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FUNGI IN RIO GRANDE GUMMOSIS LESIONS AND PATTERNS OF GUMMOSIS-AFFECTED GRAPEFRUIT TREES IN INDIAN RIVER AREA GROVES

R. M. Sonoda and R. R. Pelosi
University of Florida, IFAS
Agricultural Research and Education Center
P. O. Box 248
Ft. Pierce, Florida 34954-0248

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Abstract. **Reports of gummosis symptoms on trunks and limbs of grapefruit trees planted in the 1960's and 1970's in the Indian River citrus-growing area have increased as trees have grown older. Most affected trees have symptoms corresponding to descriptions of Rio Grande gummosis, a disease of yet unclear etiology. *Diplodia natalensis* Pole-Evans (*Lasiodiplodia theobromae* (Pat.) Griffon Maubl.) often associated with Rio Grande gummosis lesions, was not consistently isolated from gumming lesions. Also, *D. natalensis* genotypes, distinguished on the basis of vegetative incompatibility reactions on Difco Potato dextrose agar, differed within the same Rio Grande gummosis lesion and occasionally within the same area of a lesion. Analysis of a planting of grapefruit scion on different rootstocks indicated no relationship between rootstock tolerance to salinity and incidence of Rio Grande gummosis. Ordinary runs analysis, a technique used to analyze spatial patterns, indicated that Rio Grande gummosis-affected grapefruit trees were often random or sometimes aggregated downrow or across rows.**

Planting of grapefruit in the east coast citrus growing area of Florida increased in the 1960's and 1970's. As these grapefruit plantings have aged, the incidence of trunk and limb gummosis have increased. Most of the gummosis observed on grapefruit trees in groves in the Indian River area conform to descriptions of Rio Grande gummosis (RGG) (8). RGG continues to be a disease of unclear etiology, although several hypothesis have been advanced as to causal factors.

A fungus, *Diplodia natalensis* Pole-Evans, is often associated with RGG lesions in Florida (3). The fungus has a wide host range and the names applied to it have changed frequently. Currently the most acceptable name seems to be *Lasiodiplodia theobromae* (pat.) Griffon and Maubl. Another name used for the fungus when associated with citrus is *Physalospora rhodina* Cooke (7). We will use *D. natalensis*, the most commonly used name in recent literature on citrus diseases (8), in our report.

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In Texas, RGG is attributed to infection by *D. natalensis* (5). Although *D. natalensis* is frequently found in RGG cankers in Florida, Childs (4) felt that *D. natalensis* was not the initiator of the syndrome. No effort has been made to determine the make-up of *D. natalensis* in RGG lesions. The presence of different genotypes of *D. natalensis* within RGG lesions would support the hypothesis that it is a secondary invader. Voorhees (7) in 1942, reported that *D. natalensis* isolates from different sources can be distinguished on agar medium by the formation of a zone of dark colored mycelia where colonies meet. No dark zones are formed between subcultures from the same isolate. These dark zones between colonies of other fungi have been used to indicate differences in genetic makeup.

Childs (4) reviewed work by others that indicated that incidence of RGG lesions increased with increasing amounts of KCl applied to grapefruit trees. He also reported an increase in RGG symptoms with increase in applied $CaCl_2$.

Fewer RGG lesions occurred when K+ was applied as K_2SO_4 (4). Childs (4) work suggests that with increase in Cl^- applied, RGG incidence increases. It could not be determined from Childs' study (4) whether Cl^- can initiate RGG. Different rootstocks are considered to have different sensitivities to Cl^-. Observations made by the authors suggested that differences in salt tolerance may coincide with RGG tolerance. No associations of differences in RGG incidence with rootstock differences was found in a search of the literature.

Patterns of disease incidence can frequently provide clues as to etiology of a disease. No studies of RGG spatial patterns have been reported.

The objectives of this study were to determine the composition of *D. natalensis* within individual RGG lesions, document the incidence of RGG in a rootstock study and analyze patterns of RGG incidence in several Indian River area groves.

Materials and Methods

Bark or wood samples (0.5-1.5 cm x 2-3 cm) were chiseled from RGG lesions on grapefruit trunks. One lesion each on selected trees were sampled. In sample set 1, wood samples from 10 locations within each of 7 lesions, with obvious wood discoloration, were obtained from grapefruit trees in a planting on sour orange rootstock. In sample set 2, lesions were sampled from 10 trees in a grove of mixed rootstocks. In set 2, bark and wood *segments* were obtained from newly gumming lesions. The tissue samples were surface-sterilized in 0.5% NaOCl and cut into

0.25 -0.5 cm x 0.5 cm pieces and plated on acidified Difco potato dextrose agar. Hyphal tips of fungi conforming to *D. natalensis* were transferred to Difco potato dextrose agar and incubated at room temperature, about 24° C. Following fungal growth, agar blocks containing different isolates were plated out in a hexagonal pattern on Difco potato dextrose agar. In most cases, the agar blocks were placed at 1.5 cm intervals. The plates were incubated on the laboratory bench at room temperature. Colonies between which dark lines indicating vegetative incompatibility were present were assigned to different vegetative compatibility groups (vcg). Comparisons were made between isolates obtained from the same lesion and isolates obtained from the same bark or wood segment (from the same area within a lesion).

RGG symptoms were assessed in a planting of Marsh grapefruit on six rootstocks made at the Agricultural Research and Educational Center, Ft. Pierce in 1970 (1). The rootstocks were planted in three soil treatments:surface tilled (ST), deep-tilled (DT) and deep-tilled with lime incorported during tilling (DTL) (1). There were 3 replicates of each soil treatment. In each soil treatment replicate there were 12 two-tree replicates of each of the six rootstocks (1). The rootstocks in the planting were Carrizo citrange (*Poncirus trifoliata* (L.) Raf. x *C. sinensis*); Rangpur lime (*C. limonia* Osb.); Cleopatra mandarin (*C. reshni* hort. ex Tanaka); rough lemon (*C. jamhiri* Lush.); sour orange (*C. aurantium* L.); and trifoliate orange (*P. trifoliata*). Citrus blight severely affected trees on several of the rootstocks. Tree losses also occurred due to water damage as well as other causes. Of the 216 trees of 'Marsh' grapefruit on a particular rootstock planted, there were 146 Carizzo, 135 Rangpur lime, 202 Cleopatra mandarin, 120 rough lemon, 171 sour orange and 160 trifoliate orange remaining when assessments for RGG incidence took place. RGG incidence was recorded in Nov. 1990 on surviving trees, some with symptoms of citrus blight. An analysis of variance was conducted after arc-sine transformation of percent of trees affected.

Grapefruit groves in St. Lucie county were mapped to determine the incidence and spatial patterns of RGG-affected trees. Two groves (Groves 1 and 2) (Table 2) of 'Marsh' grapefruit on sour orange rootstock with a low incidence of RGG-affected trees were mapped in 1988. Grove 1 was bedded north-south and Grove 2, east-west. The other groves in the study were mapped for RGG incidence in 1990, they included: Grove 3, 'Marsh' grapefruit on Swingle rootstock, planted in 1977, beds oriented east-west; Grove 4, 'Marsh' grapefruit on sour orange, Planted in 1963, beds oriented north-south; Grove 5, 'Marsh' on sour orange planted in 1960, beds oriented north-south; Grove 6, red grapefruit on sour orange, planted in 1970, beds oriented north-south; Grove 7, red grapefruit on sour orange planted in 1974, beds oriented north-south and; Grove 8, red grapefruit on Swingle rootstock, planted about 1973, beds oriented east-west. Ordinary runs analysis (6) was conducted in the downrow and across bed directions for data obtained from each grove. Ordinary runs analysis was used to determine if the distribution of RGG-affected trees in a grove was random or aggregated in these directions. Grove 3 was separated into three blocks, Grove 5 into three blocks and Grove 6 into two blocks and runs analysis applied separately for each block.

Table 1. Presence of *D. natalensis* in wood tissue from RGG lesions on trunks of red grapefruit on sour orange rootstock and numbers of *D. natalensis* vegetative compatibility groups within each lesion.

Lesion	Wood *tissue (No.)*	*Diplodia natalensis Isolates (No.)*	*VCG*[z] *(No.)*
1	20	2	2
2	20	8	2
3	30	20	5
4	20	1	1
5	30	5	3
6	20	0	–
7	20	1	1

[z]VGG - vegetative compatibility groups of *D. natalensis* within each lesion.

Results

D. natalensis was isolated from some, but not all samples of discolored wood (Table 1) from RGG lesions in sample set 1. When the *D. natalensis* from individual lesions were plated opposite each other on the same plate of agar, several distinct genotypes, as indicated by dark lines between colonies (see plates in Voorhees (7)), were obtained from the same lesion (Table 1). In a few cases, the genotypes from the same area of a lesion were also different. In sample set 2, discoloration of tissue was often absent in wood tissue under gumming lesions. *D. natalensis* was obtained from only 2 of 10 lesions.

Significant differences in RGG incidence between rootstocks was obtained in the DTL treatment (Table 2). Trees on rough lemon had the highest RGG incidence. There was no relationship in RGG incidence among rootstocks based on their response to salinity (Table 2).

Ordinary runs analysis indicated that RGG-affected trees were present in a random pattern in four of the 8 groves (Groves 1, 3, and 8 and block 1 of Grove 6.). In Grove 2, RGG affected trees were aggregated downrow and across rows (Table 3). In Groves 4 and 5, RGG-affected trees were aggregated downrow, but not across rows and in block 2 in grove 6 and in grove 7, RGG-affected trees were aggregated across the rows but not down row.

Discussion

The results of isolations from RGG lesions suggest that RGG in Florida is not initiated by *D. natalensis*. This view

Table 2. Percent of trees affected with RGG symptoms on 'Marsh' grapefruit on six different rootstocks in 1990 in a replicated planting established in 1970 in three different soil treatments.

Rootstocks	*DTL*	*Soil Treatment*[z] *DT*	*ST*	*Salt*[x] *Tolerance*
Trifoliate orange	2.4 a[y]	2.5	6.4	poor
Rangpur lime	3.3 a	7.1	3.2	good
Cleopatra mandarin	7.0 ab	6.1	4.2	good
Sour orange	11.6 ab	5.4	4.9	interm.
Carrizo citrange	20.5 bc	13.4	16.0	poor
Rough lemon	36.2 c	15.6	12.6	interm.

[z]DTL = deep-tilled with lime incorporated; DT = deep-tilled with lime surface-applied; ST = surface-tilled with lime surface applied.
[x]Based on data from (2).
[y]Means within columns separated by Duncan's multiple range test, 5% level.

Table 3. Runs analysis of RGG incidence on grapefruit trees in eight groves in the Indian River area.

			Ordinary Runs				
				Down row		*Across row*	
Grove	No. Trees	RGG (%)	Exp.[z]	Obs.	Z	Obs.	Z
1	576	7.8	83.9	84	0.2	84	0.2
2	380	17.1	108.7	96	–2.2 *	98	–1.8 *
3–1	450	15.8	119.6	114	–0.9	138	3.3
–2	279	12.2	60.7	61	0.2	64	1.1
–3	291	15.1	75.7	79	0.9	76	0.2
4	1122	25.5	427.0	372	–4.3 *	432	0.3
5–1	270	17.0	77.3	62	–3.2 *	86	1.6
–2	270	35.9	125.3	84	–5.4 *	136	1.5
–3	270	28.9	111.9	78	–5.0 *	110	–0.2
6–1	288	74.7	110.0	106	–0.5	109	–0.1
6–2	288	76.7	103.8	108	0.8	91	–2.0 *
	768	9.3	131.5	130	0.2	120	–2.3 *
8	864	10.1	157.5	149	–1.5	154	–0.6

[z]Exp. = expected number of runs.
*Indicates nonrandom pattern, Z ≤ –1.64.

is supported by the low percent of lesions yielding *D. natalensis* and that different genotypes of *D. natalensis* occur within the same RGC canker and in some cases even within the same area of a canker. One reason that *D. natalensis* has been a prime suspect in initiating RGG may be that it is the fastest growing of several fungi recoverable from RGG lesions. *D. natalensis* as well as other fungi, *Diaporthe citri, Fusarium* spp., etc. may be important as secondary invaders that accentuate RGG severity by colonizing and destroying weakened bark and wood tissue.

Results of the survey for RGG incidence in the rootstock study do not indicate a relationship between salt tolerance and incidence of RGG (Table 2). Interpretations from the survey of this rootstock planting were difficult since the two rootstocks with the highest incidence of RGG were the two with the heaviest losses to citrus blight. However, there is no known relationship between RGG and citrus blight. RGG incidence for trees on Rangpur lime and trifoliate orange, both relatively unaffected by citrus blight in the planting, and differing in their reaction to salinity, indicate no relationship between RGG incidence and tolerance of a rootstock to salinity.

The random patterns of RGG-affected trees in Groves 1, 3, 8 and block 1 of Grove 6 suggest that if a pathogen is involved it does not actively spread in the grove. In the groves with clustered patterns of RGG affected trees downrow, the pattern could be explained by the movement of a pathogen in this direction by ongoing cultural practices. However, in groves where clustering of RGG affected trees is across the rows, with no evidence of clustering downrow, why a pathogen would move across the row and across a water furrow and not downrow would be difficult to explain.

If Cl^- acts either as an initiator of RGG or an enhancer of RGG, the random pattern of RGG-affected plants can be the result of similar edaphic and environmental conditions throughout a grove where Cl^- is evenly applied. The aggregated patterns can result from edaphic factors being different in the downrow or across row direction or in both directions. Grove 7 and 8 were immediately adjacent plantings with beds running in opposite direction. RGG affected trees were aggregated across the row, in the east-west direction, in Grove 7 and the Z value for grove 8 was nearly significant for the downrow direction, again the east-west direction. Studies on the pattern of edaphic factors in these two groves may provide further clues as to the cause of RGG.

The relatively high incidence of RGG in the relatively young Grove 3 (Table 3) prompted concern that grapefruit on Swingle rootstock may be more susceptible to RGG. However, a comparison of incidence of RGG between adjacent Groves 7 and 8, indicate little if any difference in RGG incidence between Grove 7, red grapefruit on sour orange, and Grove 8, red grapefruit on Swingle rootstock, planted within a year or so of each other.

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