

Confirmation of coffee related *Xylella fastidiosa* vectors (Cicadellidae) in Costa Rica

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FIELD COLLECTION AND EXPERIMENTAL PROCEDURE

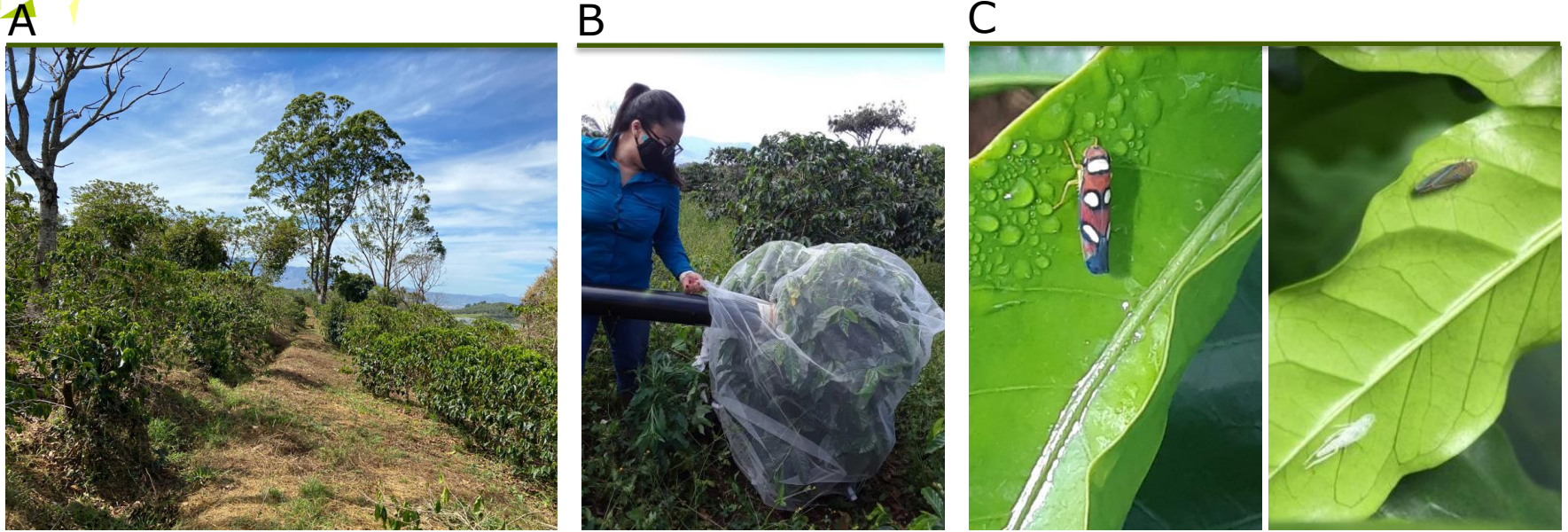


Figure 1. Field collection of cicadellids. **A.** A coffee plantation located in Alajuela, Costa Rica (N10° 4' 28" W 84° 10' 13"), was selected as the collection site. The presence of *X. fastidiosa* in plants was determined previously for this site. Sampling procedure for leafhoppers was performed using a hand-held vacuum. Each sample consisted of material collected after vacuuming approximately 20 plants, in a specific row of the plantation. **B.** As a complementary procedure to determine absolute and relative number of cicadellids per plant, 30 individual coffee plants were covered with anti-aphid previous to sampling. **C.** Photographs showing the presence of cicadellids in coffee leaves in the selected location. Samples were transported to the laboratory in a cooler with high humidity. Cicadellidae were identified and grouped for further analysis and experimental settings.

ABSOLUTE AND RELATIVE ABUNDANCE OF CICADELLIDS

A



n=30

Individuals per plant

4.3 ± 2.6

Species per plant

3 ± 1.3

B

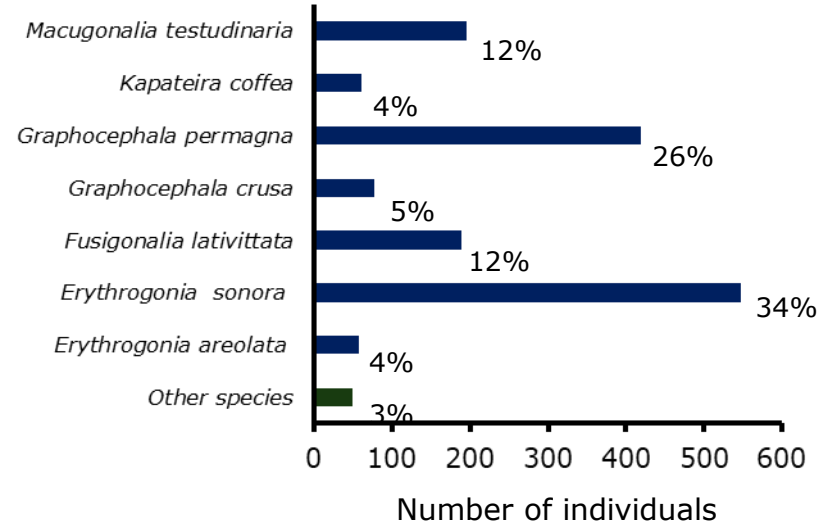


Figure 2. Cicadellidae species and abundance on coffee. A. Absolute abundance of individuals and species were determined per plant. A total of 21 different species of leafhoppers were identified. B. Relative abundance of the most frequent species of cicadellids. In total these species account for the 97% of the identified cicadellids. 14 different species represent 3% of the total population. *Xylella fastidiosa* was detected by qPCR from pooled samples of the most abundant species. Data shown represent results from sampling dates between July and December 2020. Our results suggests that despite the agricultural setting for coffee, the potential vector population is abundant and diverse.

ARTIFICIAL FEEDING TRANSMISSION TRIALS

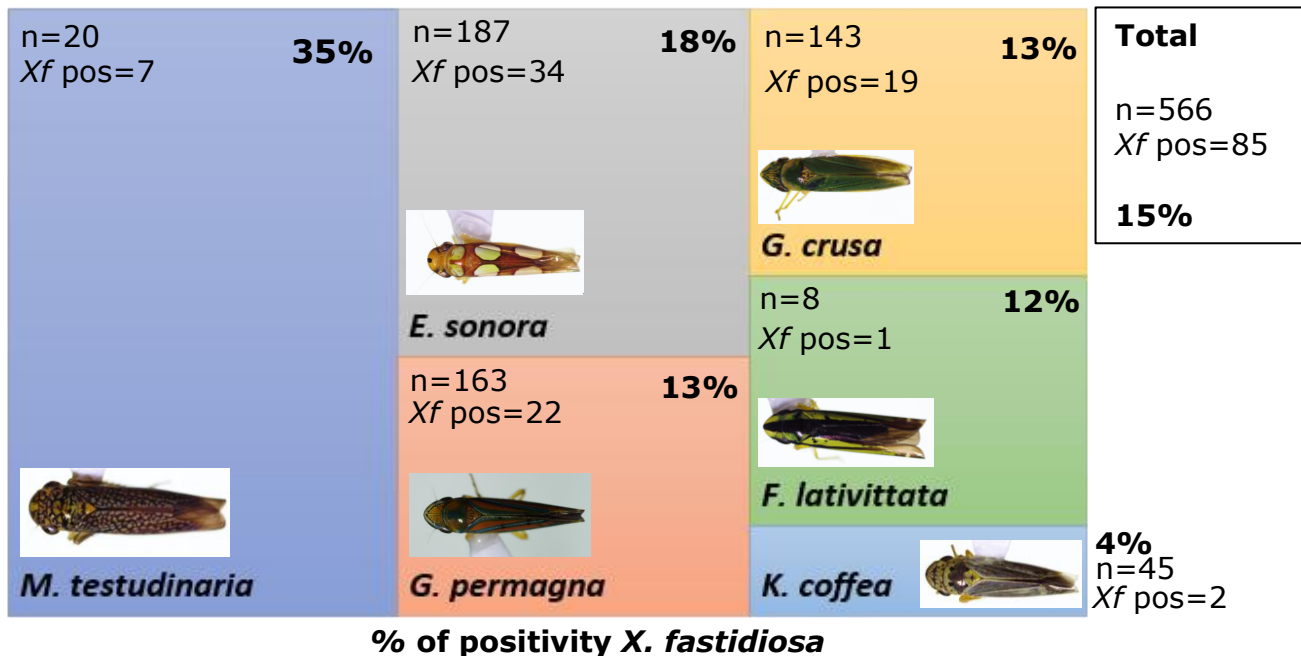


Figure 3. Percentage of *X. fastidiosa* positive potential vectors in artificial feeding transmission trials. Field collected cicadellids were individually aspirated into a 1 ml pipette tip in which the conical end was clipped and covered with a layer of fine mesh nylon fabric. The tip opposite opening was closed with a 0.5 ml microfuge tube cap. The cap contained liquid medium secured with stretched Parafilm®; the leafhoppers were able to feed through the Parafilm® layer. *X. fastidiosa* was detected by qPCR from the feeding medium in 15% of the tests (=individuals). The six species selected for trials gave positive results. *M. testudinaria* showed the highest percentage of positive results. MLST analysis was performed from a positive *Xf* feeding medium from *G. permagna*. *X. fastidiosa subsp. fastidiosa* ST20 was determined; a ST associated to coffee plants from previous work in Costa Rica.

TRANSMISSION TRIALS

A



B



Erythrogonia sonora



Kapateira coffea



Graphocephala permagna

Figure 4. Confirmation of *X. fastidiosa* vectors related to coffee in Costa Rica. Cicadellids brought from the field were aspirated by species in groups of 5-10 individuals. **A.** Each group was introduced to a coffee plant covered with a nylon mesh sleeve, for a period of 96 h. At the end of the inoculation period, the sleeve was removed, the number of live and dead leafhoppers counted. Plants were maintained in greenhouse. Plants were tested by qPCR for the presence of *X. fastidiosa* at different intervals after the inoculation period. **B.** *X. fastidiosa* vectors in coffee in Costa Rica were confirmed by transmission trial results. Further testing is required to determine the role as vectors of other cicadellids found at the coffee plantation sampled.