PROCARYOTES AND BLIGHT

Reaction of Citrus Cultivars to Graft-Inoculation of Phytoplasma aurantifolia-Infected Lime Shoots

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ABSTRACT. As observed in the Sultanate of Oman and the United Arab Emirates, Phytoplasma aurantifolia, the agent of witches' broom disease of lime, induces symptoms on the following naturally infected citrus species: lime, citron, Indian Palestine sweet lime and sweet limetta. By graft transmissions, we have shown previously that trifoliate orange, Troyer citrange, lemon and rough lemon were additional symptomatic hosts. In June 1994, several citrus species grafted on rough lemon were graft-inoculated with P. aurantifolia. By February 1996, symptomatology and ELISA have shown that the following species are susceptible: *Citrus excelsa, C. ichangensis, C. karna, C. macrophylla, C. hystrix*, rough lemon, Etrog citron, Rangpur lime and Meyer lemon.

Witches' broom disease of small-fruited acid lime (WBDL) has killed thousands of acid lime trees in the Sultanate of Oman and the United Arab Emirates (UAE) (1, 3, 4). In view of its rapid spread, the disease agent is undoubtedly transmitted by an insect vector. The leafhopper, *Hishimonus phycitis* Distant, is found exclusively on lime and is assumed to have been introduced to Oman, probably from the Indian subcontinent, and could be such a vector (2). The causal agent of the disease is a phytoplasma, a plant mollicute not available in culture and lacking helical morphology. The WBDL phytoplasma has recently been characterized and given the *candidatus* name Phytoplasma aurantifolia (6). Monoclonal antibodies and DNA probes have been obtained for its detection (2).

In Oman where citrus cultivars and species other than lime were not grown until recently, the disease has only been observed on lime trees. However, in the UAE, where the disease was first seen in 1989, not only lime, but also citron, sweet limetta and Indian Palestine sweet lime showed severe symptoms of the disease (3), and P. aurantifolia could be detected in these additional susceptible cultivars (2, 3).

Experimentally, the disease agent was graft-transmitted from lime to lime as well as to lemon, rough lemon, trifoliate orange and Troyer citrange (2, 4). The agent was also transmitted to periwinkle plants by dodder (5). In the work reported here, additional cultivars have been shown to be susceptible to P. aurantifolia.

MATERIALS AND METHODS

A number of citrus species, cultivars and related species (see Table 1 and Results and Discussion) were grafted on rough lemon rootstock seedlings in 1993 (two plants per species) at IVIA, Valencia, Spain. The grafted plants were moved to INRA Bordeaux, France and graft inoculated, about 10 cm above the budunion, with symptomatic P. aurantifolia-infected lime shoots in May 1994. The plants were grown in a temperature-controlled glasshouse at 30°C in the day (16 hr) and 25°C at night (8 hr). Plants were inspected and samples taken for ELISA detection of P. aurantifolia in May and October 1995, and in Feb-

TABLE 1
CITRUS SPECIES SHOWING SYMPTOMS OF WITCHES' BROOM DISEASE WITHIN 22 MONTHS OF GRAFT-INOCULATION WITH PHYTOPLASMA AURANTIFOLIA-INFECTED LIME SHOOTS

Species	Date symptoms first observed	ELISA (OD 405mm)
C. excelsa 167-1	May 1995	1.0 (October 1995)
C. ichangensis 235-1	February 1996	0.6 (February 1996)
Rough lemon	October 1995	0.7 (October 1995)
C. karna 242-1	February 1996	1.6 (February 1996)
Rangpur lime	February 1996	1.9 (February 1996)
C. macrophylla 288-3	May 1995	1.7 (October 1995)
Etrog citron Arizona	May 1995	2.0 (May 1995)
Meyer lemon	February 1996	1.7 (February 1996)

ruary 1996. ELISA was carried out as reported earlier (2).

RESULTS AND DISCUSSION

The grafted lime shoots used as inoculum developed into large witches' broom on all inoculated plants, and were positive for the presence of P. aurantifolia by ELISA (May 1995). On some cultivars, the lime witches' broom that developed became so large that they had to be pruned (May and October 1995).

Table 1 lists the species that developed symptoms, the periods at which symptoms were first recorded, and the ELISA readings (OD 405 nm). *C. excelsa, C. macrophylla* and Etrog citron were the first to show symptoms, one year after graft inoculation (May 1995). Rough lemon was symptomatic by October 1995. Four cultivars developed symptoms between October 1995 and February 1996: *C. ichangensis, C. karna,* Rangpur lime, and Meyer lemon. The two inoculated *C. ichangensis* plants collapsed suddenly, the leaves becoming dry within a few days. Before this shock reaction, no clear cut symptoms were observed. ELISA was carried on a *C. ichangensis* shoot that was still alive just above the budunion lime and was found to be positive (OD 405 nm: 0.6).

Four species, known to be susceptible from previous observations, had not shown symptoms by February 1996, namely sweet limetta 305, lemon (Verna 50-2 and Fino 46-1), trifoliate orange 217-1 and Troyer citrange. Previous experiments have shown that transmission to Troyer citrange (4) and trifoliate (J. Bové, unpublished data) occurs at a very low rate.

In contrast to the susceptible symptomatic species listed in Table 1, several others did not develop symptoms nor react positively with ELISA in this or previous experiments, despite the development of good witches' broom symptoms in the grafted lime shoots. They are: *C. bergamota* 254-1, *C. clementina* (Fina 39-3 and Arrufatina 58-5), *C. deliciosa* 154-1, *C. halimii* 278-1, *C. hystrix* 178, *C. junos* 335, *C. latifolia* (Bears lime) 124-1, grapefruit (Marsh 176), Cleopatra mandarin, sweet orange (Valencia 126-3, Washington navel 45-2, Sucrena 32-7 and Tarocco 271-1), *Fortunella margarita* 185-1, *Microcitrus australis* 313-2, and *Severinia buxifolia* 282. It is worthwhile noting that sweet orange, clementine and grapefruit are among the asymptomatic, P. aurantifolia-free plants. Either the phytoplasma cannot move through the graft-union from the infected lime inoculum to the cultivar, or the above cultivars are resistant to the phytoplasma. In the case of the second hypothesis, these types would remain free of phytoplasma even if insect vectors were active. Unfortu-

nately, insect transmission of P. aurantifolia to citrus has not yet been achieved experimentally. It should also be mentioned that the asymptomatic, phytoplasma-free species produced little or no new growth in comparison with the symptomatic, phytoplasma-infected cultivars which develop into large witches' brooms. It seems as if the growth of the asymptomatic, phytoplasma-free cultivars is somehow inhibited by the presence of the lime witches' broom that serves as inoculum. Pruning this witches' broom, severely, has not promoted new growth.

CONCLUSION

Several citrus species other than lime are susceptible to P. aurantifolia. Important rootstocks are among the susceptible cultivars: *C. macrophylla*, rough lemon, Rangpur lime and sweet lime which can easily be infected by graft-inoculation or, in the case of sweet lime, show severe symptoms in the field. Trifoliate orange and Troyer citrange are also susceptible, but their infection by graft inoculation is much more difficult to achieve. Up to now the major commercial scion species such as sweet orange, mandarin and grapefruit have not been experimentally infected by graft inoculation and they have shown no symptoms of infection in nature. Whether they are resistant to the WBDL phytoplasma remains to be seen.

Note added in proof. *C. hystrix* 178 developed symptoms in May 1996, with an ELISA reading of 0.910 (405 nm).

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