Lundgren (2009)

273 reported that different hybrids of corn pollen resulted in different survival rates of *Orius*

274 *insidiosus* (Say) (Hemiptera: Anthocoridae) in the absence of arthropod prey.

275

276 Predators of the genus *Macrolophus* have been observed feeding on host plants, such as tomato

277 and eggplant in times of prey scarcity (Alomar et al., 1990). Lykouressis et al. (2001) reported

278 that *M. pygmaeus* can complete its development in the absence of prey by feeding on the leaves

279 of different host plants such as tomato, pepper plant, cucumber and eggplant. In contrast to

280 several members of the Anthocoridae (Lundgren, 2009), predatory mirids are not generally

281 thought of as facultative pollen feeders. Nonetheless, Wheeler (2001) pointed out that pollinivory

282 is not uncommon in predatory Miridae. Perdikis and Lykouressis (2000) reported that adults of

283 *M. pygmaeus* have been observed inside flowers of the noncultivated plant *Ecballium elaterium*

284 (L.) A. Rich. (Cucurbitaceae) in Greece, but they made no suggestion of facultative pollinivory

285 by the predator on this plant. Latter workers investigated the effect of bee pollen on survival and

286   development of *M. pygmaeus* when offered alone or combined with eggplant foliage and/or

287   *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) as arthropod prey. They found that bee pollen

288   improved the developmental performance of *M. pygmaeus* when included in diets containing

289   other food sources. Perdikis and Lykouressis (2000) further reported that *M. pygmaeus* nymphs

290   fed bee pollen alone had longer developmental times than nymphs which were also offered *M.*

291   *persicae* and eggplant leaves. Maleki et al. (2006) stated that pollen could support development

292   and fecundity of *M. pygmaeus* nymphs reared on eggplant leaves in the absence of insect prey

293   and that the pollen improved these parameters when added to a prey diet. Both above-mentioned

294   studies used leaves of eggplant as a source of moisture when comparing the nutritional value of

295   pollen with that of insect prey. However, as certain plant materials, like eggplant leaves, may

296   support development of *M. pygmaeus* (Perdikis and Lykouressis, 2000; Lykouressis et al., 2001),

297   they arguably present the mirid with extra nutrients in addition to water, confounding the effect

298   of water source and diet (i.e. pollen and insect prey). Gillespie and McGregor (2000) showed that

299   *Dicyphus hesperus* Knight (Hemiptera: Miridae) had better fitness on prey and tomato leaves

300   than on prey and a supplementary source of free water. In order to avoid such masking effects of

301   leaf material, we chose to provide water filled Parafilm$^{®}$ domes as a moisture source. Our results

302   indicate that in the absence of leaf material *M. pygmaeus* can reach adulthood on pollen alone.

303   However, feeding the predator exclusively on pollen yielded lower survival, body weights and

304   oocyte counts compared with supplying adequate amounts of flour moth eggs.

305

306   Adding honeybee pollen to sufficient (40) or superfluous amounts (80) of *E. kuehniella* eggs did

307   not improve developmental and reproductive traits of *M. pygmaeus* in our study. Supplementing

308   a diet of *E. kuehniella* eggs with bee pollen led to a 40% increase in fecundity of the anthocorid

309   predator *Orius albidipennis* (Reuter) (Hemiptera: Anthocoridae), but did not affect fecundity of

310   *Orius laevigatus* (Fieber) (Hemiptera: Anthocordiae) (Cocuzza et al., 1997). Funao and

311   Yoshiyasu (1995) reported that corn pollen alone was not sufficient for rearing *O. sauteri*,

312   whereas a combined diet of *Aphis gossypii* Glover (Hemiptera: Aphididae) aphids and corn

313   pollen yielded an improvement of survival (from 21 to 93%), developmental time (from 17.2 to

314   13.3 days) and fecundity (from 15.4 to 30.4 oviposited eggs per female) compared with corn

315   pollen alone.

316

317   Bonte and De Clercq (2009) found that supplementing moist bee pollen to an artificial diet based

318   on egg yolk (Arijs and De Clercq, 2002) offered to nymphs of *O. laevigatus* did not fully

319   compensate for the nutritional deficiencies of the artificial diet. The artificial diet used in the

320   current study (White et al., 2000), was found to be suboptimal for the production of *M.*

321   *pygmaeus*, yielding slower development and reduced oviposition compared with insects reared on

322   *E. kuehniella* eggs (Vandekerkhove et al. 2006). Supplementing this artificial diet with bee pollen

323   had a positive effect on nymphal survival, development, adult weight and oocyte development,

324   but the predator's performance was still inferior to that on *E. kuehniella* eggs. However,

325   performance of the predator was similar on artificial diet with pollen and on pollen alone. This

326   may imply that in the combined treatment the predator nymphs largely ignored the artificial diet

327   as they were more attracted to the pollen. In visual observations during the experiment, nymphs

328   were rarely observed feeding on the artificial diet and spent most of their time near the pollen.

329

330   The current study indicates that replacing part of the costly *E. kuehniella* eggs with moist

331   honeybee pollen would not cause significant negative effects on the fitness of *M. pygmaeus*.

332   However, more study is needed to test the validity of this hypothesis for large scale production

333   systems. Further, our study confirms the ecological relevance of pollinivory for this mirid

334 predator. Like for many other arthropod natural enemies, including a range of predatory

335 heteropterans (Lundgren, 2009), pollen may be a suitable alternative food to support field

336 populations of *M. pygmaeus* in times of prey scarcity. The findings also suggest that the predator

337 may be released in pollen producing crops before pest population build-up and that its pollinivory

338 may thus play an important role in a preventative release strategy, as suggested by Cocuzza et al.

339 (1997) for *Orius* spp. Adding pollen to a crop as an alternative food source has proven successful

340 in increasing the fecundity and abundance of several predatory mites (e.g. van Rijn et al., 2002;

341 Duso et al., 2004; Hoogerbrugge et al., 2008; Nomikou et al., 2009). When *M. pygmaeus* is

342 released in a crop that does not (yet) produce pollen, the application of honeybee pollen in the

343 crop may, therefore, help establishing or sustaining populations of the predator.

344

345

350

351

352

353 References

354

355 Alomar, O., Castañe, C., Gabarra, R., Albajes, R., 1990. Mirid bugs - another strategy for IPM on

356 Mediterranean vegetable crops? Bulletin of IOBC/WPRS 13, 6-9.

357

15

358   Arijs, Y., De Clercq, P., 2002. Artificial diets for the production of natural enemies (predators

359   and parasitoids) of greenhouse pest insects. Final consolidated report, FAIR-project nr. CT-98

360   4322.

361

362   Bonte, M., De Clercq, P., 2009. Impact of artificial rearing systems on the developmental and

363   reproductive fitness of *Orius laevigatus*. Journal of Insect Science (in press).

364

365   Cocuzza, G.E., De Clercq, P., Van de Veire, M., De Cock, A., Degheele, D., Vacante, V., 1997.

366   Reproduction of *Orius laevigatus* and *Orius albidipennis* on pollen and *Ephestia kuehniella* eggs.

367   Entomologia Experimentalis et Applicata 82, 101-104.

368

369   Cook, S.M., Awmack, C.S., Murray, D.A., Williams, I.H., 2003. Are honey bees' foraging

370   preferences affected by pollen amino acid composition? Ecological Entomology 28, 622-627.

371

372   De Clercq, P., 2008. Culture of natural enemies on factitious foods and artificial diets. In:

373   Capinera, J.L. (Ed.), Encyclopedia of Entomology, 2nd edition Springer, Dordrecht, The

374   Netherlands, vol.I, pp. 1133-1136.

375

376   Duso, C., Malagnini, V., Paganelli, A., Aldegheri, L., Bottini, M., Otto, S. 2004. Pollen

377   availability and abundance of predatory phytoseiid mites on natural and secondary hedgerows.

378   BioControl 49, 397-415.

379

380   Funao, T., Yoshiyasu, Y., 1995. Development and fecundity of *Orius sauteri* (Poppius)

381   (Hemiptera, Anthocoridae) reared on *Aphis gossypii* Glover & corn pollen. Japanese Journal of

382   Applied Entomology and Zoology 39, 84-85.

383

384   Gillespie, D.R., McGregor, R.R., 2000. The functions of plant feeding in the omnivorous

385   predator *Dicyphus hesperus*: water places limits on predation. Ecological Entomology 25, 380-

386   386.

387

388   Hoogerbrugge, H., van Houten, Y., van Baal, E., Bolckmans, K., 2008. Alternative food sources

389   to enable establishment of *Amblyseius swirskii* (Athias-Henriot) on chrysanthemum without pest

390   presence. Bulletin of IOBC/WPRS 32, 79-82.

391

392   Lundgren, J.G., Wiedenmann, R.N., 2004. Nutritional suitability of corn pollen for the predator

393   *Coleomegilla maculata* (Coleoptera: Coccinellidae). Journal of Insect Physiology 50, 567-575.

394

395   Lundgren, J.G., 2009. Relationships of natural enemies and non-prey foods. Springer Science +

396   Business Media B.V., 460p.

397

398   Lykouressis, D., Perdikis, D., Michalaki, M., 2001. Nymphal development and survival of

399   *Macrolophus pygmaeus* Rambur (Hemiptera: Miridae) on two eggplant varieties as affected by

400   temperature and presence/absence of prey. Biological Control 20, 222-227.

401

402   Maleki, F., Ashouri, A., Mohaghegh, J., Bandani, A., 2006. Effects of some diets on

403   *Macrolophus pygmaeus* Rambur (Hemiptera: Miridae) fitness under laboratory conditions.

404   Communications in Applied Biological Sciences Ghent University 71, 393-397.

405

406   Margaritopoulos, J.T., Tsitsipis, J.A., Perdikis, D.C., 2003. Biological characteristics of the

407   mirids *Macrolophus costalis* and *Macrolophus pygmaeus* preying on the tobacco form of *Myzus*

408   *persicae* (Hemiptera: Aphididae). Bulletin of Entomological Research 93, 39-45.

409

410   Nomikou, M., Sabelis, M. W., Janssen, A., 2009. Pollen subsidies promote whitefly control

411   through the numerical response of predatory mites. BioControl, DOI 10.1007/s10526-009-9233-

412   x.

413

414   Patt, J.M., Wainright, S.C., Hamilton, G.C., Whittinghill, D., Bosley, K., Dietrick, J. and

415   Lashomb, J.H. 2003. Assimilation of carbon and nitrogen from pollen and nectar by a predaceous

416   larva and its effects on growth and development. Ecological Entomology 28, 717-728.

417

418   Perdikis, D., Lykouressis, D., 2000. Effects of various items, host plants, and temperature on the

419   development and survival of *Macrolophus pygmaeus* Rambur (Hemiptera: Miridae). Biological

420   Control 17, 55-60.

421

422   Perdikis, D., Lykouressis, D., 2004. *Macrolophus pygmaeus* (Hemiptera: Miridae) population

423   parameters and biological characteristics when feeding on eggplant and tomato without prey.

424   Journal of Economic Entomology 97, 1291-1298.

425

426 Perdikis, D., Lykouressis, D., Economou, L., 1999. The influence of temperature, photoperiod

427 and plant type on the predation rate of *Macrolophus pygmaeus* on *Myzus persicae*. BioControl

428 44, 281-289.

429

430 Rasmont, P., Regali, A., Ings, T.C., Lognay, G., Baudart, E., Marlier, M., Delcarte, E., Viville,

431 P., Marot, C., Falmagne, P., Verhaeghe, J-C., Chittka, L. 2005. Analysis of pollen and nectar of

432 *Arbutus unedo* as a food source for *Bombus terrestris* (Hymenoptera: Apidae). Journal of

433 Economic Entomology 98, 656-663.

434

435 Richards, P.C., Schmidt, J.M., 1996. The effect of selected dietary supplements on survival and

436 reproduction of *Orius insidiosus* (Say) (Hemiptera: Anthocoridae). Canadian Entomologist 128,

437 171-176.

438

439 Singh, V., Jain, D.K., 1987. Taxnomy of Angiosperms. Rastogi Publications, Meerut, India,

440 564p.

441

442 SPSS Inc., 1989-2009. User's guide, version 15.0. Chicago, IL.

443

444 Vandekerkhove, B., van Baal, E., Bolckmans, K., De Clercq, P., 2006. Effect of diet and mating

445 status on ovarian development and oviposition in the polyphagous predator *Macrolophus*

446 *caliginosus* (Heteroptera: Miridae). Biological Control 39, 532-538.

447

448 van Rijn, P.C.J., van Houten, Y.M., Sabelis, M.W., 2002. How plants benefit from providing

449 food to predators even when it is also edible to herbivores. Ecology 83, 2664-2679.

450

451   Wheeler, A.G., 2001. Biology of the plant bugs (Hemiptera: Miridae): Pests, Predators,

452   Opportunists. Cornell University Associates, London.

453

454   White, J.H., Stauffer, L.A., Gallagher, K.A., 2000. Methods for rearing insects, mites and other

455   beneficial organisms. United States Patent no. 6.129.935.

456

457   Yano, E., Watanabe, K., Yara, K., 2002. Life history parameters of *Orius sauteri* (Poppius) (Het.,

458   Anthocoridae) reared on *Ephestia kuehniella* eggs and the minimum amount of the diet for

459   rearing individuals. Journal of Applied Entomology 126, 389-394.

460

461

462

463

464

465

466

467

468

469

470

471

472

473 Table 1. Nymphal survival, developmental time, adult weight and weighted sum of oocytes of *M. pygmaeus* fed on

474 different amounts of *E. kuehniella* eggs

Number of *E. kuehniella* eggs/nymph	N	Nymphal survival (%)[a]	Developmental time (days)[c]	Adult weight (mg)[b]		Weighted sum of oocytes [c]
				Females	Males	
5	28	64.3 ± 9.1b	23.5 ± 0.7c	0.58 ± 0.03d	0.48 ± 0.03c	0.2 ± 0.1d
		(n=18)	(n=18)	(n=11)	(n=6)	(n=10)
10	27	96.3 ± 3.6a	19.5 ± 0.3b	0.83 ± 0.03c	0.68 ± 0.02b	3.1 ± 0.5c
		(n=26)	(n=26)	(n=11)	(n=14)	(n=10)
20	28	92.6 ± 4.9a	18.0 ± 0.2a	1.05 ± 0.02b	0.77 ± 0.03ab	9.6 ± 0.8b
		(n=26)	(n=26)	(n=15)	(n=11)	(n=14)
40	59	86.4 ± 5.4a	17.8 ± 0.1a	1.25 ± 0.03a	0.82 ± 0.02a	13.8 ± 0.6a
		(n=51)	(n=51)	(n=23)	(n=27)	(n=22)
60	30	93.3 ± 4.6a	17.9 ± 0.3a	1.21 ± 0.04a	0.79 ± 0.03ab	14.9 ± 1.4a
		(n=28)	(n=28)	(n=15)	(n=13)	(n=15)
80	33	87.9 ± 5.7a	17.9 ± 0.3a	1.32 ± 0.03a	0.83 ± 0.01a	14.4 ± 1.1a
		(n=29)	(n=29)	(n=12)	(n=17)	(n=12)

475 Means (± SE) within a column followed by the same letter are not significantly different ($P>0.05$; [a] Pearson's Chi-

476 square test, [b] Tukey's test or [c] Tamhane's T2 test for unequal variances). N = initial number of tested individuals,

477 nymphs that escaped during the experiment were dropped from analysis.

478 Table 2. Nymphal survival, developmental time, adult weight and weighted sum of oocytes of *M. pygmaeus* fed on

479 different diets

Diet	N	Nymphal survival (%)[a]	Developmental time (days) [b]	Adult weight (mg)[b]		Weighted sum of oocytes [b]
				Females	Males	
Bee pollen	29	79.3 ± 7.5a (n=23)	18.5 ± 0.3c (n=23)	1.10 ± 0.05c (n=11)	0.73 ± 0.02a (n=11)	9.4 ± 1.1b (n=11)
10 *E. kuehniella* eggs	28	71.4 ± 8.5a (n=20)	20.5 ± 0.2d (n=20)	0.84 ± 0.02d (n=11)	0.63 ± 0.02b (n=9)	2.1 ± 0.3c (n=11)
10 *E. kuehniella* eggs + bee pollen	31	87.1 ± 6.0a (n=27)	18.3 ± 0.2bc (n=27)	1.17 ± 0.02bc (n=15)	0.77 ± 0.02a (n=12)	13.5 ± 0.8a (n=14)
40 *E. kuehniella* eggs	64	89.1 ± 3.9a (n=57)	17.4 ± 0.2abc (n=57)	1.26 ± 0.02ab (n=32)	0.77 ± 0.01a (n=25)	13.8 ± 0.6a (n=28)
40 *E. kuehniella* eggs + bee pollen	26	92.3 ± 5.2a (n=24)	17.4 ± 0.2ab (n=24)	1.28 ± 0.04ab (n=12)	0.78 ± 0.03a (n=12)	13.4 ± 0.9a (n=11)
80 *E. kuehniella* eggs + bee pollen	26	88.4 ± 6.3a (n=23)	17.0 ± 0.4a (n=23)	1.39 ± 0.05a (n=8)	0.81 ± 0.02a (n=14)	14.1 ± 0.9a (n=8)

480 Means (± SE) within a column followed by the same letter are not significantly different ($P>0.05$; [a] Pearson's Chi-

481 square test, [b] Tukey's test).

482 N = initial number of tested individuals, nymphs that escaped during the experiment were dropped from analysis

483

22

484 Table 3. Nymphal survival, developmental time, adult weight and weighted sum of oocytes of *M. pygmaeus* fed on

485 different diets

Diet	N	Nymphal survival (%)[a]	Developmental time (days)[c]	Adult weight (mg)[b]		Weighted sum of oocytes [b]
				Females	Males	
40 *E. kuehniella* eggs	38	89.5 ± 5.0a (n=34)	16.3 ± 0.2a (n=34)	1.26 ± 0.04a (n=22)	0.77 ± 0.03a (n=12)	12.0 ± 0.7a (n=22)
Bee pollen	36	91.6 ± 4.6a (n=33)	17.7 ± 0.2b (n=33)	1.05 ± 0.03b (n=14)	0.68 ± 0.02b (n=17)	9.4 ± 1.0ab (n=12)
Artificial diet	36	72.2 ± 7.5a (n=26)	20.8 ± 0.6c (n=26)	0.86 ± 0.04c (n=10)	0.59 ± 0.02b (n=16)	2.1 ± 0.8c (n=9)
Bee pollen + artificial diet	39	89.7 ± 4.9a (n=35)	18.3 ± 0.2b (n=35)	1.08 ± 0.03b (n=14)	0.66 ± 0.02b (n=21)	7.8 ± 0.7b (n=14)

486 Means (± SE) within a column followed by the same letter are not significantly different ($P>0.05$; [a] Pearson's Chi-

487 square test, [b] Tukey's test or [c] Tamhane's T2 test for unequal variances).

488 N = initial number of tested individuals, nymphs that escaped during the experiment were dropped from analysis

489