

Research Article

Some Aspects of Pollination in *Ficus Rubiginosa* in Israel

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Abstract

Ficus rubiginosa Desf. (*Moraceae*) is a west Australia tree naturally pollinated by aganoid wasp *Pleistodontes imperialis*. This tree was introduced into Israel and has since become a nuisance as a result of the ripe fig droppings. We reveal its original pollinator in Israel and describe its pollination behavior as a transition between topocentric and ethodynamic. The changes in fig style length and wasp ovipositor length and the tree's ability for both selfing and cross-pollination indicate the possibility of this species' high adaptation to various climate conditions, and its ability to establish easily in new niches and its consequent potential to become an invasive plant.

Keywords: *Ficus rubiginosa*; Israel; Topocentric and ethodynamic pollination; Invasive plants.

Introduction

F. rubiginosa Desf. (*Moraceae*) is a western Australia tree, distributed along riverbanks as a native tree that produces viable seeds. The natural distribution of *P. imperialis*, its native pollinator, was once limited to Australia but was later found in the figs of *F. rubiginosa*, that were introduced into Hawaii and New Zealand [1,2]. *F. rubiginosa* was introduced into Israel ca. 50 years ago as an ornamental tree, and was very successful due to its fast growth and provision of good shade [3]. Its small, hard, green fruits would fall without causing any serious impact (soiling) on the neighborhood.

About 20 years ago ripe fleshy fruits suddenly began to appear on the trees. When these fell they soiled and damaged parking cars and sidewalks. Seedlings too began to appear in the cities, on palm tree trunks, and in rural areas.

Preliminary examination of the ripe figs revealed aganoid wasp, later identified as *Pleistodontes imperialis* Saunders. Because this plant has become a real nuisance, and because the relationship with its wasp has not yet been explored in Israel, we sought to explore the morphological and behavioral aspects of the wasp in relation to the reproductive and phenological aspects of *F. rubiginosa*. and to contribute some thoughts on the *Ficus* aganoid interaction and the potential for uncontrolled spread of this plant.

Materials and Methods

Plant material

The research took place on Tel-Aviv University campus, 32°06'5 N 034°48'13 E. The research area encompassed 59 m a.s.l. In addition, sporadic observations were carried out throughout the country.

Wasp Species Identification

Wasps from figs at stage D (male stage), while emerging from the fruits, were collected and preserved in 70% ethanol. Identification was performed in parallel by Dr. Jean-Yves Rasplus in INRA France and in our laboratory, with reference to the book *Australian Chalcidoidea (Hymenoptera)*, by S Boucek [2].

Scanning Electron Microscopy of the Whole Wasp

Twenty female wasps were collected and preserved in 70% ethanol, then mounted on metal disks, gold-coated, and scanned by a J.S.M. 840A scanning electron microscope (Joel).

Ovipositor Length Measurements

Female wasps were collected monthly and preserved in 70% ethanol. Ten wasps for each month were randomly selected and placed on glass slides, soaked in methylene blue, and mounted on glass slides immersed in Canada balsam in xylene (BDH Chemicals Ltd, Poole, England). The slides were covered by a glass cover (10 mm diameter) and the wasps were examined using "Axioplan",

two imaging microscopes, and photographed using an “Axiocam” TV 2/3” × 0.63” camera. The same wasps were photographed again under a 1 mm micrometer glass cover. The ovipositor was measured on a monitor screen (17”) with a thread and transformed into length in mm with the aid of a micrometer. Ovipositor lengths were compared among the monthly samples, by one-way ANOVA ($p < 0.05$) using the STATISTICA software (version 6.0).

Developmental stages of the figs and tree phenology

Branches of five trees were tagged. The figs were marked and monitored year round (2001-2002). The developmental stages of the figs are described according to Galil and Eisikowitch [4]. Fig diameter was assessed by electronic digital caliper and the maximum circumference was also measured. Means and standard deviations were calculated.

Wasp emergence from galls

Figs at stage D [5] were collected from the trees and opened in the lab. Emergence of the wasps from galls was recorded by a video recorder mounted on a stereoscopic microscope.

Pollen germination

Pollen grains were removed from stage D figs with the aid of an entomological pin. Pollen grains were germinated in 2.5% sucrose (BDH, England) solution. This medium was found earlier to be optimal for germination [6]. The pollen germination method constituted a modification of the hanging drop method [7]. Six drops of 5 µl germination medium were placed on the inside of the cover of a 5 cm diameter Petri dish. On each of the 6 drops, pollen grains (at least 100) were placed. To assess the role of style on germination, 10 ripe styles were dipped into these drops. In order to maintain the germination medium at a fixed concentration, 1 ml of the same medium was placed on the bottom of the Petri dish. Pollen germination was examined after 24 hours incubation at room temperature under a light microscope. Five repetitions were performed for each experiment. Germination results are expressed as an index in Table 1.

Index level	Number of pollen grains germinated
1	0-25
2	26-50
3	51-75
4	<75

Table 1: Germination index of pollen grains.

The germination results were analyzed by Sign test using the STATISTICA software (version 6.0).

Seed/gall ratio

Ten fruits at stage D were collected monthly from marked branches. Each fig was weighed and cut into four quarters. One

quarter was weighed and counted for both seeds and galls. Seed/gall ratio for the whole fig was calculated from the partial weight of the examined part.

Style length in the figs at stage B (female)

Two to six figs were collected monthly and fixed in “Histchoice” tissue fixative (Amresco, Solon, Ohio). Figs were dissected and 10 long style flowers were chosen from the distal end of the fig (opposite the ostiole end). The flowers were immersed in 1mM KOH in order to soften and separate the styles, placed on slides with a drop of distilled water, and their length was assessed with the aid of a light microscope equipped with a scaled objective. Measurements were taken from the base of the ovary up to the stigma (Figure 1).

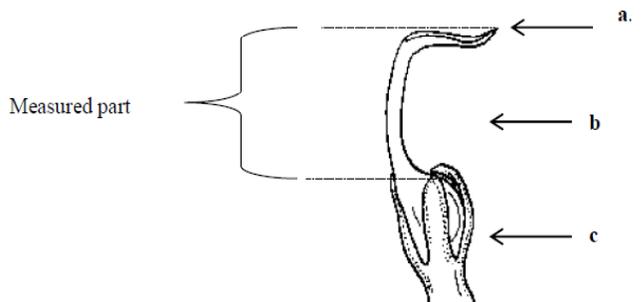


Figure 1: Parts of flower: (a) stigma, (b) style, (c) ovary. Bar= 1 mm.

The style length differences were analyzed by OneW Anova using the STATISTICA software (version 6).

Seed germination of self- pollinated and open pollinated figs

During observations, several trees were found to bloom a asynchronously, while others were found to bloom synchronically (Figure 4). In other words, selfing was hypothesized to occur in the a synchronical trees. In order to confirm this, figs of asynchronous tree number 0 at final stage A (Table 2), were bagged in organdie bags to prevent entry of the wasps and left until stage B. At stage B figs from stage D (which had released wasps), from the same tree were placed in the bags. The released wasps entered the stage B figs which were then left to develop. After reaching stage D, approximately 4 months later, the figs were harvested and 60 seeds were collected. In parallel, 60 seeds were collected from stage D figs from tree number 2 (synchronic bloom), which had been exposed to open pollination. The seeds, 60 from self-pollination and 60 from open-pollination, were separated into three groups of 20 seeds each, and placed on filter paper soaked in distilled water, with each sample then placed in a Petri dish. The dishes were incubated at 25 °C under 12 h light /12 h dark. Seed germination of each sample was examined and percentages of germination were calculated. Germination rates were examined by Sign test using the STATIATICA software (Version 6.0).

Results

The wasps inhabiting *F. rubiginosa* figs were identified as *Pleistodontes imperialis* Saunders.

Scanning electron microscope images of the female wasps revealed, in addition to a pair of pollen containers (pockets) and the corbiculae (combs) on the front legs, there was also a clear depression on the mesosternum that was filled with pollen grains (Figure 2).

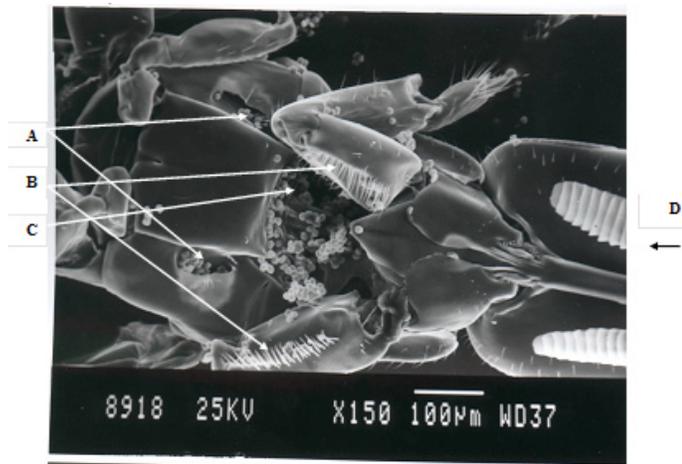


Figure 2: Ventral view of *P. imperialis* scanned by electron microscope. A= Pollen pockets; B= Corbiculae; C= Front depression in; D= Head Scale; Bar= 100 µM.

Variation in the wasp ovipositor throughout the year

Examination of ovipositor length throughout the year revealed a significant variability. ($F_{14,56} = 8.56, (P_{0.05} >)$) (Figure 3).

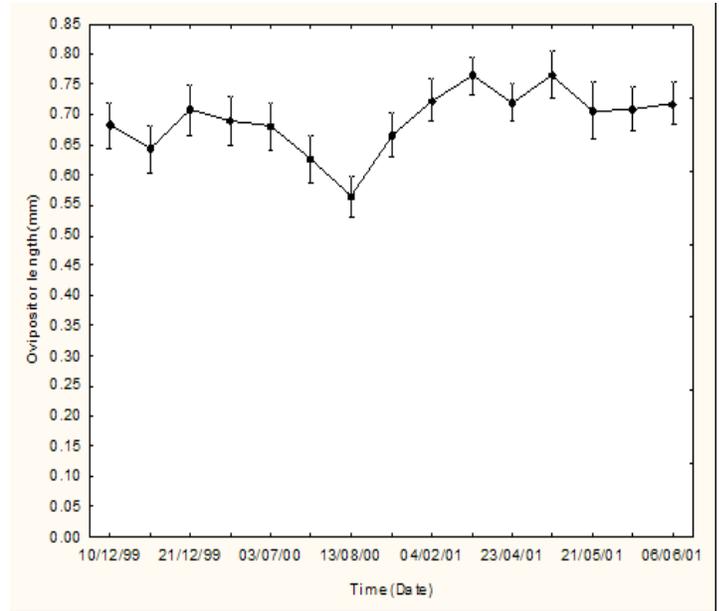


Figure 3: Changes in *P. imperialis* ovipositor length throughout the year. The graph shows mean and S.E. of ovipositor length for each month during one year.

Fig developmental stages are given in Table 2 below.

Stage	Fig description
A	Figs are small protrusions on the branch, enveloped in brackets. The hidden syconium is dark green.
B	Female stage. The syconium has left the enveloping brackets. The ostiolar scales are dark green, slightly loose, and ready to attract female wasps.
B ⁺	The syconium is in its final stage of pollinator attraction.
C	The syconium has become round and turned light green. Seeds and galls are developing.
D	Male stage. The syconium softens and turns yellow. Small emerging holes appear around the fig ostiole and female wasps, loaded with pollen grains, emerge from the fig.
E	The ripe dark syconium with ripe seeds fall to the ground.

Table 2: Developmental stages of *F. rubiginosa* figs.

Tree phenology

The Figure 4 presents the phenology of six trees on Tel-Aviv University campus. It is clear that some of the trees (nos. 2, 4, and 6) represent stages B and D simultaneously, and are thus asynchronous, and selfing, at least technically, is enabled.

In other trees, such as tree 0, stages B and D do not overlap. This tree is synchronized, and thus in order to set seed it requires cross-pollination.

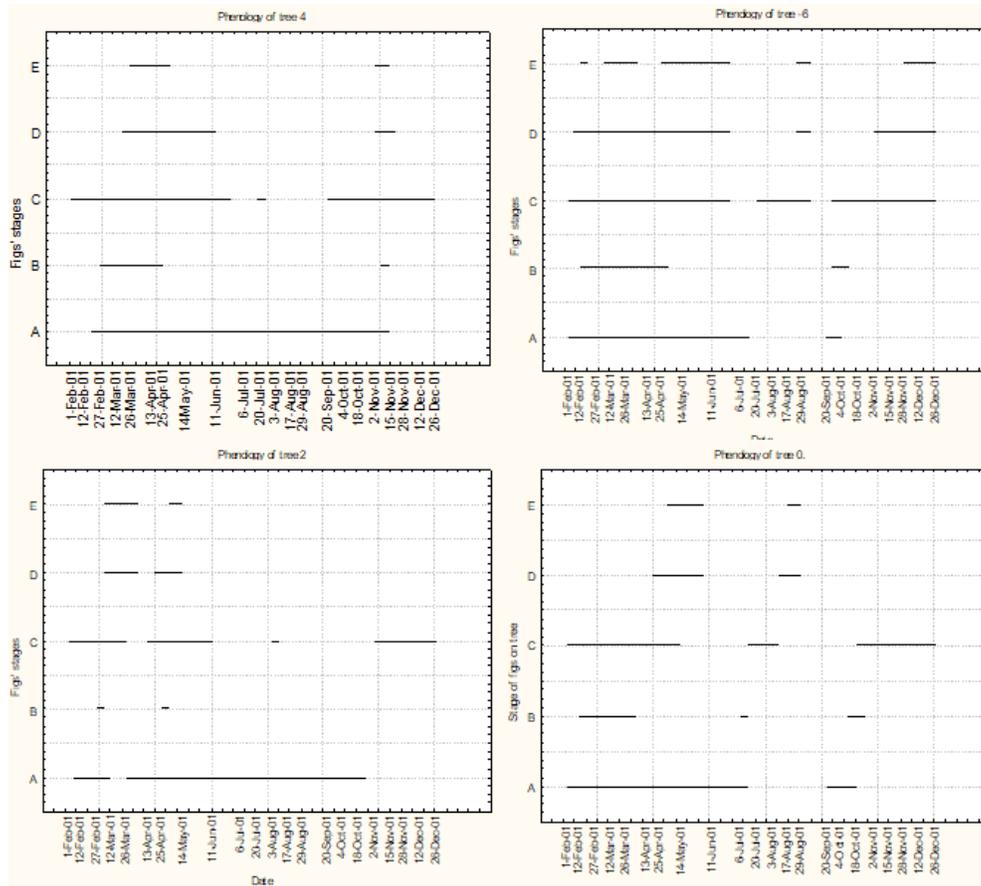


Figure 4: *F. rubiginosa* phenology on Tel-Aviv University campus.

Pollen germination

Pollen germination in standard plain sucrose medium gave the index 1.06 ± 0.27 (Table 1).

However, when 10 styles were soaked in the standard sucrose solution, germination was three-fold higher and provided an index of 2.99 ± 1.24 (Figure 5).

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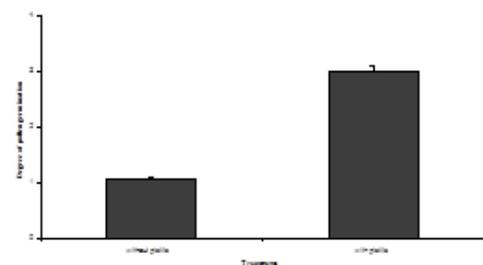


Figure 5: Pollen Germination of *F. rubiginosa* with and without pistils in sucrose solution under laboratory conditions n=(138).

Seed/gall ratios in *F. rubiginosa* throughout the year

The number of seeds or galls is not fixed and they change significantly during certain parts of the year ($F_{18,202}=3.97$ $p<0.05$). It is clear that on August 8th 2001, April 18th 2001, and August 5th 2001, the number of seeds was higher than the number of galls. For other dates, the ratio reveals that the number of galls was higher (Figure 6).

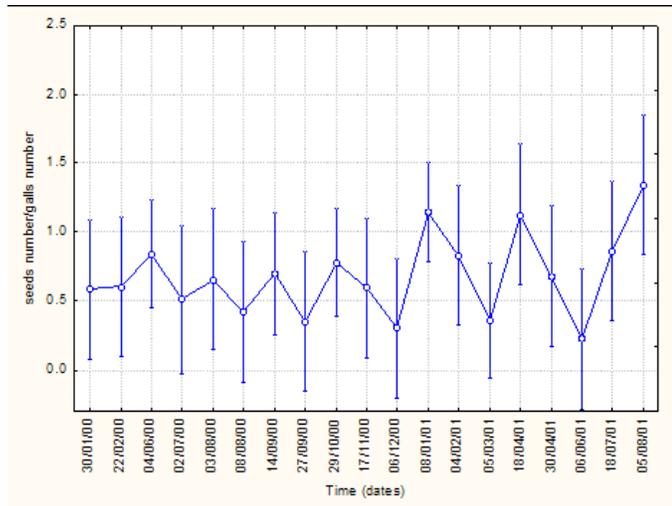


Figure 6: Seed/gall ratio in *F. rubiginosa* throughout the year. The graph shows mean and S.E. of this ratio.

Style length throughout the year

Figure 7 presents the variation in style length throughout the year. It is clear that style length differs significantly at different times of the year ($F_{11, 1184}=61.77$ $p<0.001$).

A close examination reveals that style length is at its minimum in June and maximum in January.

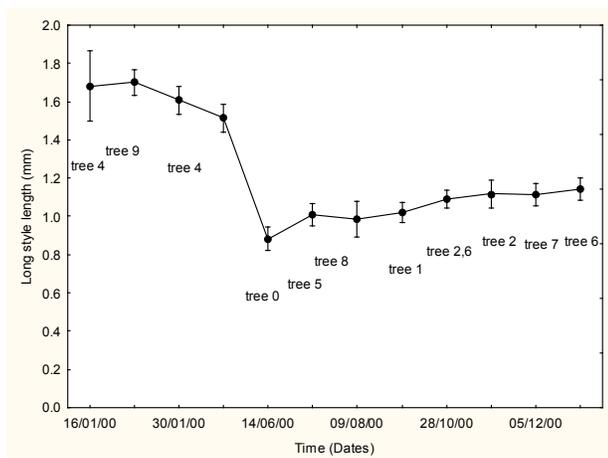


Figure 7: Style length variability in different *F. rubiginosa* trees throughout the year. The graph shows mean and S.E.

Seed germination

Germination rates of seeds resulting from self-pollination ($86.60\% \pm 10.4\%$) did not significantly differ from those of seeds resulting from open-pollination $75\% \pm 13.2\%$; $n=60$ ($F_{1,4}=1.44$, $p=0.29$).

Discussion

P. imperialis (Agaonidae, Agaoninae), the natural pollinator of the monoecious fig *F. rubiginosa*, native to Australia, is described for the first time from Israel. It should be noted that this wasp is the only one found to date in the figs of *F. rubiginosa* in Israel, whereas in Australia figs of *F. rubiginosa* can host up to six additional parasite species [8]. This means that the only roles played by this wasp in Israel are those of pollen transfer, pollination, and egg-laying, as is free from any other insect competitors [9]. It is not clear, however, how and when this wasp arrived in Israel. The phenomenon of aganoid pollinators following an introduced *Ficus* was also found in Hawaii and New Zealand in other fig trees, such as *F. macrophylla* with *Pleistodontes froggatti* [10], and *F. religiosa* with its pollinator *Ceratosolen quatraticeps* in Israel [8], as well as with other *Ficus* species introduced into Israel, [11]. Scanning electron microscope images of *P. imperialis* (Figure 2) revealed that as well as the familiar pollen pockets, similar to those found in other aganoid wasp [9,12], and the coxal curbiculae described earlier in *P. imperialis* [12], an additional frontal depression was observed filled with pollen grains. This frontal depression is described here for the first time and calls for a deeper discussion.

In 1973, Galil [13] coined the terms “Topocentric” and “Ethodynamic” pollination. Topocentric pollination, “Ensured by topography of the flower”, occurs when a pollinator enters the flower toward the targeted reward and, during this movement, pollen grains are passively “Smeared” on its body and may later be deposited on the stigma. Ethodynamic pollination, in contrast, is dictated by the active behavior of the pollinator: i.e., the pollinator actively collects pollen grains and deposits them on the stigma, as described in *F. sycomorus* [9] and earlier in *Yucca aloifolia* [14].

In *Ficus carica*, which is pollinated by *Blastophaga psenes*, pollination behavior constitutes a particular transition between ethodynamic and topocentric pollination [15]. In *F. rubiginosa*, we describe another combination between ethodynamic and topocentric pollination: female wasps actively collect and store the pollen in their pollen pockets (Figure 2) - ethodynamic; while the pollen found in the frontal depression is probably swept up and smeared passively during the wasp’s activity within the fig-topocentric.

The variability in style length (among the long-styled flowers) throughout the year (figure 7) and ovipositor length variability (Figure 3) have been little studied to date, although

the changes in number of flowers throughout the year have been previously noted and discussed [16]. These variations can explain at least partially the variability in the seed/gall ratio as presented in Figure 6 [17]. Our findings indicate the possibility that these changes are connected to climate changes throughout the year. A similar but not identical phenomenon was found by Vaknin, et al. [18], who demonstrated that seasonal changes in flower size in *Loranthus acaciae* played an important role in determining the type of pollinator. Differences in flower size in *Echium* were also found to be important for attracting bees and for seed set [19].

Our annual examination of the *Ficus rubiginosa* phenology revealed (Figure 4) that in some trees the blooming is synchronized, i.e., all figs on a certain tree are at the same stage at a certain time. In this case, pollen-carrying wasps emerging from one tree must search for another tree at the right syconium stage in order to pollinate and lay their eggs, with the result that the next generation seed set is achieved by outcrossing, [20]. On other trees, in the same research area, blooming was found to be asynchronic and thereby enabled selfing.

Table 1 illustrates that seeds derived from self-pollination demonstrate the same extent of germinability as those derived from open pollination. These findings are similar to those for *F. aurea* [21] and support the hypothesis concerning the role of self-pollination in the genus *Ficus* [22].

Pollen germination carried out under laboratory conditions with added stigmas indicates the possible role of the stigma in pollen germination [7]. The fact that the addition of stigmas to the germination medium resulted in substantially higher germination rates (Figure 5), suggests that through oviposition or biting the styles, as found in *F. sycomorus* [4], the pollinators are not merely serving as pollen conveyers but also play possible role in enhancing pollen germination, a phenomenon that deserves future study.

In conclusion, in spite of the relative complexity of the interaction between the plant and its pollinator, *F. rubiginosa* is able to disperse viable seeds far from its original habitat, which raises serious concerns about its probability of becoming an invader in settlements featuring parks and summer irrigation [11].

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