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Preliminary study of the aggregative behaviour of *Scaphoideus titanus* larvae

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Abstract: The leafhopper *Scaphoideus titanus* is the vector of the Flavescence dorée. In this study, we investigated the aggregative behaviour of the larvae. We conclude from different experiments that larval aggregation occurs at the plant scale and the age and colour of the food source could be factors cueing an aggregation. These aggregation patterns should be studied in more details in order to gain knowledge in the epidemiology of Flavescence dorée and eventually to develop control strategies based on inter-individual epideictic regulation.

Key words: *Scaphoideus titanus*, grapevine, aggregation

Introduction

Flavescence dorée, a grapevine yellows caused by phytoplasmas of the 16SrV-group, is one of the current major threats in European viticulture. The spread of the disease at a great scale is mainly due to human activities. Nevertheless, the behavior of the vector *Scaphoideus titanus*, especially its movements, is responsible of the propagation of this yellows disease from one infected vine stock to other grapevine plants in the same or nearby plots. Aggregative patterns have been observed at vineyard scales for *S. titanus* larvae (Lessio & Alma, 2006) as well as for imagos (Bosco *et al.*, 1997). Aggregative patterns of juvenile stages may influence the dispersive behavior and in the case of insect vectors, aggregation can influences some decisive parameters (e.g. acquisition and inoculation rate) for the disease spread (Zhang *et al.,* 2000). No hypothesis has yet been formulated to explain these aggregative patterns and if aggregative behaviours exist at different spatial scales (intra stock or intra plot). Field studies do not allow distinguishing between larval aggregation due to their own behaviour and aggregation due to a hypothetical aggregative egg-laying behaviour of females.

In this study, we first tried to confirm if an active aggregative behavior of the 1st larval instar of *S. titanus* may exists and secondly to investigate several stimuli that may lead to such an aggregation.

Material and methods

Insect rearing

Scaphoideus titanus larvae were obtained as described in Caudwell *et al.* (1970). Egg hatchings were obtained by placing 2 year old woody canes inside plastic hatching cages in a climatic chamber under a 16:8 (L:D) photoperiod, at 23 ± 1°C, and 65–70% RH. In order to collect neonate larvae, six cutted leaves of Cabernet-Sauvignon, maintained each in a glass

tube with water, were added in the cage, twenty days after eggs were removed from the cold room. Leaves were replaced when they began to wither. All tests were performed with 1st larval instar (L1).

Aggregation test
To test if the L1 tend to aggregate, we performed choice tests between four identical grapevine cuttings that were placed in each corner of an Altuglass® cage (60 x 60 x 60cm). A number of larvae between 140 to 350 individuals were placed in the centre of the cage at equal distance of each plant. The number of larvae on each plant was numbered after 9 hours. Six repetitions were made.

Intra-plant distribution of larvae
To study the aggregation behaviour of neonate larvae at plant scale we provided to larvae two 8/9 leaves grapevine cuttings as only food source in each hatching cage. Leaves were classified in 4 categories according to their size and position on the plant: 1) small leaves on the top which were the youngest leaves with leaf area was smaller than11 cm², 2) small leaves on the bottom which corresponds to the oldest leaves with leaf area was smaller than 23cm², 3) large leaves in intermediate position with leaf area > 60cm² and 4) we added an extra cutting category, the buds shoots because with the humidity of the hatchery cages, woody canes which carried eggs were budbursted. The numbers of L1 on each leaf were checked daily during 33 days and the leaf area index of each leaf was measured with a LAI meter. Three cage replicates were made.

Response to colour
Visual stimuli are generally important cues for the Homoptera. To test the influence of the colour on food choice, we placed on the internal side of hatching cage lid 4 coloured traps (8 x 23.6cm) representing grapevine organs (brown: bark, green: chlorophyllian organs, red and yellow: mature berries an/or symptoms of Flavescence dorée on leaves) sprayed with insect glue without any food resource,. Larvae glued on the traps were daily counted during 28 days. After each monitoring, traps were randomly rotated to avoid any position effect. Three cage replicates were made.

Odour attraction
To check the occurrence of an aggregative olfactory stimulus produced by conspecifics, we extracted 8,000 neonate larvae in cold methylene chloride during 24h. After the extraction, the solvent was evaporated under a nitrogen flow and the residues were taken up in pure water. Then, in the same experimental setup as described above the agregation test consisted in four identical plants offered in choice situation: two were sprayed with the extract, the 2 others only with pure water. The volume of sprayed extract was adjusted from 5 to one hundred larvae equivalent per leaf and 6 cage replicates per concentration were made.

Results and discussion

Aggregation test
As often observed in vineyards, the larvae tended to aggregate in our choice experiments. In spite of the occurrence of 4 identical food sources, on average, almost the half of the larvae were found feeding on the same plant (Figure 1). This result demonstrates that neonate larvae distributed by themselves among the four plant in a nonrandom pattern. Thus, aggregation of larvae in vineyards could be to some extend not solely attributed to ovipositing spatial patterns.

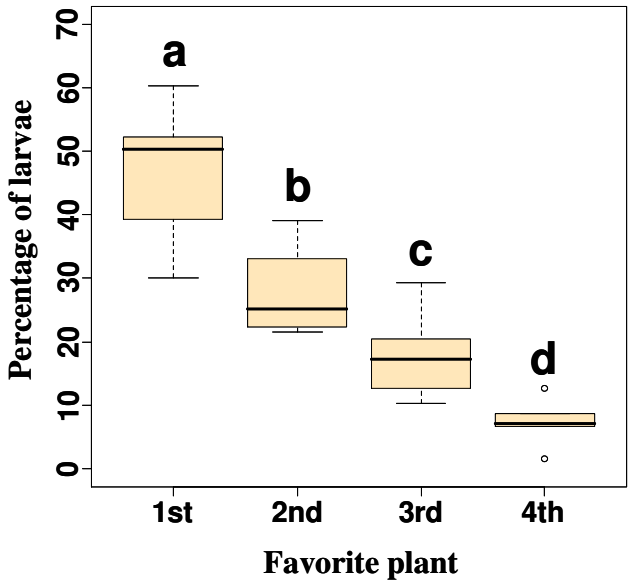

Figure 1. Percentage of larvae per grapevine cutting. (1st: plant that received most of the larvae in each choice test; 2nd: plant receiving second most of the larvae, 3rd: plant receiving third most of the larvae, 4th: plant receiving least of the larvae. Boxplots with different letters are significantly different under the Friedman Anova and LSD post hoc tests at 1 % threshold.).

Intra-plant distribution of larvae

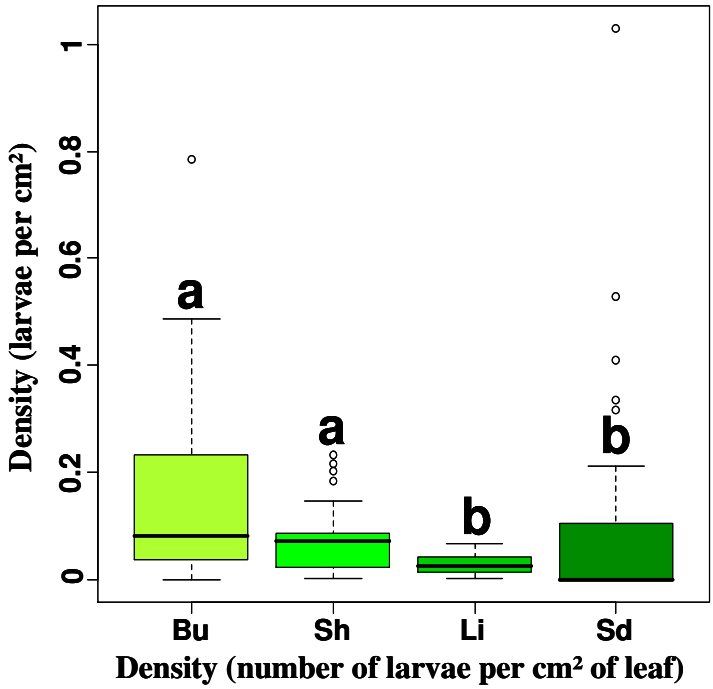

Figure 2. Larval density on different leaf classes. (Bu: bud shoots; Li: large and intermediate leaves; Sd: small and bottom leaves; Sh: small and high leaves. Boxplots with different letters are significantly different under the Friedman Anova and LSD post hoc tests at 1 % threshold.).

Densities of larvae were not uniform (Figure 2). Higher densities were observed on the youngest organs: the buds shoots and the apical leaves. The larvae prefer feeding on younger and growing leaves than on older ones.

Attraction by coloured surrogates

In the present experiment, the major part of the larvae was caught by yellow traps (Figure 3). These results are in contradiction with Lessio and Alma (2004) who caught more imagos with red traps. On the other hand, these results could be related to the L1 preferences for the youngest organs. Indeed, young leaves are yellowish and have higher nitrogen content (Mooney& Gulmon, 1982) which in turn increases the fitness of other leafhoppers (Rossi & Strong, 1991). Moreover, yellow wavelengths are known to be attractive for sap-sucking insects (Saxena *et al.*, 1974; Prokopy & Owens, 1983; Todd *et al.*, 1990).

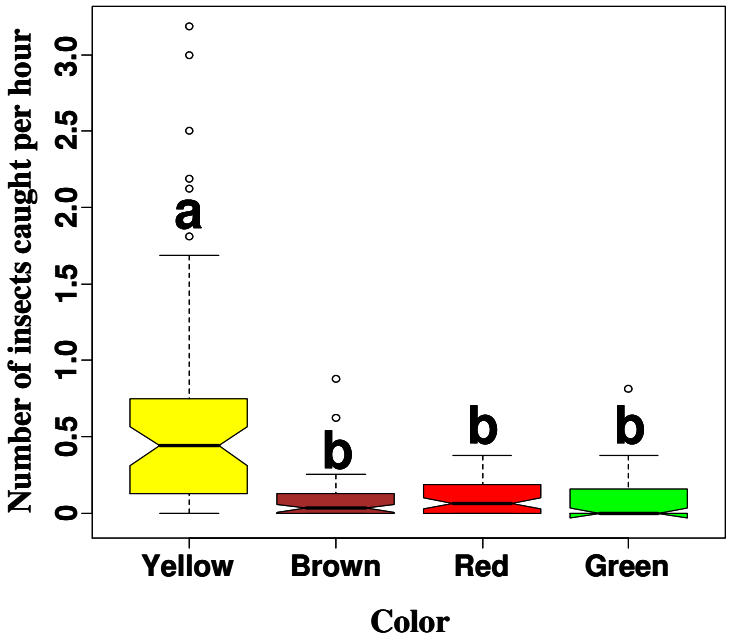

Figure 3. Colour choice by neonate larvae. Boxplots with different letters are significantly different under the Friedman Anova and LSD post hoc tests at 1 % threshold.

Attraction by odours

Whatever the concentration of the extract, the larvae showed no preference between plant sprayed with extract or with water (Figure 4). Our extract was neither attractive nor deterrent.

As a conclusion, we have demonstrated that larval aggregation in *S. titanus* occurs regardlessly of adult behaviour even if female egg laying behaviour could also contribute to aggregation. The aggregation pattern also occurs at a plant scale and the age and the colour of the leaves appears to be a key factors in the feeding site choice. This work is a first step towards understanding the aggregation behaviour of the Flavescence dorée vector and how it influences the epidemiology of the disease. Our results seem to demonstrate a "non social" aggregation for *S. titanus* but do not allow to establish yet the stimuli gathering the larvae.

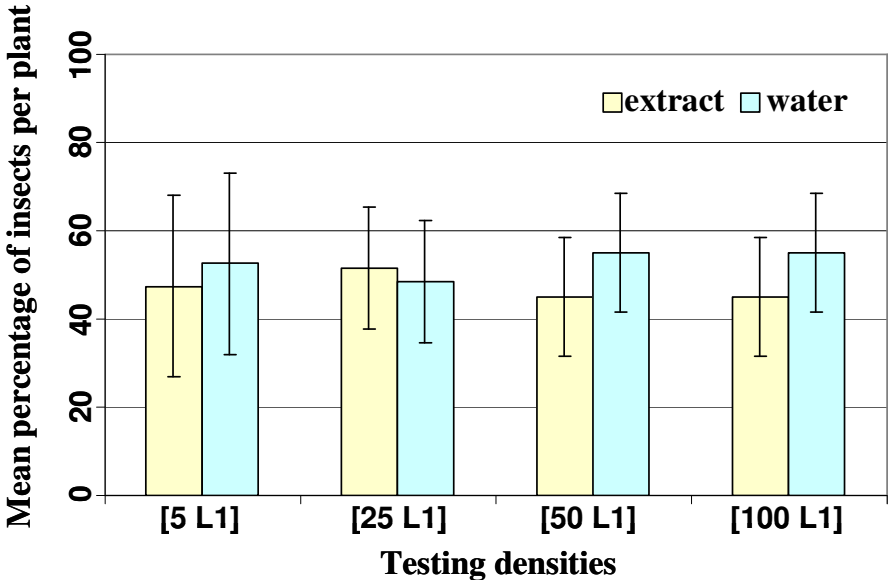

Figure 4. Response to conspecifics odour. ([X]: concentration of X larvae equivalent per leaf.)

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