Witches' Broom Disease of Lime in Iran: New Distribution Areas, Experimental Herbaceous Hosts and Transmission Trials

M. Salehi, K. Izadpanah, and M. Taghizadeh

ABSTRACT. Witches' broom disease of lime (WBDL) was observed first in the Nikshahr and Qasr-e-Qand in Sistan-Baluchistan province of Iran in 1997. Since then the disease has spread to almost all citrus growing areas of this province. In addition, the disease has been observed in Minab, Roodan, Bandarabbas and Hajiabad, the major lime producing areas of Hormozgan province. The disease is threatening Jiroft, another major citrus growing region in Kerman province, and Darab in Fars province. The phytoplasmal agent of WBDL was transmitted to lime by grafting and from lime to periwinkle and periwinkle to lime by dodder. The disease agent was transmitted to eggplant, ornamental eggplant, Jimsonweed, *Nicotiana glutinosa* L., tobacco, black nightshade and tomato by grafting and dodder. Disease symptoms consisted of yellowing, small leaves, internode shortening, virescence, phyllody, witches' broom, stunting, wilting and plant death. The type of symptoms differed from those of other phytoplasmas examined. Phytoplasmal infection of these plants was verified by PCR or back inoculation to periwinkle. Broadbean, cotton, prosopis (Syrian mesquite), sugarbeet, camel-thorn, pigweed, potato, hemp, physalis, pepper, chrysanthemum, sesame, marigold, paper-flower, Mirabilis, carrot and alfalfa were not infected via dodder or graft inoculation. Repeated inoculation with the leafhopper, *Hishimonus phycitis,* reared on WBDL infected plants did not result in transmission.

Witches' broom disease of lime (WBDL) is a lethal disease of lime caused by "*Candidatus*: Phytoplasma aurantifolia" (6). The disease was observed for the first time in the Sultanate of Oman in the late 1970s (2), in the United Arab Emirates in 1989 (3) and in Iran in 1997 (1, 4, 5). There is little information about non-citrus sources and possible herbaceous hosts of the disease agent. In the present work, new distribution areas in southern Iran and the results of studies on herbaceous hosts and transmission of the disease agent are reported.

MATERIALS AND METHODS

A survey was conducted in several southern Iranian provinces for the detection of new distribution areas. Diagnosis of WBDL in citrus orchards was based on symptom expression, graft transmission using lime as an indicator plant and polymerase chain reaction (PCR) using P1/ WB3 primers (1). A total of 25 representative samples from various areas were grafted on lime seedlings and used also in PCR.

A typical witches' broom sample from Nikshahr (Sistan-Baluchistan) was used as the source of WBDL agent in transmission and host range studies. The disease agent was maintained in lime seedlings by grafting. Non-citrus test plants were inoculated by dodder (*Cuscuta campestris* Yank.) or grafting. The latter was used to transmit WBDL from eggplant to other solanaceous species or for intraspecific transmission. Controls were set up with dodder that was previously grown on healthy periwinkle. Infection of all symptomatic test plants was verified by PCR. At least one symptomatic plant of each species was used in back inoculation to periwinkle using dodder. All inoculated plants were kept in greenhouse for 18 mo and then burned.

Hishimonus phycitis, the leafhopper suspected of being a vector of WBDL agent, was collected in an infected orchard in Minab and reared massively on infected lime seedlings. Young adults from these colonies were transferred to healthy lime seedlings for 2 mo or to periwinkles, eggplant and tomato for 2

TABLE 1
REACTION OF PLANT SPECIES TO INOCULATION WITH LIME WITCHES' BROOM PHYTOPLASMA

Plant	Inoculation method		Reaction
	Graft[a]	Dodder	
APIACEAE			
Daucus carrota L.	—	0/5[b]	NS[c]
APOCYNACEAE			
Catharanthus roseus (L.) G. Don.	15/15	10/10	FV, IS, PC, PS, SL, ST, W, WB, Y
ASTERACEAE			
Chrysanthemum parthenium L.	—	0/4	NS
Tagetes erecta L.	—	0/5	NS
CANNABIACEAE			
Cannabis sativus L.	—	0/10	NS
CHENOPODIACEAE			
Beta vulgaris L.	—	0/10	NS
Chenopodium album L.	—	0/5	NS
LEGUMINOSAE (FABACEAE)			
Alhagi camelorum Fisch.	—	0/5	NS
Medicago sativa L.	—	0/20	NS
Prosopis farcta (Banks&Sol.) J. F. Macbr	—	0/10	NS
Vicia faba L.	—	0/5	NS
MALVACEAE			
Gossypium hirsutum L.	—	0/10	NS
NYCTAGYNACEAE			
Bougainvillea spectabilis Wild.	—	0/4	NS
Mirabilis jalapa L.	—	0/5	NS
PEDALIACEAE			
Sesamum indicum L.	—	0/10	NS
SOLANACEAE			
Capsicum annuum L.	0/5	—	NS
Datura stramonium L.	2/5	—	FA, IS, PC, SL, W, Y
Lycopersicum aesculentum Mill.	8/10	—	FV, IS, PC, PS, SL, ST, W, WB, Y
Nicotiana glutinosa L.	2/5	—	FV, IS, PC, PS, SL, ST, WB, Y
N. tabacum L.	1/3	—	FV, IS, PC, PS, SL, ST, WB,Y
Physalis alkekengi L.	0/5	0/5	NS
Solanum integrifolium Poir.	5/5	—	FP, IS, PS, SL, ST, W, WB, Y
S. melongena L.	5/15	3/8	FV, IS, LS, PC, PS SL, ST, W, WB, Y
S. nigrum L.	4/5	—	FP, IS, PS, SL, ST, W, WB. Y
S. tuberosum L.	0/10	0/5	NS

[a]Grafted with scions from infected eggplant.
[b]No. of infected plants/No. of inoculated plants.
[c]FA: absence of flower, FP: floral phyllody, FV: floral virescence and phyllody, IS: internode shortening, LT: leaf twisting, NS: no symptoms, PC: proliferation of crown buds, PE: petiole elongation and twisting, PS: proliferation of stem buds, SL: small leaves, ST: stunting, W: wilting, WB: witches' broom, Y: foliar yellowing, -: not tested.

weeks. At least 20 lime seedlings and 10 of each herbaceous species were used in these studies. Usually 10 leafhoppers were used per test plant. The plants were then sprayed with an insecticide. Also, infected lime seedlings were caged with *H. phycitis* and healthy lime and periwinkle.

RESULTS

Typical symptoms of WBDL were observed in six new districts around Nikshahr and three new locations around Iranshahr, all in Sistan-Baluchistan province. In Hormozgan province, symptoms of WBDL were observed for the first time in 1998 in

Karyan near Minab, then in 14 localities in Minab and six districts in Roodan. The latter is the main lime producing area of Iran. Most recent discovery of the disease is in three regions in Bandarabbas and in Hajiabad (Fig. 1) bordering Fars province.

All lime seedlings successfully grafted with scions of diseased lime became infected. The disease agent was transmitted from lime to periwinkle and from periwinkle to lime by dodder. The incubation period in lime ranged from 5 to 14 mo. Of 25 herbaceous plant species inoculated via dodder or graft inoculation, seven developed distinct symptoms (Table 1). Disease symptoms consisted of yellowing, small leaves, internode shortening, virescence, phyllody, witches' broom, stunting, wilting and plant death. Other plants, notably broadbean, alfalfa and sesame, which are common phytoplasmal hosts in southern Iran were not infected via dodder or graft inoculation.

Repeated inoculation of lime or periwinkle with *H. phycitis* reared on WBDL-infected plants did not result in phytoplasmal transmission.

Fig. 1. Map of Iran showing citrus growing provinces (shaded areas) and current distribution sites of witches' broom disease of lime (numbers) in the order of discovery of the disease. 1, Nikshahr (original discovery site), 2, Iranshahr, 3, Minab, 4, Roodan, 5, Bandarabbas, 6, Hajiabad.

DISCUSSION

WBDL was originally discovered in Nikshahr in Sistan-Baluchistan. It is now found in six new districts around this town and four districts around Iranshahr. It is also found in several locations in the neighboring Hormozagan province. The causal phytoplasma appears to have a "jumping" pattern of spread, in which areas between old and new infection sites may be WBDL-free. Two other provinces, Fars and Kerman, are threatened.

WBDL is transmitted to several herbaceous species mainly in the family Solanaceae. However none of these plants are found to be naturally infected with WBDL phytoplasma. Alfalfa witches' broom, Bermuda grass white leaf, basil phyllody and eggplant big bud found in the lime witches' broom infected areas are etiologically different from WBDL (unpublished results).

H. phycitis is a leafhopper infesting citrus in the citrus growing areas of southern Iran. Bové et al. (2) were able to detect WBDL phytoplasma in the leafhopper by PCR, but failed to transmit WBDL under greenhouse condition in France. However, repeated inoculation of lime and periwinkle with the leafhopper reared on infected lime did not result in any infection. Despite obvious spread of the WBDL, the vector of the disease agent remains undetermined.

Note added in proof: WBDL has spread to Kerman province.

ACKNOWLEDGEMENT

This study was supported by the Iranian National Research Funds, Project No. 1581.

LITERATURE CITED

1. Bové, J. M., J. L. Danet, K. Bananej, N. Hassanzadeh, M. Taghizadeh, M. Salehi, and M. Garnier 2000. Witches' broom disease of lime (WBDL) In Iran. In: *Proc. 14th Conf. IOCV*, 207-212. IOCV, Riverside, CA.

2. Bové, J. M., L. Zreik, J. L. Danet, J. Bonfils, A. M. M. Mjeni, and M. Garnier
1993. Witches' broom disease of lime trees: Monoclonal antibody and DNA probes for the detection of the associated MLO and the identification of a possible insect vector. In: *Proc. 12th Conf. IOCV*, 342-348. IOCV, Riverside, CA.
3. Garnier, M., L. Zreik, and J. M. Bové
1991. Witches' broom, a lethal mycoplasmal disease of lime trees in the Sultanate of Oman and the United Arab Emirates. Plant Dis. 75: 546-551.
4. Salehi, M., K. Izadpanah, and H. Rahimian
1997. Witches' broom disease of lime in Sistan-Baluchistan. Iran. J. Plant Pathol. 33: 76.
5. Salehi, M., K. Izadpanah, and M. Taghizadeh
2000. A study on host range and possible vector of lime witches' broom in Iran. Proc. 14th Plant Prot. Cong. Iran. Isfahan. Univ. Technol: 146.
6. Zreik, L., P. Carle, J. M. Bové, and M. Garnier
1995. Characterization of the mycoplasmalike organism associated with witches' broom disease of lime and proposition of a *Candidatus* taxon for the organism, "*Candidatus* Phytoplasma aurantifolia". Int. J. Syst. Bacteriol. 45: 449-453.