Agrobacterium tumefaciens biovar 3 Responsible for Reduction in Yield and Vigour of Muscat d'Alexandrie

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Submitted for publication: August 1992
Accepted for publication: October 1992
Key words: *Agrobacterium tumefaciens*, crown gall, grapevine

Yield and vigour of crown gall (*Agrobacterium tumefaciens*) infected and healthy Muscat d'Alexandrie vines were compared over a five-year period. Crown-gall-infected plants exhibited a significant reduction in vigour as indicated by shoot mass and trunk diameter. A significant reduction in yield occurred during two seasons only. Gall size on vines (percentage of trunk circumference covered by gall) varied from < 10% to 75%. The highest proportion of diseased plants in the trial (52%) had galls smaller than 10% while only a few plants (6%) had galls greater than 50%. The largest reduction in vigour and yield occurred in plants with galls > 50% of trunk circumference.

Crown gall on grapevines, caused by *Agrobacterium tumefaciens* biovar 3 (AT 3), is widespread and economically important in vineyards and nurseries around the world (Goodman, Grimm & Margrit, 1992). In South Africa it is considered to be a disease of economic importance in the warmer grape-growing regions. Due to the latent infective ability of the bacteria (Lehoczky, 1968), quarantine on AT 3 is difficult. Local regulations stipulate that nursery material need only be visually free from symptoms to be regarded as crown gall free, and this has led to the distribution of the disease to many vineyards.

Loubser (1978) proved biovar 3 to be the prevalent biotype on vines in the Orange River irrigation area of the northern Cape. In this area Muscat d'Alexandrie, a cultivar very susceptible to AT 3 (Ferreira & Van Zyl, 1986), has traditionally been planted on its own root. This is believed to have caused a reduction in the area planted with this cultivar in the region. Attempts at chemical control of the disease have been unsuccessful (Ferreira, 1985).

Ferreira & Van Zyl (1986) evaluated the susceptibility of different rootstocks to five pathogenic strains of AT 3. Of these cultivars 775 Paulsen was found to be the only one resistant to all the strains used, while Jacquez was the most susceptible. The use of 775 Paulsen as a rootstock, however, is a risk because of its low growth vigour and unknown compatibility with scion cultivars, Some of the other economically important rootstock cultivars, *viz.* 110 Richter, 99 Richter, Ramsey and 143B Mgt, were classified as susceptible to crown gall. Although Schroth *et al.* (1988) found reduced yield and vigour for Zinfandel on its own root, very little scientific information concerning the effect of crown gall on yield and vigour of vines is available.

This paper reports on the effect of AT 3 infection on the vigour and yield of Muscat d'Alexandrie vines grafted onto 99 Richter in the Olifants River irrigation area.

MATERIALS AND METHODS

Muscat d'Alexandrie vines, grafted onto 99 Richter and free of crown gall visually, were planted in a virgin Hutton soil during 1985 and trellised onto a slanting system. Vines were planted 1 m apart in rows with 3 m between rows. Pruning was done according to a standard pruning system used for raisin production. Pruning shears were not sterilised after pruning an infected vine. Clean tillage, using a disk harrow, was practised between rows while herbicide (Glyphosate) was sprayed in rows to control weeds. Irrigation was applied through a microjet system.

Two years after planting twenty visually healthy vines and twenty vines with galls were randomly selected in each of six plots in the vineyard. A plot consisted of four consecutive rows 20 m long. From 1987 to 1991 the shoot masses of individual vines were determined during pruning. In 1988 and 1990 the trunk diameter of each vine was determined with a caliper approximately 50 cm above soil level. The mean of two measurements at right angles to each other was taken as representative of the trunk diameter of a vine.

Yield of individual vines was determined from 1988 to 1992. During 1990 the disease severity (gall size) for each vine was estimated by determining the percentage of the trunk circumference covered by a gall. Diseased vines were divided into four categories according to gall size [percentage of trunk circumference affected (PTC)], *viz.* 0-10, > 10-30, > 30-50, > 50, in order to determine if gall size can be used as an indication of disease severity.

Total soluble solids (sugar content) of berries was estimated with a refractometer. Analysis of variance was carried out to test for the significance of differences between healthy and affected vines.

RESULTS AND DISCUSSION

Percentage of vines affected: Galls appeared in 42% of the vines during the first season. No additional vines showed symptoms of infection during the five-year trial period. This is probably due to the fact that this area is not subjected to freezing temperatures. In addition the use of herbicides in rows, instead of mechanical practices, also prevented spread of the disease. All galls appeared at graft unions or on wounds at or above soil level where shoots had been removed. Soil from crown-gall-infected vineyards contains very low levels of AT 3 and is not an important source of infection (Burr & Katz, 1992). These results also indicated that infested propagating material was the most important factor responsible for the spread of AT 3 infection. This fact clearly illustrates that quarantine measures will remain ineffective unless a simple method by which latent infections can be detected is developed. Jäger *et al.* (1989a) suggested gas chromatographic analyses of the whole cell fatty acids of bacteria, isolated from grapevine material, as a possible routine method to examine propagating material for latent infections of AT 3. Isolations from grapevine propagating material can be made on a selective medium, but according to Stellmach (1991) colonies of other endophytic bacteria may appear similar to colonies of AT 3 and may constitute 90% of the colonies in isolations from grapevine material. However, according to Jäger *et al.* (1989b), AT 3 seems to prefer grapevine rind tissue as its main living area in the plant. This fact together with fatty acid analysis may therefore be used as a tool to detect AT 3 in vine propagating material. Important deductions can be made from the fact that the infection percentage remained static during the trial period. At this stage of the project it is clear that infection is not spead during pruning. Root damage seemingly does also not present a pathway for infection. The use of a herbicide within the row prevented damage to the rootstock trunk and clearly also prevented spread of the infection.

Disease severity according to gall size: From Table 1 it is clear that most infected vines (85%) had small galls. This percentage of vines with small galls remained approximately constant throughout the trial period (data not shown). In a large number of vines with galls < 30 PTC, infection had a stimulatory effect on shoot mass, yield and trunk circumference in comparison to mean values for healthy vines. It is also clear that the number of plants showing increased yield was higher than those showing higher shoot mass and trunk circumference. This indicates that the stimulating effect on yield was higher than on plant growth. In all vines with galls > 30 PTC stimulation was outweighed by the negative effects of the disease (Table 1). Schroth *et al.* (1988) recorded similar results for Zinfandel vines.

According to Schroth *et al.* (1988) cells infected with AT 3 produce more of the growth hormones auxin and kinetin than normal cells do. It is not clear why stimulation of the growth hormones affected only a certain percentage of the vines with galls < 30 PTC.

Trunk diameter: The mean trunk diameter of diseased vines was significantly smaller than those of healthy vines (Table 2). In spite of this significant reduction the practical effect on trunk diameter was small. From the data in Table 1 it can be calculated that for 30% of the vines in categories I and II trunk diameter was actually increased.

TABLE 2
Mean trunk diameter of crown-gall-infected and visually healthy Muscat d'Alexandrie vines.

Year	Trunk diameter/vine (cm)			
	Healthy	Diseased		Reduction (%)
1988	2,283	2,164	*	5,2
1990	3,056	2,849	*	6,7
Mean	2,669	2,507	*	6,0

A total of 120 healthy and 120 diseased vines were used per annum.
* Significantly different at $p \leq 0,05$.

TABLE 1
Effect of degree of crown gall infection on yield, shoot mass and trunk diameter of Muscat d'Alexandrie vines – 1990.

Infection category (%)	Mean shoot mass/vine (g)	[x]Percentage of diseased vines with higher shoot mass	Mean yield /vine (kg)	[y]Percentage of diseased vines with higher yield	Mean trunk diameter/ vine	[z]Percentage of diseased vines with bigger trunk diameter
I (52)	708,7	25,0	6,6	40,4	3,07	28,0
II (33)	813,9	37,2	6,4	44,2	3,15	32,4
III (9)	560,0	0,0	5,3	0,0	2,81	0
IV (6)	471,4	0,0	0,9	0,0	2,69	0

Numbers in brackets indicate percentage of vines in each category.
x – Percentage of diseased vines with higher shoot mass than the mean of healthy vines.
y – Percentage of diseased vines with higher yield than the mean of healthy vines.
z – Percentage of diseased vines with bigger trunk diameter than the mean of healthy vines.
I = < 10% of trunk covered by gall.
II = > 10-30% of trunk covered by gall.
III = > 30-50% of trunk covered by gall.
IV = > 50% of trunk covered by gall.

TABLE 3
Mean shoot mass of crown-gall-infected and visually healthy Muscat d'Alexandrie vines for each of four seasons.

Year	Shoot mass/vine (g)			
	Healthy	Diseased		Reduction (%)
1987	333,0	274,3	*	17,6
1988	482,5	417,7	*	13,4
1989	549,0	422,0	*	23,1
1990	826,0	721,6	*	12,5
1991	908,0	703,0	*	22,6
Mean	620,6	509,2	**	18,0

A total of 120 healthy and 120 diseased vines were used per annum.
* Significantly different at $p \leq 0{,}05$.
** Significantly different at $p \leq 0{,}01$.

TABLE 4
Mean yield of crown-gall-infected and visually healthy Muscat d'Alexandrie vines over three seasons.

Year	Yield/vine (kg)			
	Healthy	Diseased		Reduction (%)
1988	7,770	7,361		5,3
1989x	3,370	3,099		8,0
1990	7,266	6,175	*	15,0
1991x	6,770	5,625		16,9
1992	10,131	6,736	*	3,9
Mean	6,135	5,545	**	9,6

A total of 120 healthy and 120 diseased vines were used per annum.
* Significantly different at $p \leq 0{,}05$.
** Significantly different at $p \leq 0{,}01$.
x Mean of 3 blocks (60 healthy and 60 diseased vines).

Shoot mass: The shoot mass of diseased vines was significantly lower than that of healthy vines in all seasons (Table 3). These results further indicate the importance of crown gall in reducing the vigour of grapevines. A reduction in shoot mass was also found in Zinfandel vines infected with AT 3 (Schroth *et al.* 1988).

From the data in Table 1 it can be calculated that for 30% of the vines in categories I and II vine vigour was stimulated as is illustrated by shoot mass. Severe reduction in vigour occurred only in vines within categories III and IV.

Yield: In contrast to shoot mass, diseased vines produced a significantly lower yield only during the 1990 and 1992 season (Table 4). A large reduction in shoot mass was recorded in 1989 and 1991.

As a particular season's yield is already initiated in the buds during the previous season, this might explain the lower yield during the 1990 and 1992 season. However, percentage yield reduction showed an increasing trend from 1988 to 1992. It is interesting to note that 42% of the vines in categories I and II showed yield stimulation.

No significant differences could be found regarding sugar content of berries between diseased and healthy vines (data not shown).

CONCLUSIONS

The results of the trial clearly indicate the negative effect of crown gall on vigour and yield of Muscat d'Alexandrie vines. The relatively susceptible rootstock cultivar, 99 Richter, may have contributed to this effect.

Although vines were planted in virgin soil, 42% were infected after the first season. This indicates that the plant material must have been infected before planting. Infected propagation material is accepted to be the major cause promoting the spread of AT 3 infection. Development of a rapid detection method which would ensure that all propagation material is AT 3 free, should therefore receive urgent attention. In this specific vineyard, which is used for the production of raisins, an estimated yield loss of 4,0% or R360,00 per ha occurred. To limit the spread of the disease in an infected vineyard and thereby limit losses, the producer should minimise the risk of damage to vine trunks. This can be achieved by avoiding mechanical action in the rows by using herbicides for weed control. Removal of shoots from the rootstock section of the trunk should always be done with sterile shears.

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