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OCCURRENCE OF PHOMOPSIS CANE AND LEAF SPOT DISEASE OF GRAPES IN SRI LANKA AND ITS' MANAGEMENT

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ABSTRACT

A new disease of grapes was found to cause significant crop damage in 2008 and 2009 in Sri Lanka, where in some fields the damage was as high as 80 to 100 %. A severe outbreak of this disease was first observed in Rajanganaya and Galnewa area in Sri Lanka. Typical symptoms include brown, circular lesions surrounded by pale yellow areas on the leaves and brown to black lens-shaped lesions on the first three to four internodes of green shoots. Stems were partially or entirely girdled and the stems become stunted resulting in death. Causal organism responsible for this disease was identified as the fungus *Phomopsis viticola* (Sacc.) Sacc. and its pathogenicity was confirmed. Thus, the Phomopsis Cane and Leaf Spot (PCLS) disease caused by *P. viticola* (Sacc.) Sacc. appears to be a new record in grape cultivation in Sri Lanka. The PCLS disease can be controlled by integrating crop sanitation measures and fungicide application. Varietal screening studies showed that the grape variety 'French MI' was moderately resistant to PCLS disease. Use of pathogen-free cuttings is also important to control the disease. Satisfactory control of the disease was obtained with three applications of chlorothalonil 500 g/l SC, mancozeb 80 % WP or copper 50 % WP after pruning. A potential long term effect of application of chlorothalonil 500 g/l SC and mancozeb 80 % WP was also evident in this study.

KEYWORDS: Cane and Leaf Spot, Disease Management, Grapes, *Phomopsis viticola*

INTRODUCTION

Foreign exchange spent on the importation of grapes is increasing due to high consumer demand. During the year 2006, Sri Lanka imported 3500 mt of fresh fruit for table purposes at a cost of 326 million rupees (AgStat, 2008). Grape (*Vitis vinifera* L.)cultivation has also become popular in Sri Lanka during the past few years due to the increasing demand and hence, higher commercial value. Unfavourable climatic conditions and high incidences of diseases can result in substantial losses in grape production (Kothalawala, 1966). Fungal diseases cause severe foliage damage when weather conditions favour the disease development. *Plasmopara viticola* the causative agent of downey mildew disease, is endemic to the country and has been considered to be a major contributing factor for heavy yield losses (Anonymous, 2004). Powdery mildew caused by *Uncinula necator* has also been reported to cause severe foliage damage (Anonymous, 2004). Until 2007, these two diseases have been reported as the most serious fungal diseases affecting the grape cultivation in Sri Lanka. However, a previously unrecognized disease of grapes in Sri Lanka was found to cause significant damage in the *maha*

seasons of 2007/2008 and 2008/2009. In some fields damage was as high as 80 to 100 %. A severe outbreak of this disease was first observed in Rajanganaya and Galnewa in Anuradhapura district and Ilasalaka in Matale district during January to March 2008 and a few months later in Polonnnaruwa, Dambulla and Mahailluppallama area in Sri Lanka. Crop losses could be in the forms of shoots breaking off near the base where the lesions formed, reduced growth in shoots, loss of vigour, smaller bunches, and infected fruit bunches. The purpose of this study was therefore, to identify the disease symptoms, the causative agent responsible for this disease, and the disease control strategies.

MATERIALS AND METHODS

Isolation of pathogen

Specimens collected from affected vines were used for isolation of the pathogen. Small pieces of infected parts (5 mm^2) were surface sterilized with 1 % sodium hypochlorite (NaOCl) solution (5 % w/v) for 3 min, followed by washing in three changes of sterilized distilled water. The sterilized pieces were then aseptically transferred to Petri plates containing Potato Dextrose Agar (PDA) and incubated at 25±1°C under continuous fluorescent light for 5 to 10 days. A series of subcultures were made to obtain pure cultures of the pathogen using single spore isolation technique (Chattopadhyay, 2003).

Pathogenicity test

Spore suspension of pathogens prepared from 10 day old culture plates were used for the experiment. Spore suspension was prepared by adding 20 ml of sterilized distilled water gently to the plates, and careful scrapping to remove spores. Spore suspension was then filtered through a double layer of muslin cloth under aseptic conditions to remove the mycelial fragments and agar. The concentration of the suspension was adjusted to $1x10^6$ spores per ml.

Three-month old grape vines grown in an autoclaved soil were used for plant house inoculations. Plants were inoculated by spraying them with the spore suspension and each pot was covered with a moist polythene sheet immediately after the inoculation to ensure high humidity, followed by incubation at 25±1°C. Five plants (replicates) from each variety were used for this experiment. An equal number of plants were also inoculated with sterilized distilled water, to be used as the control. Re-isolations were made from the leading edges of lesions and the cultures were identified by inducing sporulation in the same manner as described above to fulfill Koch's postulates. Species identification was based on morphological and cultural characteristics, formation of the teleomorph in culture and disease symptoms.

Transmission studies

Transmission through crop debris

Infected crop debris was incorporated to pots containing two-month old grape plants grown in sterilized soil. Plants in pots containing only sterile soil were used as the control. Each pot was covered with a moist polythene sheet to ensure high humidity. Plants were maintained in a plant house and examined for diseases symptoms.

Transmission through cuttings

Cuttings obtained from healthy and infected plants of grape variety 'French MI', Muscut MI and Israel blue varieties were grown in pots containing sterilized soil. Ten cuttings per each variety were maintained in a plant house and examined for diseases symptoms. The disease incidence was calculated by counting the number of affected plants as follows.

$$\text{Per cent Disease Incidence} = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \quad X\ 100$$

Experiment was arranged in a complete randomized design (CRD), data were statistically analyzed, and the significance of difference was tested at p=0.05.

Variety evaluation

Three-year old grape varieties, namely French MI, Muscut MI and Israel Blue, growing in a research field at the Field Crops Research and Development Institute (FCRDI) at Mahailluppallama, Sri Lanka, were screened under natural infection. Experiment was arranged in a randomized complete block design (RCBD) with three replications. The disease scoring was done using five plants from each variety. The five basal internodes and five basal leaves on 10 shoots per vine were rated. Direct estimation of percentage of diseased area was used for internode disease assessments. The disease severity of leaves was assessed by estimating the number of lesions on each leaf using a scale with five levels (Table 1).

The disease severity was calculated using the following formula;

$$DS\ \% = \frac{\Sigma W \times N_s}{T \times N_m} \times 100$$

where, DS = disease severity, ΣW = number of affected plants, N_s = severity scale, T = total number of observations, and N_m = maximum scale number (4).

Table 1. The scale used to measure disease severity

Rank	Scale
Highly Resistant (HR)	0 : No incidence
Resistant (R)	1 : 1-5 % leaf area affected
Moderately Resistant (MR)	2 : 6-25 % leaf area affected
Susceptible (S)	3 : 26-50 % leaf area affected
Highly Susceptible (HS)	4 : > 50 % leaf area affected

Fungicide evaluation

Two grape fields infected with the disease were chosen for fungicide evaluation studies. Three-year old grape cultivation located in Rajanganaya area was used for the first experiment during *maha* season 2007/2008. Fields located at the FCRDI at Mahailluppallama, Sri Lanka were used for the second experiment during *maha* season 2008/2009. Chlorothalonil 500 g/l SC, mancozeb 80 % WP and copper 50 % WP were sprayed at the rate of 15 ml, 20 g, and 40 g per 10 litres of water, respectively. An unsprayed treatment was included in all tests. Application of fungicides commenced just after pruning and continued for further two times at 10 day intervals. Experiment was conducted in a RCBD, with three replicates. Each replicate consisted of three vines. Plants were monitored for infection. The canes were assessed for the disease and the data were analyzed as described above.

Effect of fungicides on sporulation

To assess the effects of the treatments on subsequent sporulation, three infected canes per vine were collected at the end of the growing season in 2008. Each cane was cut into 10 cm sections and then placed in a moist chamber at 25±1°C for 2 weeks to induce sporulation. Sporulation of pathogen was assessed by counting the number of pycnidia per square centimeter of cane. A total of five, 1 cm^2 sections per treatment were assessed.

RESULTS AND DISCUSSION

Disease symptoms

The disease was first observed in a luxuriously growing grape shoots just after pruning in *maha* season 2007/2008. Initial symptoms of the disease on leaves appeared as minute, irregular, light green or chlorotic spots surrounded by yellow haloes (Figure 1). The enlarged spots were dark brown. Under severe infection leaves became distorted and parts of leaves died. Severely infected leaves and leaves with heavily infected petioles resulted in

premature fall. Symptoms on shoots, stems and petioles appeared first as minute chlorotic spots which later turned brown with dark centers (Figure 2). These spots gradually enlarged into brown to black scars which cracked during cane swelling. Stems were partially or entirely girdled and the stems became stunted subsequently resulting in death. Pycnidia were evident in dead tissues. The fungus-invaded inflorescences were subjected to drying and falling off (Figure 3). Severely affected canes were weak and were prone to breakage.

Isolation

On PDA, the fungal colonies were initially hyaline and turned black as the colony reached approximately 14 days. The culture was sectored in black and white mycelium. Mycelium was septate, branched and formed a dense mat. Pycnidia were black in colour and were seen as irregular dark parts of the mycelium mat either singly or in clumps. Pycnidia were discoid in early stages of development which turned globose at maturity. Two types of pycnidiospores were produced. Alpha spores have a pointed apex, single celled, hyaline with acute ends and a large guttule at each end. Beta spores are long, curved and threadlike. Single spore isolates from pathogen yielded cultures identical to *Phomopsis viticola* (Sacc.) Sacc. (syn. *Fusicoccum viticola* Reddick) as described by Pine (1959). Identity of the causative agent was confirmed using the description of Commonwealth Mycological Institution (Punithalingam, 1979).

Pathogenicity test

Pathogenicity tests conducted in the plant house revealed that artificially inoculated plants produce symptoms identical to those in the field seven days after inoculation. All inoculated plants exhibited disease symptoms and the percentage of affected shoots ranged from 30 to 85 %. Infected shoots were removed and placed in a dark, moist chamber at 25±1°C. Within 2 weeks, Pycnidia were observed. No disease symptoms or fruiting bodies were observed on the controls. Based on the microscopic examination, morphological characteristics of pycnidia, pycnidiosopres and the disease symptoms, the pathogen was identified as *Phomopsis viticola* (Sacc.) Sacc. (Perfect stage: *Cryptosporella viticola* Shear). A similar disease has been reported from many grape growing countries and it has been described as Phomopsis Cane and Leaf Spot (PCLS) disease (Lal *et al.*, 1982; Pine, 1958; Scheper *et al.*, 2000). Thus, the PCLS disease caused by *Phomopsis viticola* (Sacc.) Sacc. appears to be a new record in grape cultivation in Sri Lanka.

Figure 1. Leaves affected with Phomopsis cane and leaf spot disease

Figure 2. Phomopsis cane and leaf spot symptoms on stem

Figure 3. Inflorescence affected with Phomopsis cane and leaf spot disease

Transmission through crop debris

In about 7-10 days the typical disease symptoms developed on cuttings grown on sterile soil incorporated with crop debris. None of the control plants exhibited disease symptoms. This suggests that *P. viticola* can successfully thrive in affected plant parts. Pearson and Goheen (1988) pointed out that removing diseased and dead wood during pruning could reduce the infection.

Transmission through cuttings

More than 25 % of the cuttings obtained from affected plants of Israel blue showed symptoms of the disease one month after potting. Nearly all cuttings (71.67 %) obtained from affected plants of the variety 'Muscut MI' showed disease symptoms. However, only 12.25 % of cuttings obtained from affected plants of the variety 'French MI' displayed disease symptoms. None of the cuttings obtained from healthy plants showed any symptoms of the disease. The results of this experiment are summarized in Table 2.

These results (Table 2) confirm that PCLS disease is transmitted by infected cuttings and the degree of transmission depends on the variety. Since, grape plants are mainly propagated by cuttings, eradication of the disease appears to be feasible. Hence, the use of disease-free cuttings is one of the key activities in preventing the disease. Clarke and others (2004) reported a varying degree of incidence of *P. viticola* in cuttings of different cultivars and from different sources.

Table 2. Percentage of cuttings of three varieties showing symptoms of PCLS disease

Variety	*Origin of cuttings*	*Percentage cutting showing symptoms*
Israel blue	Affected mature plants	31.57 b *
	Healthy mature plants	0 d
Muscut MI	Affected mature plants	71.66 a
	Healthy mature plants	0 d
French MI	Affected mature plants	12.25 c
	Healthy mature plants	0 d
CV %		10.98

* Means followed by the same letter are not significantly different at p=0.05

Varietal evaluation

Three varieties were rated for their reactions to a natural field epiphytotic of disease. No variety was completely free of symptoms, although some symptom-free plants were found in each variety. The variety 'Israel blue' showed severe symptoms in the first experiment conducted during *maha*

season 2007/2008. The varieties 'French MI' and 'Muscut MI' appeared to be moderately resistant with less than 25% of the leaf area and nodes showing symptoms. However, 'Muscut MI' developed severe symptoms in the second experiment as did the 'Israel blue'. As shown in Table 3, the disease incidence and severity of 'Israel blue' and 'Muscut MI' ranged from 30.95 - 47.3 % and 10.22 – 42.8 %, respectively, and rated as susceptible to PCLS disease. The variety 'French MI' had a significantly low disease incidence in both experiments and was rated as moderately resistant to the PCLS disease.

Table 3. Performance of grape varieties against PCLS disease under natural infection during *maha* season 2007/2008 and *maha* season 2008/2009

Variety	*Maha 2007/2008*			*Maha 2008/2009*		
	Disease severity (Leaf)	*Disease incidence (node)*	*Reaction*	*Disease severity (Leaf)*	*Disease incidence (node)*	*Reaction*
Israel blue	30.95 a *	47.1 a	S	30.04 a	47.3 a	S
Muscut MI	12.25 b	10.22 b	MR	28.41 a	42.8 a	S
French MI	7.16 b	8.56 b	MR	10.33 b	9.12 b	MR
CV%	16.84	18.3		30.83	17.3	

* Means in each column followed by the same letter are not significantly different at p=0.05.

Fungicide evaluation

In *maha* season 2007/2008, the incidence of PCLS disease was significantly less in the sprayed plots than in the unsprayed controls, but there were no differences among sprayed plots (Table 4). The disease severity observed in all the plots was higher in *maha* 2008/09 when compared to that of *maha* 2007/08. Satisfactory control of the disease was obtained with three applications of chlorothalonil 500 g/l SC or mancozeb 80 % WP or copper 50 % WP. Application of fungicides for the effective management of PCLS disease of grapes has been demonstrated by many scientists (Nita *et al.*, 2006, Cucuzza and Sall, 1982).

Table 4. Disease severity of PCLS after application of different fungicides

Fungicide	*Maha 2007/2008*		*Maha 2008/2009*	
	Location 1	*Location 2*	*Location 1*	*Location 2*
Chlorothalonil 500 g/l SC	2.84 b*	1.7 b	5.2 b	4.82 b
Mancozeb 80% WP	1.40 b	2.9 b	4.1 b	4.26 b
Copper 50% WP	2.73 b	2.33 b	4.23 b	7.8 b
Control	27.2 a	32.2 a	45.6 a	56.36 a
CV%	10.12	12.54	18.23	21.23

* Means in each column followed by the same letter are not significantly different at p=0.05.

Effect of fungicides on sporulation

There were significant reductions in the number of mature pycnidia at the end of the season with fungicides chlorothalonil 500 g/l SC and mancozeb 80 % WP applications (Table 5). Reduction in the number of pycnidia was about 45 % in the treated plants when compared to the control. The copper fungicide did not show a significant reduction ($p>0.05$) in pycnidia production compared to the control. A potential long term effect of application of chlorothalonil 500 g/l SC and mancozeb 80 % WP was also evident as it lowers inoculum density on canes.

Table 5. Number of mature pycnidia of *P. viticola* per cm^2 of grape cane after the growing season

Fungicide	*Pycnidia/ cm^2*
Chlorothalonil 500 g/l SC	4.62 b*
Mancozeb 80% WP	4.93 b
Copper 50% WP	11.29 a
Control	11.63 a
CV %	9.83

* Means in each column followed by the same letter are not significantly different at p=0.05

CONCLUSIONS

The causal organism of the Phomopsis Cane and Leaf Spot (PCLS) disease, which was reported for the first time in Sri Lanka on grapes, was identified as *Phomopsis viticola* (Sacc.) Sacc. The PCLS disease can be controlled effectively by integrating sanitation practices with application of fungicides. Shoots with typical lesions and dead wood must be removed when pruning, since these lesions provide most of the inoculum for new infections. Of the grape varieties tested, 'French MI' was moderately resistant to PCLS disease. Use of pathogen-free cuttings is also important to control the disease. Satisfactory control of the disease was obtained with three applications of chlorothalonil 500 g/l SC, mancozeb 80 % WP or copper 50 % WP, after pruning. A potential long-term effect of application of chlorothalonil 500 g/l SC and mancozeb 80 % WP was also evident in this study.

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