The midge *Dasineura mali* Kieffer (Diptera: Cecidomyiidae) is an important pest of apple (*Malus domestica* Borkh.) and a potential fresh fruit contaminant, causing quarantine concerns. The phenological dynamics of *D. mali* and its egg parasitoid *Platygaster demades* Walker (Hymenoptera: Platygasteridae) were studied in the field in Palmerston North, New Zealand, for 2 yr. Both shoot infestation rate by *D. mali* and *D. mali* density per shoot sharply increased in the second generation, reaching ≈65% and 100–200 eggs, respectively. However, although the infestation rate in the third generation remained as high as in the second generation, the pest density per shoot significantly decreased to 40–60 eggs in the third generation. In the fourth generation, both infestation rate and pest density per shoot decreased to ≈30% and 10 eggs. Due to the simultaneous decline of the apple shoot number and *D. mali* density in the third and fourth *D. mali* generations, the absolute number of *D. mali* in the orchard also has declined proportionally during the same period. The parasitism and superparasitism rates significantly increased as the season progressed, from 45 to 55 and 37% in the first generation to 87 and 82% in the fourth generation, respectively. Our results suggest that *P. demades* contributes to the continuous decline of *D. mali* numbers in the field; it is a good searcher, particularly when its hosts become increasingly scarcer over the season, and it avoids overshooting the host population later in the season by increasing superparasitism. The frequency of *P. demades* aestivation increases from late spring to midsummer and then decreases during the late summer and early autumn. Although the emergence of *P. demades* was ≈2 to 3 wk behind that of *D. mali* in each generation, the increasing parasitism rates from the first to the fourth generations indicate that *P. demades* is synchronized with *D. mali* in the field.

**KEY WORDS** *Dasineura mali*, *Platygaster demades*, phenology, parasitism, synchronization
de Vere Graham and Gijswijt 1998). It is a solitary endoparasitoid of *D. mali* eggs, and its eggs hatch after the mature *D. mali* larvae start spinning their cocoons in the soil; its larvae feed and complete their development in the cocooned *D. mali* larvae (Todd 1956, He et al. 2010). Adult females carry >1,300 eggs at emergence, and can live for 20–35 d under 13–20°C (Sandanayaka and Ramankutty 2007). Therefore, this parasitoid can be a highly effective biological control agent of *D. mali*. However, previous fieldwork (Todd 1959, Tomkins et al. 2000, Shaw et al. 2005) suggests that high parasitism occurs only in the early (up to 82%) and late (up to 96%) generations of *D. mali*, whereas in the second generation only 1–3% of *D. mali* are parasitized, leaving almost the entire second generation out of control by the parasitoid. Todd (1959) and Shaw et al. (2005) attribute the low parasitism rate in the second generation to the asynchronized emergence of *D. mali* and *P. demades* adults. However, the parasitism rates recorded in these observations may be questionable due to small sample sizes: only one sample was collected for each generation and a small number of individuals were dissected by these authors. Parasitism rate may be a poor measure of the impact of parasitoids on the host population dynamics (Kidd and Jervis 2005) if the number of samples taken is inadequate (van Driesche 1983). Hassell and Waage (1984) and Kidd and Jervis (2005) suggest that to evaluate the efficiency of a parasitic wasp in suppressing host populations, average levels of parasitism for each generation and variability of parasitism between generations must be determined. Furthermore, superparasitism (more than one parasitoid egg laid in a host) is an important factor that governs host-parasitoid interactions and host population dynamics (Taylor 1988, van Alphen and Visser 1990). So far, superparasitism in the *D. mali*- *P. demades* system is still not well known. Therefore, more frequent sampling and larger sample size are required to obtain reliable information on parasitism and superparasitism rates throughout the season and the role the parasitoid may play in *D. mali* population suppression.

Our recent work (He et al. 2010) shows that *P. demades* has one to four generations a year because it produces aestivating and nonaestivating phenotypes over the season. However, so far the development and population dynamics of *P. demades* associated with *D. mali* are poorly understood, making it difficult to develop measures for the improvement of biological control of *D. mali* by using *P. demades*.

Here, we report our findings over 2 yr on the phenology of *D. mali* and *P. demades* in the field, with two objectives: 1) to determine rates of infestation by *D. mali* (percentage of growing apple shoots infested), dynamics of *D. mali* population density, and rates of parasitism and superparasitism by *P. demades* throughout the season; and 2) to identify the developmental dynamics of *D. mali* and *P. demades*. Knowledge from this work provides essential information for the understanding of phenological dynamics of *D. mali* and *P. demades* and for the development of *D. mali* monitoring measures and effective biological control programs for *D. mali* using *P. demades*. The methodology developed in the current study may be useful for studies on other similar pest-parasitoid systems.

### Materials and Methods

**Study Site.** New Zealand has a mild summer from December to February and winter from June to August. For example, in Palmerston North (40.3°S, 175.6°E), New Zealand, mean temperature ranges from 15.8 to 18.0°C for summer and from 8.5 to 9.5°C for winter (New Zealand Meteorological Service 2005–2007). This study was carried out in a mature organic apple orchard (0.85 ha) with ‘Tenroy’ (‘Royal Gala’) for two seasons from 2005 to 2007 in Palmerston North, with more extensive investigations performed in the 2006–2007 season. Due to our inadequate understanding of *D. mali* population dynamics data for the fourth generation was not collected in the 2005–2006 season.

**Phenological Dynamics of Infestation by *D. mali* and Parasitism by *P. demades*.** When *D. mali* adults were first observed on 10 September 2005, we started daily monitoring of their oviposition by examining the infested shoots in the orchard. Five growing shoots infested by *D. mali* eggs were randomly cut from different trees each day during the oviposition peaks of the first (6–9 October 2005), second (29 November–4 December 2005) and third (7–11 January and 17–19 February 2006) generations (He et al. 2010), and brought to the laboratory for examination. The number of *D. mali* eggs per shoot was recorded under a stereomicroscope (SZ51, Olympus, Tokyo, Japan).

On the basis of our work in the 2005–2006 season, more extensive investigation was carried out in the 2006–2007 season. After apple trees started budding in early September 2006, we randomly sampled 60 shoots (examining all young leaves on each shoot by using a hand lens) in the orchard once every 3 d to determine the dynamics of infestation rates by *D. mali* in the field until early April 2007. On each sampling day, we randomly cut ten infested shoots from those 60 shoots examined in the field and brought them to the laboratory to determine the dynamics of *D. mali* population density in the infested shoots by counting the number of eggs under the stereomicroscope.

To determine the parasitism and superparasitism rates by *P. demades* throughout the season in the field, we collected and dissected a large number of mature *D. mali* larvae (third instar) in both years, with more frequent sampling and larger samples collected and dissected in the 2006–2007 season. In the 2005–2006 season, mature *D. mali* larvae were randomly collected from rolled leaves in the orchard on 1 November 2005 (*n* = 120, first generation), 24 December 2005 (*n* = 265, second generation), and 30 January 2006 and 8 March 2006 (*n* = 150 and 200, early and late third generation, respectively). In the 2006–2007 season, 50 mature *D. mali* larvae were randomly collected from ten infested shoots every 3 d between 17 October 2006 and 9 April 2007. Larvae were brought to the laboratory and dissected individually in a drop of Ringer’s solution on a glass slide under the stereomicroscope.
For each sample in both 2005–2006 and 2006–2007 seasons, larvae were equally divided into five groups to obtain the mean parasitism and superparasitism rates and the number of parasitoid eggs per parasitized D. mali larva for analysis.

Developmental Dynamics of D. mali and P. demades in the Field. This experiment was designed to determine how developmental rates differed in different generations of both D. mali and P. demades and whether the different developmental rates affected synchronization between these two species in the field.

In the 2005–2006 season, we collected mature D. mali larvae from rolled apple leaves in the field, placed them in metal mesh dishes (2 cm in height by 8.5 cm in diameter, with aperture size of 0.25 mm) with soil and then buried these dishes in the soil (2 cm in depth) horizontally under the apple trees. The larval collection and burial were conducted on four separate occasions: 1 November 2005 (first generation), 24 December 2005 (second generation), and 30 January 2006 and 8 March 2006 (early and later third generation, respectively). Forty dishes were buried on each occasion with 20 having 100 mature D. mali larvae each for observation of adult emergence of both species and 20 containing 50 mature D. mali larvae each for examination of developmental stages of both species. Immediately after burial, a transparent plastic cylinder was pushed into the soil on the top of each dish and fastened by two 5-cm steel nails. The cylinder had the same diameter as the dish and 10.5 cm in height, with a metal mesh top and two metal mesh holes (3 cm in diameter) on opposite walls for ventilation (mesh aperture of 0.25 mm).

In the 2006–2007 season, we collected mature D. mali larvae and buried 448 dishes in total by using the above-mentioned method at fortnightly intervals for each generation of D. mali. Each dish contained 50 mature larvae. The burial schedule was 1) first generation: 18 October, 1 November, and 15 November 2006 with 58, 56, and 54 dishes, respectively; 2) second generation: 1 and 16 January 2007 with 48 and 46 dishes, respectively; 3) third generation: 6 February, 20 February, and 6 March 2007 with 42, 40, and 38 dishes, respectively; and 4) fourth generation: 2 and 16 April 2007 with 34 and 32 dishes, respectively. In total, 10 burials were made in the 2006–2007 season.

In both seasons, adult emergence of D. mali and P. demades in the field was examined daily at 11:00 a.m., 1 h after the emergence peak between 6:00 and 10:00 a.m. (Harris et al. 1999), to record daily emergence of both species. Plastic cylinders on buried dishes were lifted up individually. Adults of both species found in the cylinders and on the dishes were counted. The counted adults were removed and discarded, and the cylinders were refastened to dishes. We report the mean emergence per dish weekly (see Fig. 3). The developmental duration between burial time and adult emergence was recorded for both species.

To determine developmental stages of both species we brought two dishes with 50 mature D. mali larvae each from each burial in the 2005–2006 season to the laboratory for examination every week. The exception was the fourth burial (i.e., late third generation) where only one dish per week was examined. The contents of each dish were put in a sieve (4 cm in height by 10 cm in diameter, with aperture size of 0.75 mm) on top of another sieve of the same diameter with aperture size = 0.25 mm and gently rinsed under running tap water until all existing D. mali cocoons were exposed. The cocoons were then individually dissected. Based on our dissections all P. demades eggs had developed to embryos when D. mali larvae became mature. Thus, P. demades individuals were considered as having entered aestivation if they were at embryonic stage with some development of the head, after all other individuals of the same burial had developed to adults and emerged (He et al. 2010).

In the 2005–2006 season, due to an unexpectedly high proportion of P. demades that entered aestivation in the second and third generations and prolonged aestivation duration, all 20 dishes with 50 mature D. mali larvae each from these generations had been dissected within 10 weeks. Three hundred and 250 D. mali cocoons from the second and early third generations, respectively, were extracted from dishes originally containing 100 mature D. mali larvae each and reburied as described above in new dishes in the field. To continue the examination of development and overwintering, we dissected seven and five cocoons weekly during the winter for the second and third generations, respectively. The remaining dishes were observed as mentioned above to record emergence of both D. mali and P. demades in these dishes daily until all emerged in the next spring.

In the 2006–2007 season, we brought one dish from each burial to the laboratory each week for dissecting, and we recorded the emergence of both species as mentioned above.

Statistical Analysis. A goodness-of-fit test was used to test the distribution of data residuals. Data on the parasitism in both seasons were normally distributed and thus analyzed using analysis of variance (ANOVA) followed by a Tukey’s studentized range (ANOVA) followed by a Tukey’s studentized range test (KWT) followed by Dunn’s procedure for multiple comparisons. Data on the frequency of parasitoids entering aestivation were analyzed using a chi-square test followed by Marascuilo procedure for multiple comparisons (Daniel 1990).

Results

Phenological Dynamics of Infestation by D. mali and Parasitism by P. demades. The percentage of apple shoots infested by the second and third generation D. mali was significantly higher than that by the first and fourth generations in the 2006–2007 season (KWT: $\chi^2 = 47.81 > \chi^2_{3,0.05} = 7.82, P < 0.001$) (Fig. 1). In both
seasons, the number of *D. mali* eggs per infested shoot was significantly higher in the second generation than in the first and third generations (KWT; $\chi^2 = 14.74 > \chi^2_{2,0.05} = 5.99$ for the 2005–2006 season; $\chi^2 = 23.30 > \chi^2_{3,0.05}$ for the 2006–2007 season; $P < 0.0001$) (Fig. 2).

The mean parasitism and superparasitism rates and number of parasitoid eggs per parasitized *D. mali* larva significantly increased as the season progressed in both seasons (ANOVA: $F = 28.84$, 12.92, and 15.45 for parasitism and superparasitism rates and number of parasitoid eggs per *D. mali* larva, respectively (df = 6, 58; $P < 0.0001$) (Table 1).

**Developmental Dynamics of *D. mali* and *P. demades* in the Field.** Healthy *D. mali* continued to develop throughout the season. However, the development of parasitized *D. mali* larvae was arrested and these did not pupate. Some *P. demades* entered aestivation at the embryonic stage if the parasitized *D. mali* larvae became mature between late spring (mid-November) and late summer (late February), with the highest percentage of parasitoids entering aestivation during early and midsummer (18.8, 64.8, and 47.1% of parasitoids entered aestivation in late spring, early to midsummer, and late summer, respectively) ($\chi^2$ test: $\chi^2 = 22.76 > \chi^2_{6,0.05} = 12.59, P < 0.01$). None of the aestivated *P. demades* individuals emerged until the next spring.

In each generation, the developmental duration of *D. mali* was significantly shorter than that of nonaestivating *P. demades* without significant difference between sexes (Table 2). Our data, particularly those in the 2006–2007 season where more frequent sampling took place, show that although *P. demades* required significantly more time than *D. mali* to develop to adults (Table 2), emergence of *D. mali* and *P. demades* during the season was still more or less synchronized within and between generations (Fig. 3).

*D. mali* larvae in the late third generation and all individuals in the fourth generation overwintered: they remained as larvae during the winter and pupated during the early spring (early September). The aestivated *P. demades* in the first, second, and third generations and the nonaestivated *P. demades* in the fourth generation overwintered. At the beginning of the winter (early June), all live aestivated and nonaestivated *P. demades* individuals from different generations developed to larval stage simultaneously. They continued to grow, develop, and start pupation approximately mid-winter (early July). In the next spring adult emergence of both *P. demades* and *D. mali* were completely synchronized (Fig. 3).

**Discussion**

Due to insufficient knowledge of the *D. mali*–parasitoid–apple system in the earlier phase of this study, sample size and sampling frequency were different in the two seasons with much larger sample size and higher sampling frequency occurring in the 2006–2007 season. Despite these differences, our data show a very
similar trend of phenological dynamics in both seasons (Tables 1 and 2; Figs. 2 and 3).

Our results demonstrate that both shoot infestation rate by *D. mali* (Fig. 1) and *D. mali* density per shoot (Fig. 2) sharply increased in the second generation, supporting previous studies (Todd 1956, Shaw et al. 2005). However, although the infestation rate in the third generation remained as high as in the second generation (Fig. 1), the pest density per shoot significantly decreased in the third generation (Fig. 2). In the fourth generation, both infestation rate (Fig. 1) and pest density per shoot (Fig. 2) significantly decreased. This pattern of population dynamics over the season may be attributed to 1) the seasonal variation of availability of apple shoots, the only food source for *D. mali*; and 2) the action by *P. demades*, the only parasitoid of *D. mali*, in the field.

According to Shaw et al. (2005), the number of growing shoots peaks in mid- to late spring (the second *D. mali* generation) and then declines in early and

<table>
<thead>
<tr>
<th>Year</th>
<th>Generation</th>
<th>Male</th>
<th>Female</th>
<th>Male</th>
<th>Female</th>
<th>χ²</th>
<th>P</th>
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</thead>
<tbody>
<tr>
<td>2005–2006</td>
<td>First generation</td>
<td>30.3 ± 1.6b</td>
<td>30.6 ± 1.6b</td>
<td>49.0 ± 3.0a</td>
<td>49.5 ± 3.1a</td>
<td>667.5</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Second generation</td>
<td>21.6 ± 2.2b</td>
<td>21.8 ± 2.4b</td>
<td>40.5 ± 4.2a</td>
<td>42.0 ± 4.7a</td>
<td>391.1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Third generation</td>
<td>23.2 ± 1.5b</td>
<td>23.4 ± 1.4b</td>
<td>44.0 ± 7.1a</td>
<td>45.4 ± 7.7a</td>
<td>314.8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>2006–2007</td>
<td>First generation</td>
<td>34.9 ± 6.2b</td>
<td>35.3 ± 5.8b</td>
<td>56.1 ± 7.6a</td>
<td>55.2 ± 6.9a</td>
<td>2590.2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Second generation</td>
<td>22.6 ± 4.5b</td>
<td>22.5 ± 4.4b</td>
<td>39.5 ± 5.0a</td>
<td>38.6 ± 4.2a</td>
<td>1,111.8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Third generation</td>
<td>24.7 ± 3.9b</td>
<td>24.8 ± 3.9b</td>
<td>44.6 ± 8.1a</td>
<td>43.3 ± 6.3a</td>
<td>723.7</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Means ± SE followed by the same lowercase letters in rows are not significantly different (*P* > 0.05; KWT). Data for the aestivated and overwintered populations were excluded for analysis as those individuals would not emerge until the next spring (see Fig. 3).

Fig. 3. Mean weekly number of emerged adults of *D. mali* and *P. demades* per buried dish in the 2005–2006 and 2006–2007 seasons. The first week started on 19 November when the earliest *D. mali* adult was observed to emerge from the buried dishes. Data for the fourth generation were not collected for 2005–2006 season.
midsummer (second and third *D. mali* generations), and reaches the minimum level in late summer and early autumn (the fourth *D. mali* generation). Therefore, the population dynamics of *D. mali* are well synchronized with those of apple shoots. The synchronization between herbivores and their hosts is particularly important in monophagous cecidomyiids that are responsible for young leaf or bud galls of tree species (Yukawa 2000, Yukawa and Akimoto 2006).

Due to the simultaneous decrease of apple shoot numbers and *D. mali* density and increase of parasitism rate in third and fourth *D. mali* generations, the absolute number of *D. mali* in the orchard also must decline proportionally during the same period.

Todd (1959) and Shaw et al. (2005) reported an extremely low parasitism rate in the second generation (1–3%). However, in those studies they collected only one sample for each generation and dissected only a small number of individuals. In the current study we carried out much more frequent sampling and dissected ≈3,000 mature *D. mali* larvae. Our results show that the parasitism and superparasitism rates significantly increased as the season progressed (Table 1), the implication of which may be three folds. First, *P. demades* contributes to the continuous decline of *D. mali* numbers in the field. Second, this parasitoid is a good searcher, particularly when its hosts become increasingly scarce over the season (Figs. 1 and 2). Third, the increasing superparasitism may serve to avoid overexploiting the host population later in the season. As Umbanhowar and Hastings (2002) predicted, food limitation would reduce herbivore densities, and subsequently reduce the parasitoid population growth. Therefore, overexploiting the host population may result in the extinction of both parasitoid and host populations (Boots and Sasaki 2001).

Many platygastrid species, such as *Platygaster herrickii* Pack and *Platygaster vernalis* Myers, egg parasitoids of Hessian fly, *Phytophaga destructor* (Say) in North America (Hill and Smith 1928), have only one generation per year although their hosts may have several generations (Faculty 2008). *P. demades* is multivoltine in Europe (Gruys 1982, Trapman 1988). In the current study, a proportion of *P. demades* entered embryonic aestivation in the first, second and third generations, resulting in univoltine, bivoltine, trivoltine, and quadrivoltine populations (He et al. 2010). The percentage of *P. demades* entering aestivation increases from late spring to midsummer and then decreases during the late summer and early autumn. In the meantime, superparasitism rate increases over the season (Table 1). According to Seger and Brockmann (1987) and He et al. (2010), the dynamic superparasitism and aestivation rates over the season may be a temporal risk-spreading strategy in response to the decline of *D. mali* eggs (Figs. 1 and 2) in the summer and autumn, to maintain high overwintering population size for the next season.

The synchronization between parasitoids and their hosts is critical to the successful biological control of pests in the field (Kidd and Jervis 2005). The emergence of *P. demades* was ≈2–3 wk behind that of *D. mali* in each generation (Table 2), suggesting that these two species are not synchronized as suggested by Shaw et al. (2005). However, the increasingly high parasitism rates from 45 to 55% in the first generation to over 80% in the fourth generation (Table 1) indicate that *P. demades* is synchronized with *D. mali* in the field. Emergence patterns of *D. mali* and *P. demades* during the season overlapped within and between generations (Fig. 3). Therefore, the synchrony between the two species is probably achieved through within-generation and between-generation parasitism, i.e., in each generation some *D. mali* individuals are parasitized by parasitoids of the same generation and others by those of the earlier generation. Furthermore, the long longevity and high reproductive potential of *P. demades* adult females (Sandanayaka and Ramankutty 2007) further enhance its synchronization with *D. mali* in the field.

Acknowledgments

We thank Plant Growth Unit in Massey University, New Zealand, for allowing us to carry out experiments in their orchards. We also thank Mike Butcher of Pipfruit NZ and two anonymous reviewers for constructive comments on the earlier version of the manuscript. We thank Pipfruit NZ and MURF for funding.

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Received 24 March 2011; accepted 14 July 2011.