

## **Supplementary material**

Virulence of *Melissococcus plutonius* and secondary invaders associated with European foulbrood disease of the honey bee

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**Table S1:** Sample overview (number of individuals) per colony for each assay of experiment 1 – ‘Impact of host background’: survival, larval weight on day 7, relative *M. plutonius* load per larvae; and CFUs used for infection for each treatment group (mean  $\pm$  SE).

| Colony | Treatment | Survival | Larval weight | <i>M. plutonius</i> load | CFUs per ml                           | CFU per larvae   |
|--------|-----------|----------|---------------|--------------------------|---------------------------------------|------------------|
| A      | control   | 70       | 65            | 8                        | 0                                     | 0                |
|        | 49.3      | 70       | 59            | 12                       | $4.8 \times 10^6 \pm 1.6 \times 10^6$ | $1206 \pm 398$   |
|        | 119       | 66       | 57            | 12                       | $3.2 \times 10^6 \pm 1.5 \times 10^6$ | $797 \pm 380$    |
|        | 4-127     | 71       | 65            | 12                       | $7.0 \times 10^7 \pm 3.4 \times 10^7$ | $17512 \pm 8424$ |
|        | total     | 277      | 246           | 44                       |                                       |                  |
| B      | control   | 47       | 44            | 8                        | 0                                     | 0                |
|        | 49.3      | 84       | 76            | 12                       | $4.8 \times 10^6 \pm 1.6 \times 10^6$ | $1206 \pm 398$   |
|        | 119       | 42       | 38            | 8                        | $3.2 \times 10^6 \pm 1.5 \times 10^6$ | $797 \pm 380$    |
|        | 4-127     | 42       | 39            | 8                        | $7.0 \times 10^7 \pm 3.4 \times 10^7$ | $17512 \pm 8424$ |
|        | total     | 215      | 197           | 36                       |                                       |                  |

**Table S2:** Sample overview (number of individuals) for each assay of experiment 2 – ‘Impact of co-infection’: survival, larval weight on day 8; and CFUs used for infection for each treatment group (mean  $\pm$  SE).

| Treatment      | Survival | Larval weight | CFUs per ml                           | CFU per larvae                        |
|----------------|----------|---------------|---------------------------------------|---------------------------------------|
| control        | 73       | 83            | 0                                     | 0                                     |
| <i>M. p.</i>   | 59       | 62            | $1.4 \times 10^8 \pm 6.3 \times 10^7$ | $34605 \pm 15654$                     |
| <i>E. f.</i> * | 65       | 40            | $1.1 \times 10^9 \pm 5.2 \times 10^8$ | $2.7 \times 10^5 \pm 1.3 \times 10^5$ |
| <i>P. a.</i> * | 69       | 42            | $3.4 \times 10^7 \pm 1.4 \times 10^7$ | $8387 \pm 3474$                       |
| total          | 266      | 227           |                                       |                                       |

\* *M. p.* treatment on day 1

**Table S3:** Larval weight (mean  $\pm$  SD) on day 8 (day 7 post-infection) from the preliminary infection experiment. Controls and *M. plutonius* (strain LMG 20360, ST1, CC13) inoculated larvae.

| Colony | Treatment | Weight (mg)      | N  |
|--------|-----------|------------------|----|
| A      | control   | 139.6 $\pm$ 23.2 | 23 |
|        | LMG 20360 | 148.1 $\pm$ 20.3 | 66 |
|        | total     |                  | 89 |
| B      | control   | 124.4 $\pm$ 33.3 | 23 |
|        | LMG 20360 | 134.7 $\pm$ 21.0 | 64 |
|        | total     |                  | 87 |

**Fig. S1.** Larval survival in preliminary infection experiment (*M. plutonius* strain LMG 20360, ST1, CC13) with colony A and colony B (cox regression model:  $P > 0.05$ ).

