

## Observation of Insect *Coccinella undecimpunctata* Parasitizing on the Pathogen of Powdery Mildew in Barley and Wheat

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### ABSTRACT

Powdery mildew caused by *Blumeria graminis* f. sp. *hordei* and *Blumeria graminis* f. sp. *tritici* infects barley and wheat, respectively. The use of chemical pesticides to combat diseases has led to many problems, such as environmental pollution, the residual effect of pesticides on agricultural products, the emergence of new strains of pathogens resistant to pesticides, and disruption of the natural balance between pests and their biological enemies. Therefore, interest began in biological control to reduce the role of pesticides in the process of resisting pathogens. In this study, the eleven-spotted ladybird (*Coccinella undecimpunctata*) or eleven-spotted beetle was used to resistance powdery mildew in barley and wheat, which is a type of insects belonging to one of the genera of the ladybug family of the order of beetles. The insect was observed feeding on mycelium and spores of the fungus *Blumeria graminis* f. sp. *hordei*, and *B. g. f. sp. tritici* which causes powdery mildew in barley and wheat. Therefore, it can be used to resistance disease as a type of biological control. Microscopic examination of the intestine *Coccinella undecimpunctata* revealed the presence of powdery mildew fungus spores in the guts of ladybugs when they were fed on powdery mildew.

**Keywords:** powdery mildew, *Blumeria graminis* f. sp. *hordei*, *Blumeria graminis* f. sp. *tritici*, biological control, *Coccinella undecimpunctata*.

### INTRODUCTION

Wheat and barley are two of the founding crops that started the agricultural revolution about 10,000 years ago in the Fertile Crescent (Zohary et al., 2012). Many of the wild progenitors of these crops still exist in this region (Harlan and Zohary, 1966). Cultivated barley (*Hordeum vulgare* L. ssp. *vulgare*) is remarkably morphologically like its wild progenitor, *Hordeum vulgare* ssp. *Spontaneous*, Hexaploid bread wheat (*Triticum aestivum* L. ssp. *aestivum*) derives its three genomes (A, B, and D) from three diploid wild ancestors (Marcussen et al., 2014; Pont and Salse, 2017; Glemin et al., 2018). Powdery mildews (*Erysiphales*) of

plant pathogens are it may reach epidemic proportions that attack many cereal crops (Glawe, 2008). It is a widespread fungal disease of many plant species in temperate and humid climates. Powdery mildew fungi cause severe yield loss in a wide range of crops in many countries, including Egypt. They also occur regularly in northern Egypt in the Nile Delta region, where they are common humidity and temperature are suitable for the occurrence and development of the disease, furthermore, asexual conidia are the main sources of disease spread during the growing season of plants (Aist and Bushnell, 1991; Arab et al., 2021). However, the sexual structure ascocarp (*cleistothecium*) occurs when conditions are unfavorable for

the formation of conidia, early infection affects crop density and the number of seeds per ear. Yield loss increases to 25% and may cause approximately 10% reduction in production in cold climates in case of late infection Jorgensen (1988). Consequently, these fungi are among the most economically important plant pathogens (Amano, 1986), and treatments with chemical fungicides are most effective but are not economically feasible and may be ineffective when weather conditions are conducive to epidemics. The use of fungicides is challenged by the effects of contamination with chemical fungicides and concerns about effects on non-target organisms, necessitating the search for safer alternatives for disease control management (Razdan and Sabitha, 2009), including biological control by arthropods (Hijwegen, 1992). Mycophagous ladybird beetles (*Coleoptera: Coccinellidae*) are potentially attractive agents for the biological control of pathogens powdery mildew (Sutherland and Parrella, 2009a), guide beetles to mildew-infected plants, foraging especially in the absence of aphids. *Coccinellids* are a heterogeneous group of insects divided into three major categories according to their food preferences: zoophagous (predating), *phytophagous* (plant-eating), and *mycophagous* (fungus-eating) (Giorgi et al., 2009). Predatory ladybirds have evolved from *mycophagous* ladybirds that first were adapted for feeding on sooty molds but then accepted the insects that produce honeydew such as aphids (Leschen, 2000; Weber and Lundgren, 2009), DNA was extracted from the intestine of *Coccinella undecimpunctata* and analyzed using PCR for the presence of *Blumeria graminis* of gut content have fungal spores can be taken up by, *Coccinella*, and even be more frequently present than prey body parts, especially when aphids are low or unavailable (Triltsch, 1999). We used similar methods to assess beetle responses to individual components of pathogens powdery mildew on barley and wheat for presenting a study of powdery mildew infection and the foraging environment of fungal beetles, which have an important role in developing new strategies for integrated disease management.

In this study, we were unable to determine the nutritional potential of *Coccinella undecimpunctata* against powdery mildew in barley and wheat. Therefore, further studies are needed to clarify the potential of using this type of biological control, such as mass breeding, and control techniques against powdery mildew in agroecosystems.

## MATERIAL AND METHODS

Barley and wheat are affected by a group of diseases that cause serious damage and loss of a percentage of the crop annually. These diseases include powdery mildew caused by the fungus *Blumeria graminis* f. sp. *hordei* on barley and *Blumeria graminis* f. sp. *tritici* on wheat.

### Plants and powdery mildew:

#### 1. Experiment with seedling

Isolates of the fungus *Blumeria graminis* f. sp. *hordei* and *Blumeria graminis* f. sp. *tritici* that were collected from the previous season (cleistothecium) were used as a source of experimental infection, on young seedlings of the barley cultivar Giza 123 and wheat cultivar Morocco. High susceptibility to infection, were used to maintain and propagate vaccine source. Plants were grown in greenhouse at plant Plant Pathology Research Institute, Agriculture Research Center, Giza, Egypt during the 2022/2023 growing season, with 16 hour light and 8 hour dark at 16-22°C. Plants were grown in plastic pots (9 cm upper diameter). Inoculation was carried out when plants were 10 days old (the second leaf started to develop) and inoculated with a mixture of conidiospores by brushing conidia or shaking infested plants above healthy plants.

#### 2. Adult stage experiments

Grains were cultivated into 25 cm pots containing clay soil (3 kg per each plot). Pots were placed in a greenhouse with a maximum temperature of 25°C, falling to a minimum of 18°C at night, under 14 h natural daylight, and relative humidity ranging between 60 and 80% [after Sabri and Clark (1995) method].

The experimental plants were inoculated with a mixture of conidiospores by shaking infested plants above healthy plants at the growth stage (days 30) for 5-7 days until new sporulating lesions of powdery mildew formed on the leaves; the plants were exploited for experiments.

### **Insects:**

Eleven-spotted ladybird, *Coccinella undecimpunctata*, adults were collected from barley and wheat fields naturally and before they were used in experiments, the ladybird bug (*Coccinella undecimpunctata*). It eleven-spotted (Figure 1) (Linnaeus, 1758) has been defined at the Plant Protection Research Institute, A.R.C, Giza, Egypt. Ladybirds were reared in Glass jars (30 × 50 cm) closed with a piece of gauze and sealed with plastic parts. They fed on a mixed diet consisting of barley aphids (*Rhopalosiphum padi*) and wheat aphids (*Diuraphis anoxia*), aphids were reared (*R. padi*) on barley plants Giza variety 123 and wheat cultivar morocco with Powdery mildew (*Blumeria graminis* f. sp. *hordei* and *Blumeria graminis* f. sp. *tritici*) in the presence a piece of cotton soaked in water is a source of insects.

### **Experience of ladybird survival in four different treatments (Industrial infection of barley and wheat):**

- 1 - plants infected by powdery mildew;
- 2 - plants infected by aphids (10 aphids per plant);
- 3 - plants infected by powdery mildew and aphids;

4 - control of plants without aphids and powdery mildew.

After placing the treated plants inside a glass cylinder, 10 ladybirds were inserted into each cylinder, every five days, we recorded alive or dead ladybirds during an experimental period of 25 days. All ladybirds were kept there until DNA analysis of gut content.

### **Calculating the rate of ladybird feeding:**

Ladybird (*Coccinella undecimpunctata*) were placed in Petri dishes perforated after isolation and starvation (24 h) and then fed on wheat and barley leaves (pieces 4 cm long) infected with powdery mildew, Leaves of plants infected with powdery mildew were placed on filter paper saturated with water through certain time and under certain conditions (Mohdly et al., 2017), temperature (12-20°C), relative humidity (50 ± 8) and photoperiod (14: 8 h) lighting to darkness (Dharpur et al., 1990).

## **RESULTS AND DISCUSSION**

*Coccinella undecimpunctata* (Figure 1) were observed from the beginning of January feeding on powdery mildew fungi (*Blumeria graminis* f. sp. *hordei* and *Blumeria graminis* f. sp. *tritici*) and were collected from barley and wheat plants grown in the Sakha, Nubariya, Giza and Fayoum research stations. The odors emanating from plants infected with powdery mildew in the field were more attractive to beetles compared to those plants that were not infected, especially in the absence of aphids in the open fields.

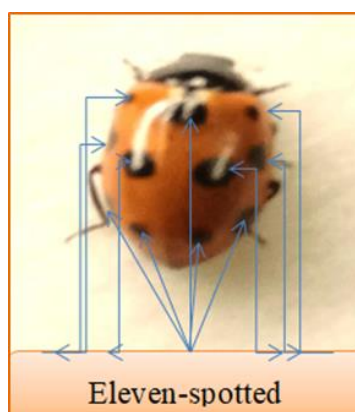


Figure 1. Clarifies that the ladybird bug (*Coccinella undecimpunctata*) is eleven-spotted

**Ladybird survival:**

Showed a ladybird survival rate test by comparing the four treatments that the ladybird survival rate depended on the type of diet provided to it (Figure 2). The experiment showed that the survival rate was higher for ladybirds with the diet mixed (powdery mildew and aphids) on barley and wheat, up to 100% up to day 10, then the survival rate of ladybirds decreased at 20 days by 10% indeed after 10 days, the survival rate of ladybirds with feeding on powdery mildew alone was reduced to 70% which was significant compared to on the aphids alone by percentage 90% when it was the control percentage 0% (Table 1, Figure 3). These results clearly demonstrate that feeding only on powdery mildew strongly reduced the survival rate of the ladybirds to 50% in day 15 compared to 90% still alive on the mixed diet and 70% on the aphids alone. Until day

15 compared to the control group 0% that fed on plants only.

The diet of entomophagous *coccinellids* is mainly based on aphids and other food sources such as pollen or fungal spores, insect falls under order (*Coleoptera*), family (*Coccinellidae*), genus (*Coccinella*), species (*C. undecimpunctata*). In this experiment, we investigated the feeding rate of *Coccinella undecimpunctata* on barley and wheat plants infected by powdery mildew in the absence of aphids (Figure 4). The rate of ladybirds feeding on powdery mildew in infected wheat and barley leaves in Petri dishes after starving her for 24 hours the highest percentage was (40.00 colony/24 hours), lower percentage (28.33 colony/24 hours), the average rate of consumption of *Coccinella* insect was by 35.42 and 28.14 fungal colonies/24 hours for wheat, respectively, barley (Table 2).

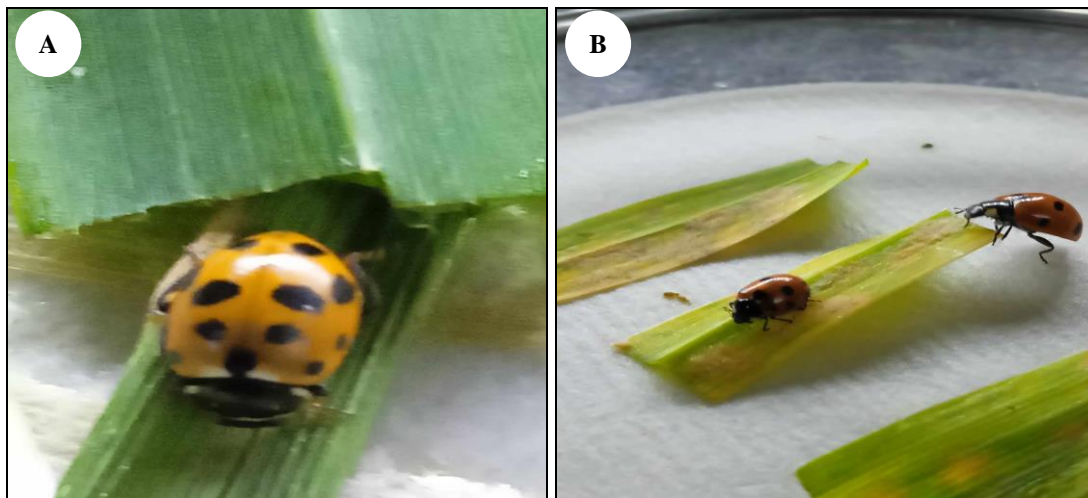


Figure 2. Ladybirds with feeding on plants only (A) and ladybirds with feeding on powdery mildew (B)

Table 1. Ladybug survival rate of *Coccinella undecimpunctata* through follow a different diet

| Feeding methods  | Survival rate of ladybirds reared (day %) |     |     |    |    |    |
|--|---|-----|-----|----|----|----|
|  | 1   | 5   | 10  | 15 | 20 | 25 |
| Aphids ( <i>R.padi</i> and <i>D. anoxia</i> ) and Powdery mildew ( <i>Bgh</i> and <i>Bgt</i> ) | 100                                       | 100 | 100 | 90 | 10 | 0  |
| Aphids ( <i>R.padi</i> and <i>D. anoxia</i> )  | 100                                       | 100 | 90  | 70 | 0  | 0  |
| Powdery mildew (( <i>Bgh</i> and <i>Bgt</i> )  | 100                                       | 100 | 90  | 70 | 0  | 0  |
| Control (barley and wheat)   | 100                                       | 100 | 70  | 50 | 0  | 0  |
| Aphids ( <i>R.padi</i> and <i>D. anoxia</i> ) and Powdery mildew ( <i>Bgh</i> and <i>Bgt</i> ) | 100                                       | 50  | 0   | 0  | 0  | 0  |

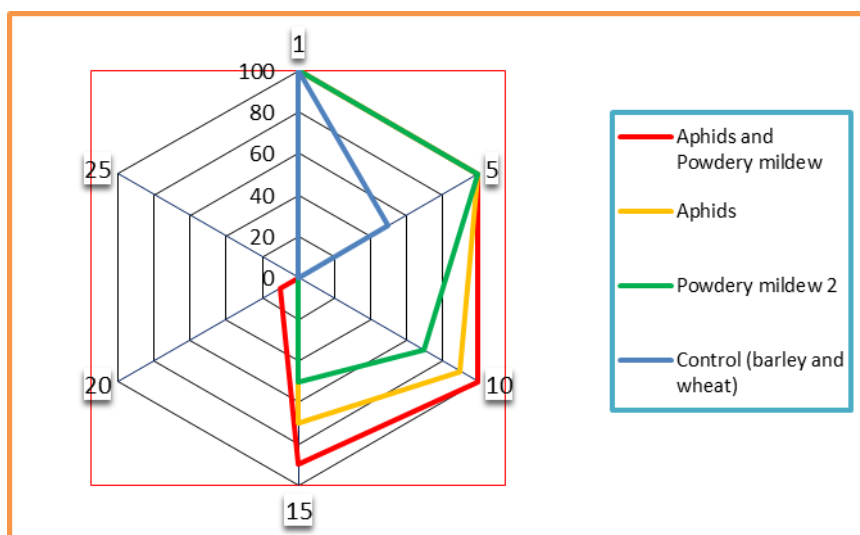


Figure 3. Ladybug survival rate by comparing the four nutritional treatments

### Calculating the rate of ladybird feeding:

Table 2. The feeding rate of *Coccinella undecimpunctata* on barley and wheat plants infected with powdery mildew in the absence of aphids

| Cultivate  | No. of colony/24 hours |       |       |       | The mean rate of ladybird feeding |
|--|------------------------|-------|-------|-------|-----------------------------------|
|  | EX.1                   | EX.2  | EX.2  | EX.4  |                                   |
| Wheat powdery mildew ( <i>Blumeria graminis</i> f. sp. <i>hordei</i> )   | 31.67                  | 33.33 | 36.67 | 40.00 | 35.42                             |
| Barley powdery mildew ( <i>Blumeria graminis</i> f. sp. <i>tritici</i> ) | 28.33                  | 36.67 | 40.00 | 31.67 | 28.14                             |

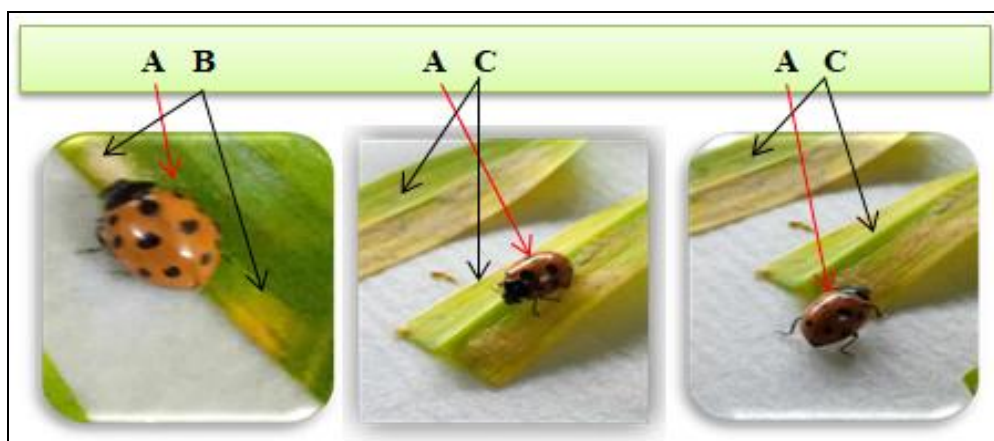


Figure 4. *Coccinella undecimpunctata* (A), *Blumeria graminis* f. sp. *tritici* on wheat (B), and *Blumeria graminis* f. sp. *hordei* on barley (C)

An observation of a high number of *Coccinella undecimpunctata* ladybirds in barley and wheat fields especially on plants infected by powdery mildew disease, with extremely low aphid abundance, prompted us to observation the mechanism her capacity on nutrition and possibility of their survival on powdery mildew colony and use it as a type

of biological control. Our study are with this is what Tabata et al. (2011) reported, saying that fungal ladybirds are attracted by odors that have been reported as fungal volatiles, and are not attracted to the same extent to other compounds found in healthy plants. This is what the study indicated, as *Coccinella undecimpunctata* ladybirds that

fed on plants infected with powdery mildew fungi remained alive for up to 15 days, while they remained alive on uninfected plants (control) for only 5 days, it was also confirmed Ninkovic et al. (2001) that the smell of barley plants infested with *R. padi* makes them attractive to *Coccinella*. Ninkovic et al. (2001) and de Vos and Jander (2010) indicated that suitable feeding for predatory insects is partly directed by the emission of plant volatiles resulting from insect feeding, interestingly. Sutherland and Parrella (2009b) reported that induced odors may be important cues to ladybirds in their foraging behavior, and colonies of powdery mildew fungi that appeared on the leaf surface are thought to have been mistakenly consumed by ladybirds. In a study by Leschen (2000), he explained that predatory ladybirds evolved from fungal ladybirds that were initially adapted to feed on colonies of sooty mold fungi, but then accepted honeydew-producing insects such as aphids. Weber and Lundgren 2009 that most predatory ladybugs feed on honeydew-producing insects from the order *Hemiptera* suborder *Sternorrhyncha*, which they need to complete evolution. He reported that, to date, no *Coccinellid* species is known for the full breadth of its nutritional range.

## CONCLUSIONS

The present study therefore underlines the potential of *Coccinella undecimpunctata* for application in biological control against *Blumeria graminis*, the causal agent of powdery mildew in barley and wheat. Since the result of this research shows the ladybird ingesting fungal spores, it supports the reduced application of chemical fungicides, which contribute to environmental pollution, pesticide resistance, and ecosystem imbalance. Integrating mycophagous insects into pest management practices is an approach that rises to the call of sustainable agriculture and biodiversity conservation and offers environmentally friendly alternatives to chemical treatments. Results highlight that further studies related to large-scale applications, mass-rearing techniques, and ecological impacts are very

important for enhanced efficiency in biological control. Promotion of natural regulation of pests makes this approach highly contributive to global efforts of sustainable farming, food security, and environmental preservation. Ultimately, this research provides valuable insights into minimizing agrochemical dependency while maintaining effective disease management, reinforcing the critical role of eco-friendly solutions in modern agriculture.

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