

# *Drosophila suzukii* response to *Leptopilina boulandi* and *Ganaspis brasiliensis* parasitism

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## Abstract

The spotted wing drosophila, *Drosophila suzukii* (Matsumura) (Diptera Drosophilidae), is considered one of the world's most important pests on berries because of the direct damage it causes. Thus, inclusion of biological control agents is being explored as part of the IPM program. There are two species of larval parasitoids of *Drosophila* spp. currently registered in Mexico: *Leptopilina boulandi* (Barbotin, Carton et Keiner-Pillault) (Hymenoptera Figitidae) and *Ganaspis brasiliensis* (Ihering) (Hymenoptera Figitidae), but the response of *D. suzukii* against the attack of these parasitoids is unknown. The objective of this study was to determine if Mexican populations of *L. boulandi* and *G. brasiliensis* could parasitize and complete their development on *D. suzukii*; as well as determining if there is a preference to parasite either *D. suzukii* or *Drosophila melanogaster* Meigen. In laboratory tests, both species succeeded in parasitizing *D. suzukii*, and even though the larvae did not complete their development, they killed the host. Additionally, in choice test, both species had a 2:1 ratio of preference for using *D. melanogaster* as a host rather than *D. suzukii*. Although neither of the parasitoids complete their development on *D. suzukii*, they could contribute to pest management since they cause death by oviposition and could maintain their populations on *D. melanogaster*, since both drosophilid species are present in the field.

**Key words:** berries, larval parasitoids, encapsulation, melanisations, *Drosophila melanogaster*, host-preference.

## Introduction

The spotted wing *Drosophila*, *Drosophila suzukii* (Matsumura) (Diptera Drosophilidae), is considered one of the world's most important pests for berries. The adults of *D. suzukii*, unlike other species of the genus, can oviposit on immature fruits of several Rosaceae plants including berries (Walsh *et al.*, 2011; Cini *et al.*, 2012). It makes this species a direct pest of these crops, and could cause losses of up to 50% of the total crop value if prevention or control measures are not implemented timely (Bolda *et al.*, 2010). The polyphagous potential of this pest, its cryptic nature and the global berry trade have contributed to its dispersion throughout the world (Calabria *et al.*, 2012), and turns this species as a key pest in Asia, America and Europe (Lee *et al.*, 2011; Cini *et al.*, 2012; Asplen, 2015). In Africa and Oceania, its distribution and importance are still limited (CABI, 2019).

Cultural, ethological (traps with attractants) and, more frequently, chemical control is used worldwide to prevent damage by *D. suzukii* to berry crops (Cini *et al.*, 2012; Haye *et al.*, 2016). Alternative management techniques have been explored to reduce dependence on insecticides and minimize the risks of contamination in berries, among those alternatives is the use of its natural enemies.

So far, at least 14 species of *D. suzukii* parasitoids are known, including two for pupae, *Pachycrepoideus vindemmiae* (Rondani) (Hymenoptera Pteromalidae) and *Trichopria drosophilae* (Perkins) (Hymenoptera Diapriidae), and at least twelve larval parasitoids belonging to the *Asobara*, *Ganaspis* and *Leptopilina* genera (García-Cancino *et al.*, 2015; Moreno-Carrillo *et al.*, 2015; Haye *et al.*, 2016; Daane *et al.*, 2016; Wang *et al.*, 2018). Among these species, *T. drosophilae* seems to have the

greater potential for control of *D. suzukii* because of its high reproductive potential and adaptability to different environmental conditions (Haye *et al.*, 2016; Wang *et al.*, 2016; Kaçar *et al.*, 2017; Rossi-Stacconi *et al.*, 2017), and promising results in greenhouse tests (Trotin *et al.*, 2014). However, under field conditions, this parasitoid has not yet achieved satisfactory control of the pest (Rossi-Stacconi *et al.*, 2018; 2019; González-Cabrera *et al.*, 2019). Then, several researchers suggest conducting laboratory and field experiments with larval parasitoids along with *T. drosophilae*, on the hypothesis that each would attack a different state of this pest and would probably obtain an additive effect in controlling *D. suzukii* (Rossi-Stacconi *et al.*, 2018; 2019).

From the worldwide larvae-parasitoid pool, *Leptopilina boulandi* (Barbotin, Carton et Keiner-Pillault) (Hymenoptera Figitidae), and *Ganaspis brasiliensis* (Ihering) (Hymenoptera Figitidae) stand out for their prevalence in the field, reproductive capacity and because they attack *Drosophila* spp. larvae under natural conditions (Kopelman and Chabora, 1992; Buffington and Forshage, 2016). However, there are discrepancies regarding their effectiveness on *D. suzukii* because some parasitoid populations have limitations to overcome the host's immune system (Gatti *et al.*, 2012; Kacsoh and Schlenke, 2012; Giorgini *et al.*, 2019; Girod *et al.*, 2018; Wang *et al.*, 2018).

Several species of drosophilids have high hemocytes production in their larval stage, which has been linked to its ability to encapsulate larval parasitoid eggs, particularly figitids (Rizki *et al.*, 1990; Rizki and Rizki, 1992; Kraaijeveld *et al.*, 2001; Eslin and Prevost, 1998; Sorrentino *et al.*, 2004; Moreau *et al.*, 2005). In this group, co-evolution has allowed hemocyte production to be a form

of defense against the most common parasitoids (Kacsoh and Schlenke, 2012), but this may differ between species or populations, as reported in *G. brasiliensis* by Nomano *et al.* (2017). They found *G. brasiliensis* lineages highly specialized to *D. suzukii*, but at the same time, they found generalist populations parasitizing a variety of Drosophilidae and Tephritidae, and lineages in which the females did not lay eggs in *D. suzukii*.

Mexico grows more than 33,000 hectares of berries each year (SIAP, 2017), and *D. suzukii* has been present in some production areas since 2011 (SENASICA, 2013). Therefore, to contribute to the protection of the national berry industry, field explorations for natural enemies were conducted since 2013, and two larval parasitoids were collected: *L. boulandi* and *G. brasiliensis* (Moreno-Carrillo *et al.*, 2015; García-Cancino *et al.*, 2015; González-Cabrera *et al.*, 2020). However, laboratory and field studies are needed to assess if this species could be used as part of a biological control program. Thus, the first objective of this work was to determine the ability of these species to parasitize and complete their development on *D. suzukii* as a host; the second was to determine the preference to parasitize either *D. suzukii* or *D. melanogaster*, since both species are commonly found together in berry fields in Mexico (García-Cancino *et al.* 2015).

## Materials and methods

### Biological material

The parasitoids *L. boulandi* and *G. brasiliensis*, as well as the hosts *D. suzukii* and *D. melanogaster*, were provided by the “Centro Nacional de Referencia de Control Biológico” (CNRCB), of the National Service for Food Safety and Agrifood Quality-Ministry of Agriculture (SADER-SENASICA), located in Tecmán, Colima. All insect colonies, and the experiments were performed at  $25 \pm 1$  °C, 60% HR and 12:12 photoperiod at CNRCB facilities.

The laboratory colony of *L. boulandi* started with material collected in March 2013 in a blackberry production area, *Rubus fruticosus* L. (Rosales Rosaceae), in Colima (19°24'28.8"N 103°35'34.8"W), following the methodology of García-Cancino *et al.* (2015). The colony of *G. brasiliensis* started with material collected from the same type of crop, but in Ciudad Guzmán, Jalisco (19°40.6'92.7"N 103°32.4'49.5"W) in December 2017. Both colonies have been maintained on *D. melanogaster* produced in an artificial diet described by González-Cabrera *et al.* (2018a; 2018b).

### Parasitoids survival on different host species

Larvae of *D. suzukii* and *D. melanogaster* that were less than 24 hours old were used in the experiments. They were fed on an artificial diet reported by González-Cabrera *et al.* (2018a; 2018b) and reared in 9 cm diameter Petri dishes inside an entomological cage (40 × 40 × 40 cm). Parasitoids used in the trials were two days old and had no previous contact with hosts. 200 larvae of *D. suzukii* and *D. melanogaster* in separate Petri dishes were exposed to 50 parasitoids of each species for 24 hours,

respectively. After that, the parasitoids of each of the four treatments were removed (treatments were defined by the combination of the two host species with two species of parasitoids) and the larvae were allowed to continue their development on the artificial diet.

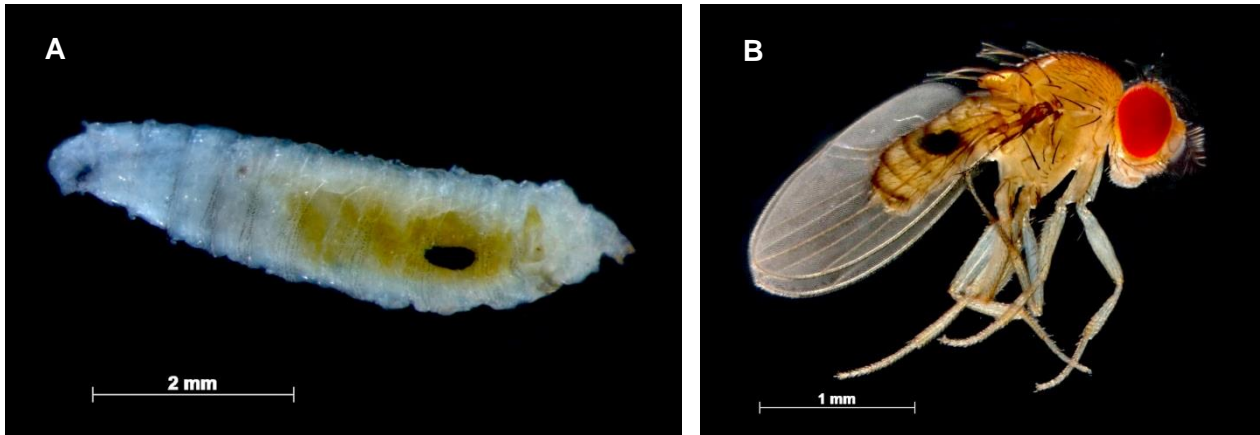
After 48 hours and 144 hours, 50 randomly selected larvae (i.e., replicates per measurement for each treatment) were extracted from the 200 initial pool, and, using a stereoscopic microscope Stemi 305 (Carl Zeiss), they were dissected and recorded for the current parasitoid life stage (normal and encapsulated egg as well as normal and encapsulated larva). The larvae were dissected by using entomological pins, opening the abdominal contents and directly observing the immature stages of the parasitoids. Finally, (when all the parasitoids had emerged and died), 50 pupae per treatment of each *Drosophila* species were collected and emergence hole of adults (flies or parasitoids) was recorded. To avoid duplication in the data collection, all extracted specimens were discarded at the end of the observation. Finally, 50 pupae per treatment were collected and emergence hole of parasitoids was recorded once more. Due to logistical issues, especially for material and risk of contamination in simultaneous trials, each parasitoid species was evaluated independently four times, and the entire evaluation was repeated four times at different dates (16 repetitions per treatment).

### Choice tests

An election test of *L. boulandi* and *G. brasiliensis* was performed on the two *Drosophila* species. Twenty eggs, less than 4 hours old, of *D. suzukii* and twenty of *D. melanogaster* were manually extracted from the colonies, and were evenly distributed into a new Petri dish ( $\varnothing = 9$  cm) with a corresponding diet inside a 500-mL plastic container. A couple of *L. boulandi* or *G. brasiliensis* were introduced in each Petri dish for 24 hours. The same number of eggs was used in another Petri dish, this time without parasitoids as a control group, to determine mortality by handling. Each treatment had four repetitions and the entire evaluation was repeated four times on different dates (16 repetitions in total per treatment). The number of adult parasitoid or flies per treatment was recorded as a response variable, to assess the mortality percentage.

### Data analysis

The survival of *L. boulandi* and *G. brasiliensis* was recorded in each stage of development, as well as the percentage of encapsulated-melanised eggs. Both variables were compared through a two-factor analysis of variance (ANOVA), where factor 1 was species of parasitoids and factor 2 species of host. The Tukey test was used to perform the adjustment by multiple comparisons. In the oviposition preference tests, a two-factor ANOVA test was also performed, where the response variable was the number of flies emerged in the four treatments and the Tukey multiple comparisons test was used to find the statistical differences between treatments. All analyses were performed according to the SAS program (v9.0) (SAS Institute, 2002), and using a significance value of  $p \leq 0.05$ .



**Figure 1.** A) Larva of *D. melanogaster* third instar with a melanic egg of *G. brasiliensis* in its abdomen; B) Adult male of *D. melanogaster*, where a melanic part corresponding to the egg of the encapsulated parasitoid is visible.

## Results

### Parasitoids survival on different host species

*L. boulandi* and *G. brasiliensis* were able to parasitize *D. suzukii* and *D. melanogaster*; however, when the host was *D. suzukii*, neither of the two parasitoid species managed to complete its development. Egg encapsulation was detected in both host species, but the percentage was notably different between species ( $F_{15,48} = 191.19$ ,  $p < 0.0001$ ).

The percentage of encapsulation and melanisation of parasitoid eggs in *D. melanogaster* was less than 2% for *L. boulandi* and less than 4% for *G. brasiliensis* (figure 1); while in *D. suzukii*, encapsulation was recorded in  $15.21 \pm 4.15\%$  for *L. boulandi* and  $76.59 \pm 5.13\%$  for *G. brasiliensis*, respectively. Parasitized *D. suzuki* individuals, regardless of the parasitoid species, did not reach the adult stage, in some cases presenting melanised or obscure body parts (figure 2C).

The hatching egg percentage of *L. boulandi* on *D. suzukii* was  $76.24 \pm 5.29\%$  and for *G. brasiliensis*  $17.47 \pm 4.82\%$ , but no first instar larva of either parasitoid species managed to survive (figure 3). On the contrary, on *D. melanogaster*, the average egg hatching for *L. boulandi* was  $95.39 \pm 1.05\%$  and the survival rate of larvae and pupae was  $89.57 \pm 2.16\%$  and  $77.45 \pm 4.34\%$ , respectively. For *G. brasiliensis* a  $96.15 \pm 1.34\%$  hatching eggs was

recorded, and a survival rate of  $91.68 \pm 2.54\%$  and  $86.89 \pm 4.53\%$  of larvae and pupae, respectively (figure 4).

### Choice tests

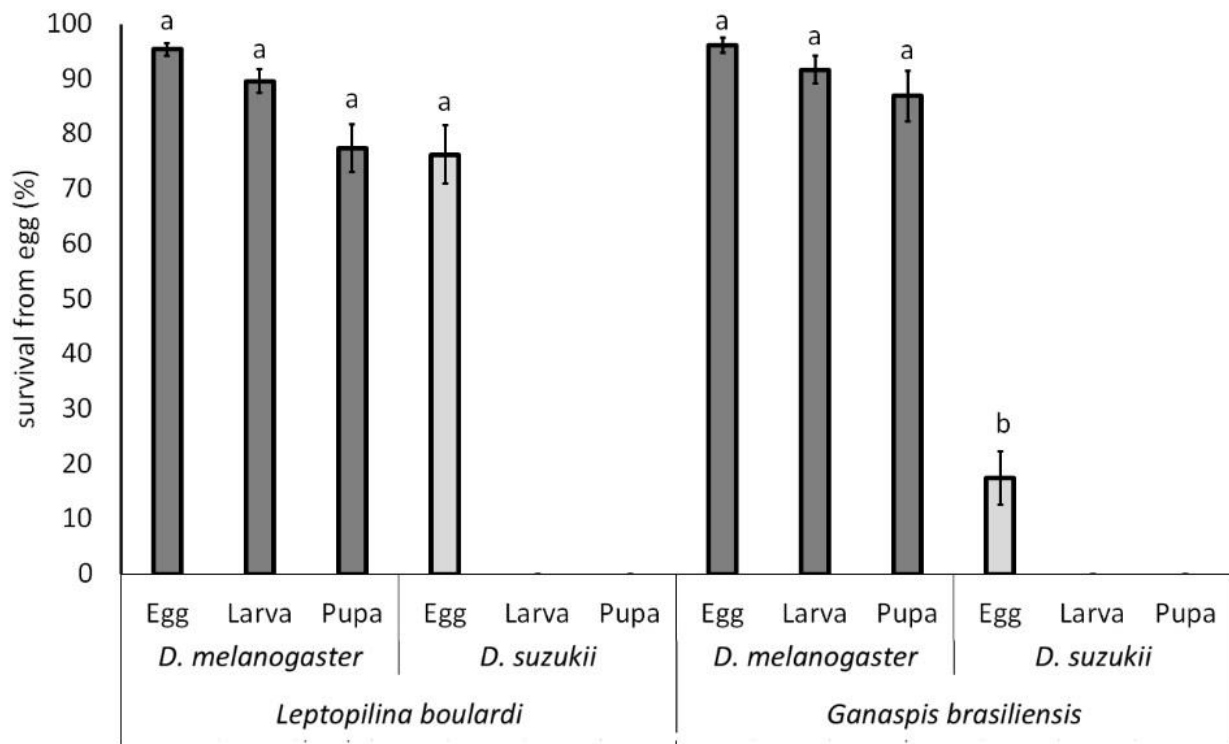
Both parasitoid species preferred to parasitize larvae of *D. melanogaster* than *D. suzukii* ( $F_{5,90} = 13.12$ ,  $p \leq 0.04$ ). Of the 20 individuals of each species offered, both parasitoid species eliminated about 40% of adults from *D. melanogaster*, but that percentage was reduced by half in the case of *D. suzukii* (figure 4).

## Discussion

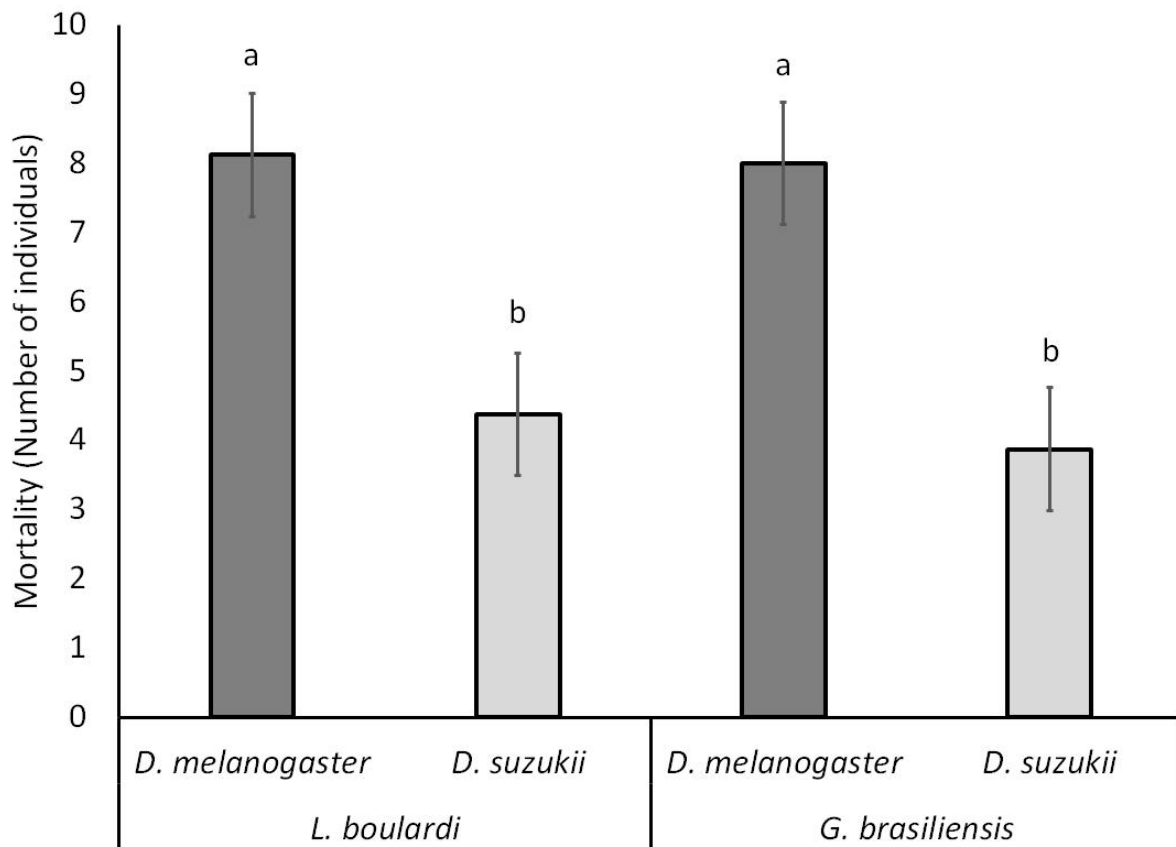
As previously stated, among the Figitidae parasitoids there are discrepancies regarding their effectiveness on *D. suzukii*, ranging from specialization up to total rejection. So, our findings that *L. boulandi* and *G. brasiliensis* were able to parasitize *D. suzukii*, but did not reach their adult stage, indicate that the Mexican phenotypes belong to an intermediate category of effectiveness. This could be due to *D. suzukii* producing a great amount of hemocytes (plasmocytes, podocytes and crystal cells), meaning it has a great capacity to encapsulate, melanise and consequently survive parasitoid attacks (Eslin and Prevost, 1998; Carton and Nappi, 2001; Kacsóh and Schlenke, 2012; Chabert *et al.*, 2013).



**Figure 2.** A) Normal *G. brasiliensis* egg; B) Eucoiliform *G. brasiliensis* larva of first instar; C) failed larval emergence as a result of encapsulation and melanisation of the egg.



**Figure 3.** Survival percentage of different development stages of *L. boulardi* and *G. brasiliensis* over *D. suzukii* and *D. melanogaster*. Means with the same letter between developmental states and parasitoids are not statistically different (Tukey,  $p \leq 0.05$ ).



**Figure 4.** *L. boulardi* and *G. brasiliensis* preference over *D. melanogaster* or *D. suzukii*, expressed in difference of adult mortality in regard to the control. Means with the same letter between hosts are not statistically different (Tukey,  $p \leq 0.05$ ).

Parasitism of the two species (*L. boulandi* / *G. brasiliensis*) did not prosper on *D. suzukii* but killed the host. Some authors have pointed out that the death of *D. suzukii*, by these parasitoids, could be associated with the host high energy cost of their immune response and a low feeding rate (Fellowes *et al.*, 1999; Kacsoh and Schlenke, 2012; Lynch *et al.*, 2016; Mazzetto *et al.*, 2016; Knoll *et al.*, 2017).

A hypothesis to explain survival in both the host and the larval parasitoids, depends on the specificity of each strain of flies and parasitoids. Between populations of the same region there may be differences in the behaviour, effectiveness and survival of organisms (Kraaijeveld and Godfray, 1999; Fellowes and Godfray, 2000; Rossi-Stacconi *et al.*, 2015; Mazzetto *et al.*, 2016). The wild populations of *L. boulandi* and *G. brasiliensis* collected in Mexico have probably been reproduced for longer in *D. melanogaster* than in any other drosophilids, since its presence has been known since 1921. Consequently, there has not yet been sufficient selection pressure to see if some individuals of the parasitoid can adapt to exploit this new host (*D. suzukii*) in the face of the abundance of alternate hosts. This could be a more acceptable explanation because *D. suzukii* is an exotic and recently introduced pest in Mexico.

Although neither of the parasitoids completed their development on *D. suzukii* and prefer ovipositing on *D. melanogaster* in an approximate ratio of 1:2, they could apparently contribute to the control of *D. suzukii* by killing through oviposition, as shown by Gonzalez-Cabrera *et al.* (2019). They found that *L. boulandi* increased control of *T. drosophilae* on the spotted wing drosophila by 5% in field experiments. This contribution could look small in statistical terms, but to the farmers, as verbally expressed by them, it means a lot of saving in farmer operating cost. Additionally, it is important to consider that in field conditions, larval populations of both *D. suzukii* and *D. melanogaster* are present at the same time as well as other alternate hosts (SENASICA, 2014), and that condition also favours the presence of those parasitoids.

Apparently, there are at least two scenarios to continue looking for larval parasitoids as biological alternatives for the control of *D. suzukii*. The first is exploring more places to find others local lineages of parasitoids of *D. suzukii*. The above is based on the work of several authors who found populations of *G. brasiliensis* that developed normally on *D. suzukii* (Buffington and Forshage, 2016; Nomano *et al.*, 2017; Girod *et al.*, 2018; Giorgini *et al.*, 2019). The second route is to explore the possibility of massive releases of *L. boulandi* and *G. brasiliensis* to contribute to the control of *D. suzukii* populations in the field, considering their role as a single mortality agent or combined with another type of parasitoid (i.e., *T. drosophilae*), even if there is no establishment on this host. Both suggestions will have to first consider the biological and economic feasibility of the proposals through field studies, before being offered as viable alternatives to be incorporated into an Integrated Pest Management program of this pest.

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