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Short communication

Disease reaction of apple germplasm to white root rot (*Dematophora necatrix*)

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ABSTRACT

Rooted suckers of 22 *Malus* species were screened for their reaction to white root rot (*Dematophora necatrix*) under artificially inoculated field conditions. Observation on the rate of disease development comprised of foliage chlorosis, wilting and defoliation was recorded *in situ*. The plants were uprooted from the sick plot 30 days after the inoculation and measurement for necrosis on stem bark, wood, vascular region and mycelial colonization on the infected parts were recorded. Results showed that none of the species exhibited immune reaction. However, some of the species like *M. prunifolia* var. *ringoasami* and *M. purpurea* could delay the expression of disease symptoms indicating the tolerance to disease. *Malus baccata* from Kashmir and Shillong restricted the disease development to some extent better than the other indigenous crab apples. These three *Malus* species were further evaluated at variable disease pressure. Marked differences with respect to disease progress curve and mortality rate was observed at lower disease pressure. Maximum tolerance was recorded in *M. prunifolia* var. *ringoasami* followed by *M. purpurea* at low disease pressure.

Key words: White root rot, *Dematophora necatrix*, *Malus baccata*.

White root rot caused by *Dematophora necatrix* (*Rosellinia necatrix*) is a serious threat to the economic cultivation of temperate fruits. The incidence of white root rot in Himachal Pradesh has been reported to be 15% in adult trees and 33% in nurseries and had caused a loss of 1.3 million rupees in 1966 (Agarwala and Sharma, 1). The fungus has a broad host range of 144 plant species including all the temperate fruits (Behdad, 3). The disease is soil borne and mortality varied from 47.2 to 51.2% in M9 and crab apple rootstock, respectively when planted in the newly reclaimed forest soils (Ram and Randhawa, 3). Efforts are being made throughout the world to identify resistance source and develop resistant rootstock to combat the disease. Different species of *Malus* native to Himalayas and different rootstocks of Malling (M) and Malling Merton (MM) series have been collected and maintained at the Indian Agricultural Research Institute's Regional Station at Shimla. Among these, 22 *Malus* species/ rootstocks were evaluated for their reaction to white root rot under field conditions.

An isolated field plot was selected and rooted suckers of 22 *Malus* species and rootstocks were planted during their late dormancy stage (February). A distance of 60 cm and 30 cm between rows and plants respectively was maintained. In each species two replications were maintained comprising of seven rooted suckers in each. Inoculation was made during July when plants were well established and had attained normal growth. To maintain uniform quantity of inoculum mycelium of *D. necatrix* was cultured on wheat grain and introduced in the rhizosphere of each plant by opening the furrows on both sides of planted suckers (Sharma and Kishore, 10). Field was irrigated after inoculation and moisture was maintained above the field capacity. Un-inoculated check plants were also maintained. Observations on the development of disease symptoms (syndrome) comprising of leaf chlorosis, wilting and defoliation were recorded *in-situ* at 2 day interval. To measure the diseased area on different *Malus* species, suckers were uprooted from the inoculated field plots 30 days after inoculation. Soil debris was removed by washing in running water. Observations on the necrotic area on the stem bark and wood (after peeling of the bark) were recorded. To record the colonized area by fungal mycelium on the infected part, uprooted suckers were incubated in moist environment chamber at 25-30°C for 48 h to promote regrowth of root rot mycelium to facilitate the observation of colonized area of the host. The species exhibiting significant delay in expression of various disease syndromes were further evaluated under variable disease pressure which was created by varying the inoculum quantity, *i.e.*, 5, 10, 25, 50 and 100 wheat grain inoculum per rooted sucker. The experiment was conducted with three replications with three plants under each treatment. Leaf chlorosis, rate of defoliation was recorded at different time intervals. Mortality of affected suckers was recorded

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in succeeding spring season by observing the appearance of new leaves.

Observations revealed that mycelial growth from wheat grain inoculum colonized the root and collar region within a week. The first symptom of the disease development consisted of chlorosis on the leaf margins and interveinal area was noticed 10 days after the inoculation. Gradually affected leaves showed complete necrosis, rolled upwards, and exhibited wilting and defoliation which varied with the different species. Later the bark near the collar region showed necrosis, which turned brown, and finally the plants collapsed.

Perusal of results showed that none of the tested germplasm (*Malus* species) exhibited immune reaction. However, different species varied with respect to incubation period required for the initiation and progress of disease syndrome comprising of leaf chlorosis, wilting and defoliation (Table 1). Among different species, *M. prunifolia* var. *ringoasami*) and *Malus purpurea* took longer time for the expression of these symptoms. On 12th day of inoculation no chlorosis was observed on these species whereas on the 18th day the extent of chlorosis was appreciably lower as compared to the other species. Similar pattern was observed in the appearance of wilting and defoliation. The extent of wilting and defoliation in these two species varied from 78.5 to 85.7% as compared to 100% in other species. It was interesting to observe the mycelium of *D. necatrix* growing on the stem bark as well as on the wood when bark was peeled off and shoots were incubated in the

Table 1. Rate of chlorosis, wilting and defoliation symptoms in different *Malus* spp. and rootstocks after inoculation with *Dematophora necatrix*.

Species	Chlorosis (% population)			Wilting (% population)			Defoliation (% population)		
	Days after inoculation			Days after inoculation			Days after inoculation		
	12th	18th	22nd	12th	18th	26th	18th	22nd	26th
Malus baccata (Kashmir)	28.6	64.3	100	0.0	15.4	100	7.1	14.3	100
M. baccata (Khrot)	21.4	85.7	100	0.0	64.3	100	7.1	50.0	100
M. baccata (Dhak)	0.0	85.7	100	0.0	14.3	100	0.0	14.3	100
M. baccata (USA)	78.6	92.8	100	21.4	78.6	100	64.3	100	100
M. baccata (Shillong)	26.7	66.5	100	0.0	16.3	100	8.7	15.8	100
M. baccata (Kinnaur)	44.3	100	100	24.3	78.6	100	14.3	58.7	100
M. crimson brilliant	62.0	91.5	100	25.0	55.8	100	14.3	35.7	100
M. spectabilis	28.6	100	100	7.1	100	100	100	100	100
M. purpurea	0.0	57.1	100	0.0	14.3	78.5	0.0	14.3	78.5
M. prunifolia var *ringoasami* (Saishi-E)	0.0	35.7	100	0.0	0.0	78.5	0.0	14.3	85.7
M. prunifolia	42.9	85.7	100	14.3	71.4	100	0.0	42.8	100
M. micromalus (Nagasaki zumi)	39.2	100	100	16.6	71.4	100	7.1	35.7	100
M. zumi	21.4	71.4	100	0.0	50.0	100	7.0	21.4	100
M. prunifolia (Maruba)	22.6	68.9	100	0.0	47.5	100	14.3	35.7	100
M. eseltine	21.4	71.4	100	21.4	42.9	100	14.3	42.9	100
M. sieboldii (Sanashi-62)	30.9	100	100	0.0	22.6	92.8	21.4	57.1	100
MM-106	35.7	85.7	100	21.4	42.9	92.8	14.3	28.6	100
M-4	39.2	100	100	14.3	42.9	100	14.3	64.3	100
M-9	85.7	100	100	75.7	100	100	35.7	100	100
M-26	30.9	100	100	16.6	47.5	100	0.0	48.6	100
M-27	47.9	100	100	62.4	100	100	14.3	64.3	100
M-7	84.4	100	100	74.9	100	100	21.4	100	100

moist chamber for 48 h. Data on stem area colonized by the mycelium (Table 2) revealed that different tested species do not exhibit significant differences as far as mycelium growth on the infected part is concerned. Therefore, mycelium colonization cannot be considered as an index for relating the tolerance level of different *Malus* species. Growth of mycelium below the stem bark further reveals that plants could be killed not only by attacking the roots but also by infecting stem bark as well as cambium (phloem) of the plants.

The white root rot fungus produces toxic metabolites which are translocated to different plant parts to cause necrosis (Gupta and Gohain, 5). On the bark and vascular region black coloured mark varying in its width from hairline to a narrow strip was observed to run parallel to each other. The length of black line varied with the tested species. Observations recorded on the necrosis on the stem bark and vascular region showed that different tested species exhibited significant difference whereas necrosis on the wood varied non-significantly among different species (Table 2).

Though screening was conducted at high disease pressure yet these species exhibited differential disease reaction. Subsequently, these were evaluated under variable disease pressure created by inoculating 5, 10, 25, 50 and 100 grains of inoculum/plant. Comparison of disease progress curve showed that host species do exhibit differential reaction at variable disease pressure, maximum delay in the expression of disease symptoms were found in *M. prunifolia* var. *ringoasami* followed by *M. purpurea* at each inoculum density. Amongst the indigenous crab apples, *M.*

Table 2. Mycelial colonization, necrosis on stem, wood and vascular bundles (mm) in different apple germplasm caused by field inoculation with *Dematophora necatrix*.

Species	Mycelial growth	Necrosis on		
		Stem bark	Wood	Vascular region
Malus baccata (Kashmir)	161.3	169.9	269.9	255.7
M. baccata (khrot)	180.7	183.3	264.3	288.6
M. baccata (Dhak)	202.3	177.3	322.2	352.0
M. baccata (USA)	178.7	186.8	334.6	289.5
M. baccata (Shilong)	204.8	253.3	392.7	422.5
M. baccata (Kinnaur)	177.9	159.9	307.7	378.8
M. crimson (Brilliant)	219.8	205.3	331.4	436.0
M. spectabilis	174.5	257.5	269.8	278.7
M. purpurea	178.9	178.4	346.1	403.0
M. prunifolia (var. *ringoasami*) (Saishi-E)	159.0	168.3	325.7	311.1
M. prunifolia	157.3	184.5	272.7	231.2
M. micromalus (Nagasaki zumi)	154.7	184.3	300.0	334.3
M. zumi	173.1	167.6	365.6	396.1
M. prunifolia (Maruba)	168.4	177.4	329.6	333.3
M. eseltine	157.5	161.4	291.3	292.4
M. sieboldii (Sanashi 62)	189.2	191.7	368.9	432.5
MM 106	181.3	195.6	328.9	321.0
M 4	165.1	180.1	240.3	270.1
M 9	201.8	255.8	267.0	247.6
M 26	176.7	185.5	358.3	331.5
M 27	123.7	168.8	287.4	257.9
M 7	117.7	221.7	276.5	291.1
CD at 5%	NS	51	108	103

baccata (Kashmir) and *M. baccata* Shillong showed tolerance to some extent as compared to others. Delay period with respect to disease expression was more pronounced at lower inoculum densities in all the three species. However, *M. prunifolia* var*. ringoasami* was found to possess better tolerance than the other species. Data on the mortality rate revealed that both inoculum density and host species reaction affected the mortality rate. Maximum mortality rate (66.6 - 100%) was observed at 100 grain inoculum/plant. Mortality rate had shown a decreasing trend with the reduction of inoculum density in all the host species (Table 3). It is evident from the results that some of the host species *M. prunifolia* var*. ringoasami* and *M. purpurea* possess tolerance against white root rot at lower disease pressure.

No significant success in host plant resistance has been reported against this disease so far. However, *M. floribunda* and *M. toringoides* had shown considerable resistance (Anderson, 20). The *Malus* species, *viz*., *M. baccata* (Rohru) and *M. baccata* (Khrot) reported as non-congenial resistant host by Ram (7) exhibited susceptible reaction in the present studies. It might be due to appropriate disease pressure created near the rhizosphere exhibiting disease reaction within 40 days, where as field evaluation method mentioned by Ram (7) took six years to conclude the results in sick plot. Gupta and Verma (5) reported the reaction of eight Malling (M) and Malling Merton (MM) series rootstocks under pot/ field conditions. None was reported to possess immune reaction but M7 and MM109 were reported as field resistant as these escape the mortality up to 500 days under field condition. Where as M2, M9, M13 and MM 106 were found susceptible in pot evaluation but under field conditions MM 106, MM 109 and MM 111 showed low mortality rate up to 200 days. The present studies revealed that all the six Malling and Malling Merton series rootstocks were found susceptible (Tables 1 & 2). It is evident from the results in certain instances of low inoculum level, species like Saishi-E (*M. prunifolia* var. *ringoasami*), *M. purpurea*, *M. baccata* from Kashmir and Shillong and can be utilized as tolerant rootstock in white root rot endemic areas and these can be included in the integrated disease management practices (Lopez-Herrera *et al*., 6; Rana and Gupta, 9) to over come this dreaded disease of apple.

It emanates from this that the reaction of a host species might have been related to its ability to tolerate the toxic affect of metabolites produced by the fungus which requires further investigation. Toxin produced by this fungus is thermostable and its production was found maximum from 7th to 11th day of fungal growth and its concentration was more in woody parts than diseased roots and bark (Gupta and Gohain, 4). The present studies also revealed that disease symptom expression initiates 10th day onward and delayed appearance of the symptoms exhibited by different species could be related with the tolerance of the host species.

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Table 3. Mortality rate of different *Malus* species which are tolerant to *D. necatrix* at variable disease pressure.

Number of grain inoculum	Mortality (%)			
	M. baccata (Kashmir)	*M. purpurea*	*M. prunifolia* (var. *ringoasami*)	*M. baccata* (Shillong)
100	100.0	100.0	88.8	100.0
50	100.0	88.8	44.4	100.0
25	88.8	55.5	22.2	88.8
10	66.6	22.2	0.0	66.6
5	0.0	0.0	0.0	0.0
Check (no inoculum)	0.0	0.0	0.0	0.0

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