Citrus Chlorotic Dwarf, a New Whitefly-Transmitted Disease in the Eastern Mediterranean Region of Turkey

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ABSTRACT. A new virus-like disease of citrus was observed in the eastern Mediterranean region of Turkey at the end of the 1980s. Field symptoms consist of a V-shaped notch and chlorotic flecking on young leaves, and warping, crinkling, inverted cupping, and variegation on mature leaves. This disease has been named "Citrus Chlorotic Dwarf (CCD)." CCD has been observed on 12 citrus varieties including sweet orange, mandarin, grapefruit, and lemon. Sweet orange was the least affected. The highest disease incidence (49%) was found in the Içel area, whereas only a few diseased trees were observed in Adana or Hatay. In the laboratory, the pathogen was transmitted frequently by the Japanese bayberry whitefly, *Parabemisia myricae*. The transmission rate increased from 18% to 46% with an increase in the inoculation access period from 24 to 48 hr. The pathogen was transmitted to rough lemon by stem-slash inoculation at a rate of 5% (5 cuts) to 72% (100 cuts). The causal agent was not transmitted mechanically by leaf inoculation to citrus or herbaceous plants and did not react in ELISA to antisera against citrus variegation, citrus leaf rugose, and satsuma dwarf viruses.

Index words. Citrus viruses, *Parabemisia myricae*, stem slash inoculation, disease survey, rough lemon, ELISA.

A new virus-like disorder was observed in the eastern Mediterranean region of Turkey during citrus orchard inspections. This disease spread rapidly along the south coast and reached epidemic levels at the beginning of the 1990s. Currently, this disorder is considered the most serious citrus disease occurring in the eastern Mediterranean region of Turkey (1, 2).

Field symptoms resembled those caused by citrus variegation virus (CVV) (1). Subsequent studies in the greenhouse, however, revealed that it differed in important characteristics from CVV (11). Since chlorosis was particularly strong, and leaf size was reduced, the name "Citrus Chlorotic Dwarf" (CCD) was proposed for this new disease (11).

The causal agent was graft-transmitted to 20 of 36 citrus cultivars and species inoculated. The best symptoms developed on rough lemon 5 to 8 weeks after inoculation. Symptoms occurred at 20 to 25°C, but even better symptoms were observed at 30 to 35°C (10).

Because of the intense and rapid outbreak of CCD, vector transmission was suspected. This suspicion was strengthened when CCD was found on rough lemon seedlings used as host plants for a *Parabemisia myricae* (Kuwana) mass culture maintained at the Department of Plant Protection in Adana. In preliminary laboratory experiments, the putative pathogen was transmitted by *P. myricae* from infected rough lemon to half of the 20 exposed rough lemon seedlings (1).

In the present paper we report further investigations on distribution and transmission of CCD.

MATERIALS AND METHODS

Field survey. The distribution of CCD was studied in the provinces of Içel, Adana, and Hatay, in the eastern Mediterranean region of Turkey (Fig. 1), from September 1993 to March 1994. The survey included 55 orchards ranging in size from 1 to 10 ha with a planting density of about 210 trees/ha. Plantations consisted of 4- to 25-yr-old citrus trees and were mixed plantings of grapefruit, mandarin, sweet orange, and lemon. Lemons were especially common in

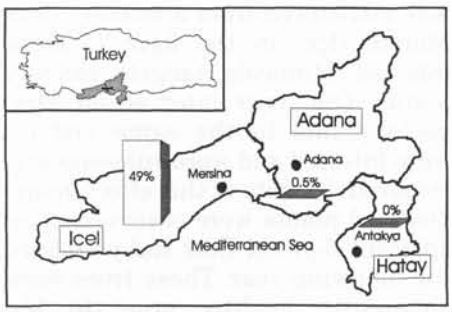

Fig. 1. Distribution and incidence of Citrus Chlorotic Dwarf (CCD) in the eastern Mediterranean region of Turkey.

the province of İçel. In total, 4,407 systematically selected trees were visually examined for symptoms of CCD.

Laboratory experiments and plant material. Rough lemon plants were grown from seeds in sterilized potting mixture and kept in a partially shaded greenhouse cooled by evaporative coolers. The temperature ranged from 20 to 25°C and the relative humidity was about 65% ± 5%.

The source of CCD used in all experiments was obtained originally from a rough lemon seedling inoculated with CCD by *P. myricae* and was found free of any other detectable citrus virus or virus-like disease by biological indexing (13). Other virus sources used for comparison were Strain 401A of CVV supplied by C. N. Roistacher, University of Riverside, California, CVV-1 from the USDA Horticultural Research Laboratory, Orlando, Florida, citrus leaf rugose virus (CLRV) obtained from the University of Catania, Italy, and satsuma dwarf virus (SDV 58) provided by Dr. T. Iwanami, Okitsu Research Station, Japan. All virus sources were maintained on rough lemon seedlings in the greenhouse.

Transmission tests. *P. myricae* were reared on rough lemon seedlings in a climate-controlled room as described previously (17). For each experiment about 500 adult whiteflies were transferred onto 1-yr-old CCD-infected rough lemon for an acquisition access period (AAP) of 24 hr. Without any latent period, groups of 15 to 20 *P. myricae* were caged on 6-month-old rough lemon seedlings having many young flushes. Inoculation access periods (IAP) of 24 and 48 hr were tested. After inoculation, the plants were sprayed twice with insecticides and were cut back after one month.

Mechanical inoculations to red kidney bean, cowpea, and white sesame were made by conventional leaf-inoculation procedures. Inocula were prepared from young, symptomatic citrus leaves in cold 0.05 M neutral potassium phosphate buffer, and applied with sterile cotton swabs to leaves dusted with 500-mesh Carborundum (6). A minimum of five plants was used for each inoculation experiment. In addition, the causal agent of CCD was inoculated by the stem-slash technique (8, 12) to 6-month-old rough lemon seedlings. Inoculum was prepared in the same way without further concentration of extracts. Test plants were inoculated with a razor blade to which a single drop of inoculum was applied. New inoculum was added after each three to five cuts. Treatments consisted of plants slashed 5, 10, 25, 50 or 100 times. About 10 days after inoculation all leaves were removed and the plants were cut back above the inoculation site.

All inoculated plants (insect and mechanical transmission tests) were kept at 20 to 25°C in the greenhouse and examined periodically for CCD symptoms for at least 6 months. Usually the plants were cut back after each flush (every second month).

ELISA. Young leaf samples of rough lemon, infected with CCD, CVV, CLRV, and SDV were assayed by conventional DAS-ELISA procedure (3) using specific antisera for CVV (4), CLRV (6), and SDV. Anti-

serum for SDV (SDV 58) was kindly provided by Dr. T. Iwanami, Okitsu Research Station, Japan. Dilutions of IgGs varied between 1/400 (CVV, CLRV) and 1/1000 (SDV) and were prepared from 1 mg/ml stock solutions of purified IgG. Conjugates were used in the same dilutions as the IgGs. For each ELISA test at least 10 replications were done with plant extracts 1/10 (w/v) prepared in phosphate saline (pH 7.4) using leaves obtained from plants infected with CCD, CCV, CLRV, and SDV and leaves from healthy plants.

RESULTS AND DISCUSSION

Field survey. In the eastern Mediterranean region an average of 20.3% of all citrus trees revealed symptoms of CCD. However, almost all infected trees were found in İçel whereas some infected grapefruit and Minneola tangelo were observed only in one small area in Adana (Fig. 1, Table 1). No diseased plants were found in the Hatay province. The high incidence of CCD in İçel suggests it may be the possible origin of this disease and that there is a slow movement over longer distances.

Field symptoms consist of a V-shaped notch and chlorotic flecking on young leaves, and warping, crinkling, inverted cupping, and variegation on mature leaves. Sweet orange was much less affected than grapefruit, lemon, or mandarin. Not only was incidence lower on sweet orange, but symptoms were less severe. The following citrus varieties were found infected during this survey: Aydin, Interdonata, Lamas, and Kütdiken lemons, Minneola tangelo, Fremont, and satsuma mandarins, Marsh seedless, Star Ruby, and Redblush grapefruit, Valencia, Washington Navel, and local cultivars of sweet oranges.

A few CCD-diseased citrus plants were found in Adana, but at only one location. The history of this infection focus is known. The first inoculum was introduced from a nursery near Mersin (İçel) in the form of three infected Minneola tangelo nursery plants. One year later seven Minneola plants in the same orchard were infected and were subsequently removed. In spite of this effort, many diseased plants were observed in an adjacent 6-yr-old Star Ruby orchard the following year. These trees were apparently healthy prior to the introduction of the CCD-infected Minneola trees.

Vector transmission. *P. myricae* transmitted the putative agent of CCD from rough lemon to rough lemon. In general, symptoms occurred in the first flush after inoculation (as early as 14 days), and they were much stronger than those observed after graft-transmission of the causal agent. However, in a few plants, symptoms did not appear until 3 to 6 mo. after inoculation. By increasing the IAP, from 24 to 48 hr, the transmission rate was increased from 18% to 46%. These findings indicate that CCD is probably transmitted in a semi-persistent or persistent-circulative manner rather than in a non-persistent manner where the agent is only retained for a few hours (5).

The role of *P. myricae* in the spread of CCD in the field is difficult to judge. According to our field observations, and statements of local citrus growers, CCD outbreaks occurred after a heavy infestation of citrus by the Japanese bayberry whitefly (2). However, since 1990, the populations of *P. myricae* in the field have been very low (<0.1 life stage/leaf). A program for biological control by releasing the aphelinid wasp *Eretmocerus debachi* Rose and Rosen was launched in 1988, and this has proved to be extremely successful (14, 15, 16). However, natural transmission of CCD in the field still occurs (9). This indicates that low populations of *P. myricae* can spread CCD or that other whitefly species are also vectoring it. Due to effective

TABLE 1
INCIDENCE OF CITRUS CHLOROTIC DWARF (CCD) ON DIFFERENT CITRUS SPECIES IN THREE PROVINCES IN THE EASTERN MEDITERRANEAN REGION OF TURKEY

	Number of trees inspected and percentage of diseased trees									
	Grapefruit		Mandarin[z]		Sweet orange		Lemon		Total	
Province	Trees inspected	% CCD[y]	Trees inspected	% CCD	Trees inspected	% CCD	Trees inspected	% CCD	Trees inspected	% CCD
Adana	639	0.6	588	1.2	399	0.0	526	0.0	2,152	0.5
Hatay	101	0.0	133	0.0	147	0.0	71	0.0	452	0.0
İçel	317	59.6	400	46.8	176	25.6	904	50.9	1,797	49.0
Total	1,057	18.2	1,121	17.4	722	6.2	1,501	30.7	4,401	20.3

[z]Mandarins and mandarin hybrids
[y]Plants showing field symptoms of CCD infection

control of *P. myricae*, the citrus whitefly, *Dialeurodes citri* (Ashmead), is now the predominant species on citrus (18). Whether *D. citri* is also a vector of CCD and should be implicated in the rapid spread of this disease in the eastern Mediterranean region is yet unknown.

Mechanical transmission. CCD was not transmitted mechanically to red kidney bean, cowpea, or sesame by leaf-inoculation, while CIVV and SDV were readily transmitted in the same tests. In contrast, CCD was transmitted by stem-slash inoculation from rough lemon to rough lemon at a rate of 5% when five to ten cuts per plant were made and a rate of 72% when 100 cuts were made (Table 2). Only a few plants developed symptoms in the first flush; most of the symptomatic plants were observed in the second flush after a 3 to 4 mo. incubation period. CCD is apparently not transmitted in the field by knife cuts during pruning, since several attempts to transmit CCD from citrus to citrus by knife cuts to simulate pruning failed (S. Korkmaz, unpublished data). The transmission efficiency of CCD by stem-slash inoculation was comparable to that obtained in similar transmission studies for citrus tristeza virus (CTV) using concentrated bark extracts (7). CTV is a phloem-restricted, semi-persistently transmitted virus and is not transmissible to citrus and herbaceous plants by conventional leaf-inoculation (7, 12). Because of these similarities, our results suggest that CCD might be a phloem-restricted pathogen. The lack of mechanic transmission to herbaceous plants and citrus by leaf-inoculation are further indications that CCD is not related to CVV, CLRV, and SDV.

ELISA. Although some symptoms of CCD resemble those caused by CVV, CLRV, and SDV, no antigenic relationship between these viruses and CCD was observed in DAS-ELISA using CVV, CLRV, and SDV antisera. The OD_{405} readings in ELISA for extracts from CCD-infected plants were no different than those from healthy citrus. In each case, positive controls for CVV, CLRV, and SDV reacted strongly to their respective antibodies.

TABLE 2
TRANSMISSION OF CITRUS CHLOROTIC DWARF (CCD) BY STEM-SLASH INOCULATION TO ROUGH LEMON

Treatment	Number of cuts[z]	Transmission: Plants infected
1	5	1/20[y]
2	10	1/20
3	25	2/20
4	50	6/20
5	100	36/50

[z]Number of plants infected/number of plants inoculated.
[y]Inoculum was extract of young infected leaves prepared in neutral 0.05M potassium phosphate buffer.

FUTURE CONCERNS

CCD is the first whitefly-transmitted disease reported on citrus. It is apparent from the situation in İçel that this disease has the potential to greatly reduce production of susceptible cultivars in an entire region, especially if trees become infected when young. While the source of infection is now localized, we are concerned that this disease might move quickly once introduced into other citrus areas where *P. myricae* is prevalent (such as the Mediterranean basin and the USA) and cause severe losses. Further attempts are in progress to identify and characterize the causal agent of CCD and to develop suitable detection tools to study its epidemiology.

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